

Guidelines to the Contributors

The journal publishes original research papers, review articles and short communications in all areas of agricultural sciences. The manuscripts will be accepted for publication from the members of the society. All the manuscripts will be referred. Though responsibility for any statement in the article rests entirely with the concerning authors. The manuscripts should be submitted to www.indianjournals.com and the copy of manuscript should also be mailed to progressiveagriculture@gmail.com. **A paper will not be accepted for publication unless all the authors are the fellow or the member of the Society for Recent Development in Agriculture.** A paper will not be published without printing/processing charges which will be @ Rs. 100/- per page. The authors are advised to prepare their manuscripts according to the following guidelines.

Preparation of the Text: Each full length research paper must not exceed 8-10 typed pages including tables and illustrations. However, the review articles may contain 15-20 typed pages. Short communication should not exceed 3-5 typed pages in which no separate abstract and other sections are needed. Manuscripts should be typed on one side of bond paper of 22x28 cm, double space and thoroughly revised before submission. Numerical data and calculations should be carefully checked. No editing or material changes at the proof state will be permitted unless the extra cost is paid by the authors involved.

Title: Title should be brief, specific, informative, typed in all capitals and scientific name in italics/underlined.

Address: Address of the institution where the work has carried out should be given below the name(s) of the authors).

Abstract: A brief abstract not exceeding 250 words, of the principal points and important conclusions should be typed.

Key words: The abstract should be followed by not exceeding ten key words indicating the contents of the research article.

Introduction : This should be brief and without heading, related to the aim of the study. The review of the literature should be pertinent to the theme of the paper. Extensive review and unnecessary detail of earlier work should be avoided.

Materials and Methods: It should inform the reader about appropriate methodology etc. but if known methods have been adopted only references be cited. It should comprise the experimental design and techniques.

Results and Discussion: It should be combined to avoid repetition. The results should not be repeated in both tables and figures. The discussion should relate to the significance of the observations.

Tables and Figures: Tables should be typed on separate sheet with first latter capital, table numbers followed by the title of the table. For each figure, a glossy print or original drawing should be submitted. The size of the figure should not exceed 8.5 cm x 15 cm Captions and legends to illustrations should be typed on separate sheet of the paper. Line drawings and photographs should contain figure number and authors name on the back with soft lead pencil. Colour photographs will be printed on special request and on payment of extra printing charges which will be communicated after receiving the specific request.

Acknowledgment: It should be mentioned only assistance received in real terms, and financial grant provided by an agency.

References: References should be cited in text by numbers in brackets (for review articles with author name will be cited in text). Listing of references by S. No. in accordance with their order of text citation. Author's names to be written in normal sequence, with surname first, followed by initials (in double space) and year.

Journal name/book name, volume number (underlined/italic) and page number. The following style of the references should be followed:

1. Johnson HW, HE Robinson, and RE Comstock (1955). Estimate of genetic and environmental variability in soyabean. *Agronomy. Journal*. 47:314-318.
2. Allard RW (1960). *Principles of Plant Breeding*, John : Wiley and Sons Inc. New York.
3. Palmer JD (1992). In *Cell Organelles* (R.O. Herman, Editor) springeru Verlag, Vienna. 99-133
4. Weber DR (1967). On the Intractions of Nohomologous Segments of Chromosomes in *Zea mays*. Unpubl. *Ph.D, thesis* Indiana University. Bloomington, Indiana USA (For Indian Universities, Country's name not to be indicated)
5. Chandola, R.P., Bhatnagar, C.P. and Sah. Sudha 1971. Nature of variability induced by radiations, In : *Proc. Int. Symp on use of Isotopes. Radiations in Agriculture and Animal Husbandry Research, New Delhi* 46-50

Reprints : No free reprints will be supplied. However, the reprints may be supplied to the author/authors on charges @ Rs. 175/-per printed page for 50 reprints. Extra reprints, if needed, should be ordered which will be supplied on advance payment. Reprints requirments should be indicated at the time of acceptance of manuscripts.

Published by : **Dr. Bijendra Singh**, Secretary and Chief Editor
on behalf of **Society for Recent Development in Agriculture**
B-94, European Estates, Near Best Price, Kankerkhhera Bypass, Meerut-250001, U.P. India
Mob.: 09149281328, 09412701340 Website : www.progressiveagriculture.in, www.indianjournals.com
E-mail: progressiveagriculture@gmail.com & drbijendrasingh66@gmail.com

R.N.I. No. 5368/2001

PRINT ISSN 0972-6152
ONLINE ISSN 0976-4615

Progressive Agriculture

An International Journal

Vol. 25, No. 2
August 2025



SOCIETY FOR RECENT DEVELOPMENT IN AGRICULTURE

Website :
www.progressiveagriculture.in
www.indianjournals.com

E-mail :
progressiveagriculture@gmail.com
drbijendrasingh66@gmail.com

Society For Recent Development in Agriculture
(Registered under Societies Registration Act XXI of 1860, Regd. No. 1004)
[Founded in August 2001]

The main object of the Society is to cultivate and promote research, education and development in the field of agriculture and allied branches.

EXECUTIVE COUNCIL FOR 2024-2025

Patron in Chief	—	Prof. Ramesh Chand
Patrons	—	Dr. D.V. Singh, Dr. J.P. Srivastava
President	—	Dr. P.S. Pandey
Vice President	—	Dr. A.K. Shukla
Secretary	—	Dr. Bijendra Singh
Joint Secretaries	—	Dr. Arvind Kumar, Dr. Vaishali
Assistant Secretary	—	Dr. AK Chauhan
Treasurer	—	Dr. Geeta Singh
Members	—	Dr. S.R. Singh, Dr. Sanjive Kumar Singh

ADVISORY BOARD

Dr. Mangala Rai, Ex. D.G., I.CAR., New Delhi	Dr. John Farrington, England
Dr. M.P. Yadav, Ex. V.C., S.V.P.U.A. & T., Meerut	Dr. Bijendra Pal, Hyderabad
Dr. H.S. Gaur, Ex. V.C., S.V.P.U.A. & T., Meerut	Dr. R.J. Hagger, England
Dr. Gaya Prasad, Ex. V.C., S.V.P.U.A. & T., Meerut	Dr. R.K. Chaudhary, New Delhi
Dr. S.K. Malhotra, Ex. Commissioner Agri., New Delhi	Dr. H. Eswaran, USA
Dr. R.P.S. Verma, Morrocco	Dr. A.R. Singh, Kanpur

EXECUTIVE COUNSELLORS

Dr. Mahak Singh, Kanpur	Dr. Ashok Kumar, Meerut	Dr. Vijay Kumar Umrao, Meerut
Dr. B.R. Singh, Meerut	Dr. Neelima Kunwar, Kanpur	Dr. Anju Singh, Meerut
Dr. Anju Verma, Meerut	Dr. Sangita Bansal, New Delhi	Prof. Samsher, Meerut
Dr. R.G. Somkuwar, Pune	Dr. Monee Verma, Meerut	Dr. Aarti Bhardwaj, Meerut

EDITORIAL BOARD

Chief Editor	—	Dr. Bijendra Singh, Meerut
Managing Editor	—	Dr. R.S. Sengar, Meerut
Managing Editor	—	Dr. Subhash Kumar, CCR(PG) College, Muzaffarnagar

EDITORS

Dr. Nidhi Verma, New Delhi	Dr. Vaishali, Meerut	Dr. Satish K. Yadav, New Delhi
Dr. Saurabh Sharma, Beed	Dr. A.K. Sharma, Karnal	Dr. Ramesh Kumar, Ludhiana
Dr. S.S. Gaurav, Meerut	Dr. Prerna Singh, Meerut	Dr. Rashmi Yadav, New Delhi
Dr. R.G. Somkuwar, Pune	Dr. R.K. Yadav, Kanpur	Dr. S.R. Singh, Jaipur
Dr. D.R. Singh, Kanpur	Dr. Deepak Kumar, Moradabad	Dr. Yeshpal Singh, Muzaffarnagar
Dr. Rakesh Kumar, Jaipur	Dr. Aditya Singh, Meerut	Dr. R.K. Naresh, Meerut
Dr. Reshu Chaudhary, Meerut	Dr. Shweta Tyagi, Muzaffarnagar	Dr. Moni Verma, Meerut
Dr. Pushpendra Singh, Noida	Dr. Poonam Kashyap, SS, ICAR-IIFSR, Meerut	Dr. A.K. Prusty, SS, ICAR-IIFSR, Meerut
Dr. Pratima Gupta, Meerut	Dr. Vipin Kumar, Meerut	

JOURNAL SUBSCRIPTION

Ordinary (Annual) Membership	—	Rs. 1000/-	100 US \$
Life Membership	—	Rs. 5000/-	400 US \$
Institutional (Annual) Membership	—	Rs. 2000/-	200 US \$
Institutional (Life) Membership	—	Rs. 15,000/-	1500 US \$
Corporate Membership	—	Rs. 15,000/-	1500 US \$
Admission Fee	—	Rs. 100/-	12 US \$

The life membership fee may be paid either in lump sum or in two/three equal installments within one year of enrolment, membership fee must be paid through Bank Draft and in favour of the **Society for Recent Development in Agriculture (SRDA)** and payable at **SBI, SVBP Uni. of Agri & Tech., Meerut and for e-payment A/c No.: 34054706599, IFCS Code: SBIN0010653, Branch Code: 10653.** Correspondence regarding membership subscription and any other matters on the journal should be addressed to Secretary, Society for Recent Development in Agriculture. B-94, European Estate, Near Best Price, Kankerkhhera By-pass, Meerut-250 001 (U.P.) India or e-mail to : progressiveagriculture@gmail.com & drbijendrasingh66@gmail.com

Advertisement Tariff (For one volume)

Size : Full Page Rs. 2000.00 (India) US\$ 50.00 (Abroad), Half Page Rs. 1000.00 (India) US\$ 25.00 (Abroad)

MEMBERSHIP FORM

Progressive Agriculture - An International Journal
Society For Recent Development in Agriculture

To
Dr. Bijendra Singh,
Secretary (SRDA)
B-94, European Estate, Near Best Price, Kankerkhhera, By-pass, Meerut-250 001, U.P. INDIA

Dear Sir,
Please enrol me as a Life/Annual Member of I am enclosing herewith my Biodata and a draft of Rs. (in words.....), having draft No..... Dated or Online transaction ID Dated Bank Name for Life/Annual Subscription & if enrolled, agree to abide by its rules and regulations.

1. Full Name (In capital letters) :
2. Designation :
3. Academic and professional qualification :
4. Specialization :
5. Institute/Organization where employed :

6. Address for correspondence :

City State
Pin Code Telephone no. With STD code
Office Residence
Mobile No. E-Mail
7. Permanent Home Address.....

City State
Pin Code Telephone no. With STD code
Office Residence
Mobile No. E-Mail

Dated Signature

The life membership fee may be paid either in lump sum or in twolthree equal installments within one year of enrolment, membership fee must be paid through Bank Draft and in favour of the **Society for Recent Development in Agriculture (SRDA)** and payable at **SBI, SVBP Uni. of Agri & Tech., Meerut and for e-payment A/c No.: 34054706599, IFCS Code: SBIN0010653, Branch Code: 10653.** Correspondence regarding membership subscription and any other matters on the journal should be addressed to Secretary, Society for Recent Development in Agriculture. B-94, European Estate, Near Best Price, Kankerkhhera By-pass, Meerut-250 001 (U.P.) India or e-mail to : progressiveagriculture@gmail.com & drbijendrasingh66@gmail.com

Journal Subscription

	Inland	Foreign
Ordinary (Annual) Membership	— Rs. 1000/-	100 US \$
Life Membership	— Rs. 5000/-	400 US \$
Institutional (Annual) Membership	— Rs. 2000/-	200 US \$
Institutional (Life) Membership	— Rs. 15,000/-	1500 US \$
Corporate Membership	— Rs. 15,000/-	1500 US \$
Admission Fee	— Rs. 100/-	12 US \$

R.N.I. No. 5368/2001

PRINT ISSN 0972-6152
ONLINE ISSN 0976-4615

Progressive Agriculture

An International Journal

VOLUME 25, NUMBER (2), AUGUST 2025



SOCIETY FOR RECENT DEVELOPMENT IN AGRICULTURE

Website : www.progressiveagriculture.in,
Email : progressiveagriculture@gmail.com,

www.indianjournals.com
drbijendrasingh66@gmail.com

PROGRESSIVE AGRICULTURE

Volume 25 (2)

August 2025

CONTENTS

RESEARCH/REVIEW PAPERS

1. Integrated Approaches for Screening Salt Tolerance in Sugarcane: From in Vitro Selection to Field Evaluation 118-133
Priyanka Chaudhary, Amit Kumar, Bijendra Singh and R. P. Singh
2. Enhancement in Yield and Quality Attributes of Litchi through the Foliar Application of NAA, Boron and Zinc Sulphate 134-140
Shivani, V.K. Tripathi and Akhilesh Mishra
3. Genetic Transformation of Grapevine using Agrobacterium-Mediated Gene Transfer 141-149
Aakansha Goswami, Bijendra Singh and R. P. Singh
4. Path Coefficient and Character Association Analysis of Yield-Contributing Traits in Cucumber (*Cucumis Sativus* L.) 150-155
Jahnvi Pal, Bijendra Singh, Satya Prakash, LK Gangwar, Vipin Kumar, Vaishali, Mayuri Gupta
5. Economic Feasibility of Wheat Cultivation under the Treatment of Pusa Hydrogel and Vermi-Compost 156-163
Sunil Kumar, Avinash Kumar Singh, Amit Kumar, Anuj Yadav, Bijendra Singh and R.P.Singh
6. Optimization of Plant Growth Regulators for in Vitro Callus Induction and Shoot Proliferation in the Natural Sweetener Stevia Rebaudiana (Bertoni) 164-168
Amit Kuma, Priyanka Chaudhary, Reshu Chaudhary, Bijendra Singh and R. P. Singh
7. Synergistic Effects of Selected Herbal Plant Extracts with Antibiotics Against Carbapenem-Resistant Escherichia Coli: A Systematic Review 169-177
Sandhya, Amit Kuma, Bijendra Singh and R. P. Singh
8. Enhancement in Fruit Retention, Yield and Quality Attributes of Aonla cv. NA-7 through the Foliar Application of Boron and Zinc 178-184
Gopal Gond and V.K. Tripathi
9. Economic Assessment of Agroforestry Systems in Saharanpur District of Western Uttar Pradesh 185-195
Divya Yadav, Charan Singh and Harikesh Singh
10. Genomics-Assisted Breeding for Drought Tolerance in Wheat (*Triticum Aestivum* L.): Recent Advances and Future Prospects 196-199
Amit Kuma, Shivani, Reshu Chaudhary, Bijendra Singh and R.P.Singh

11. Genetic Divergence Analysis in Indian Mustard [*Brassica Juncea* (L.) Czern & Coss] Genotypes 200-205
Sharad Kumar Singh Redu and Tejbir Singh
12. Ultra High-Density Plantation (UHDP) in India: Current Status, Opportunities, and Future Prospects 206-210
Sandeep Kumar and Everest Shiwach
13. Impact of Electric Vehicles on Traffic and Emissions 211-215
Aakansha Goswami, Bijendra Singh and R. P. Singh



INTEGRATED APPROACHES FOR SCREENING SALT TOLERANCE IN SUGARCANE: FROM IN VITRO SELECTION TO FIELD EVALUATION

Priyanka Chaudhary¹, Amit Kumar^{*1}, Bijendra Singh³ and R. P. Singh⁴

¹Department of Microbiology, KVSCOS, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh, India

³College of Horticulture, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India

⁴Former Vice Chancellor, Swami Keshwanand Rajasthan Agriculture University, Bikaner

***Corresponding Author Email-**amit.agbiotech1581@gmail.com

Abstract

Salt is one of the biggest threats to sugarcane (*Saccharum officinarum* L.) farming globally because millions of acres of farmland have salt issues that affect their production of sugarcane. Sugarcane as a glycophyte (a plant that needs to grow in a freshwater environment) is at great risk from the ionic and osmotic stresses associated with salinity, which reduce the amount of sugar cane produced and inhibit the efficiency of photosynthesis. The solution to this serious problem is to develop a coordinated, multi-pronged research program that integrates controlled in vitro screening, characterization of salt-tolerant genotypes physiologically and biochemically, evaluation in the field, molecular omics, and artificial intelligence-based decision support systems. This review highlights recent advances in these areas. Salt-tolerant genotypes can be identified rapidly and in high-throughput form using in vitro selection of callus and shoot tip cultures, temporary immersion bioreactor systems, and polyethylene glycol-induced osmotic stress. Physiological and biochemical research has elucidated the major adaptive mechanisms of sugarcane, which include osmotic adjustment, activation of antioxidant enzyme activity (*superoxide dismutase*, *catalase*, and *ascorbate peroxidase*), ion homeostasis through the SOS pathway, and the accumulation of compatible solutes such as proline. Through multi-location field trial data evaluation (using the AMMI and GGE statistical models), stable high-yielding cultivars (such as CoG 7) that are tolerant to salt have been identified. Molecular omic studies that employ genomics, transcriptomics, proteomics, metabolomics, and GWAS data analyses along with genomic selection and the CRISPR/Cas9 genome editing technique are being used to improve the accuracy of developing salt-tolerant crops. Additionally, phenotyping and agricultural management in saline environments are changing due to Technology advancements, such as AI, Machine Learning, and Remote Sensing. Finally, the review identifies three primary translational gaps associated with Breeding Salt Tolerant Varieties (disconnect between In vitro tolerance and Field performance, no standard phenotyping protocol, and the complex polyploid Sugarcane Genome) and highlights Priority Areas for the development of Climate-resilient high-yielding Salt Tolerant Varieties.

Keywords: *Saccharum officinarum*; salt tolerance; in vitro selection; genomic selection; CRISPR/Cas9; precision agriculture

Introduction

Sugarcane (*Saccharum officinarum*) represents one of the most important C (carbon)4 crops on the globe with ~80% of the world's total sugar produced from it, as well as being used as a

major source of feedstock for both bio-ethanol and lignocellulosic biomass. Its importance to both food security and to the transition to renewable energy systems is well documented. Nevertheless, deteriorating soil salinity, which is expected to

impact over 800 million hectares of arable lands by the year 2050, threatens sugarcane's yields globally, and more particularly gives cause for concern in irrigated agro-ecosystems in South Asia, Southeast-Asia and the Americas (14; 6).

Sugarcane is a glycophyte, making it very susceptible to high levels of NaCl (Sodium Chloride) and other salts found in the root zone. Salt stress produces two types of physiological damage: 1) Osmotic stress mimics drought conditions because soil water potential is decreased, limiting the ability of roots to take up water; 2) Ionic toxicity is caused by the accumulation of Na and Cl ions, which interfere with enzyme function, protein conformation (structure), and overall metabolism within the plant's cells. The combined effects lead to stunted growth, reduced leaf area, decreased chlorophyll, reduced photosynthesis-related productivity, and, finally, reduced cane and sucrose production. (41; 66).

The process of developing salt-resistant sugarcane varieties through traditional breeding methods has been extremely difficult because of the extreme complexity of the sugarcane genome; its high degree of heterozygosity, its allopolyploid and aneuploid genetics (with a genome size of $2N=100-130$), as well as its high ploidy levels (greater than $10x$), in addition to low fertility and long growth cycles, make it difficult to breed for salt-tolerant sugarcane (81; 75). Moreover, the complex nature of the sugarcane genome presents several problems that impede the use of conventional MAS/QTL mapping methodologies, thus requiring collaborative approaches that utilize molecular technologies, physiological characterization, and sophisticated analysis are needed to develop salt-tolerant varieties.

In light of the challenges associated with sugarcane production, an overall research approach to these problems has developed; and includes: (1) in vitro selection methods to quickly and precisely identify large quantities of plants; (2) identification of the physiological and biochemical basis of stress tolerance; (3) multi-yr/multi-env field experimentation with statistically sound design; (4) use of molecular/omic and precision genomic analyses in understanding the genetic architecture of species,

and the development of predictive tools for breeding; and (5) utilizing artificial intelligence and digital decision-support systems to monitor production and manage practices in real time. The objective of this review is to evaluate recent advances across each component of the five areas listed above, review knowledge gaps and to outline future research needs in order to develop high-quality, climate-resilient sugarcane varieties that are suited to saline environments.

Methodology

PRISMA's guidelines have been used to report on how we used these principles to conduct a systematic review of all available research pertaining to this topic as they relate to systematic reviews or meta-analyses. Thus, it provides a framework to demonstrate our transparency and ability to reliably identify literature related to the above areas by having adherence to PRISMA's guidelines.

A systematic review of the scientific literature was conducted using four online databases (PubMed, Scopus, Web of Science, and Google Scholar) covering the years from January 2018 to 2026. Articles were retrieved through the use of Boolean operators (AND, OR), and keywords: (sugarcane, *Saccharum officinarum*, salt stress, salinity tolerance, in vitro selection, tissue culture, genomic selection, genome-wide association study (GWAS), CRISPR, transcriptomics, metabolomics, field testing, artificial intelligence and machine learning) were used as search terms to create the search strings. For the purposes of providing an evidence base of high quality and rigor, priority was assigned to articles published in Q1 ranked journals as indicated by SCImago Journal Rank (SJR) and Journal Citation Reports (JCR).

Studies that qualified for inclusion were those that examined the response of *Saccharum* spp. and closely related species to salinity or sodium stress using quantitative and validated experimental methods. In addition to using these methods, they must have reported an answer using one of three approaches: cellular (or anatomical), physiological, molecular, or agricultural. Studies conducted using model organisms (*Arabidopsis thaliana* or *Oryza sativa*) could only be included if the results provided mechanistic information relevant to

sugarcane. Literature reviews, meta-analyses, and book chapters were included to help establish context and concepts for the primary literature. Two exclusion criteria were used to eliminate studies from consideration: 1) studies presenting only descriptive screening results without mechanistic characterization; and 2) studies that were based on unpublished sources (that is, gray literature).

Study selection. Following the removal of duplicates and the application of the eligibility criteria, more than 120 primary research articles and 30 review papers were assessed for relevance. Of these, approximately 90 publications met the inclusion criteria and were incorporated into the present review.

Literature Review

In Vitro Physiological Approaches for Salt Stress Screening

Tissue Culture and Callus-Based Screening Systems

In vitro selection is, therefore, an indispensable tool for a harmonious and quick identification of a reasonably tolerant sugarcane genotype to salt. It is basically a callus established from several types of explant sources like immature leaf sheaths, young whorled leaves, and shoot tips, growing in Murashige and Skoog (MS) basal medium supplied with plant growth regulators, usually the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) at concentrations of 1-3 mg/L (Iqbal et al., 2022; Maruprolu et al., 2022; Chen et al., 2024). The cultured callus is then exposed to graded concentrations of NaCl (generally between 50-300 mM) or mannitol as osmotic stress agents so that cell lines tolerant to osmotic stress can be selected based on their respective growth and regeneration potential along with biochemical markers (43; 77; 1).

Callus-based systems have the main advantage of being able to test thousands of explants at the same time in a controlled environment with respect to stress which is a large logistical constraint and subjected to environmental variability when doing field tests. Several studies have demonstrated that the rate of callus growth or the efficiency of regeneration of

callus from parent plant tissue under NaCl stress strongly correlates to the salt tolerance of whole plant phenotypes including but not limited to the Thai sugarcane cultivar KK3 (43). Callus cultures as well as shoot tip cultures have been used to measure salinity tolerance for elite varieties in relative terms by comparing the activities of three antioxidant enzymes; superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), between salt tolerant and susceptible varieties (77). Shoot tip cultures provide a more stable source of materials in comparison to somaclonal variants regenerated through a callus (Gajjar et al., 2022).

Advanced In Vitro Systems: Bioreactors and Osmotic Stress Simulation

Temporary immersion bioreactor (TIB) systems have made a major impact methodologically on sugarcane in vitro research, so that large-scale production may now be readily accomplished while providing programmable stress regimes. (47) demonstrated very strong correlations between NaCl and mannitol responses of sugarcane using TIB systems, demonstrating the value of osmotic stress surrogates and further establishing TIBs as highly-reproducible, high-throughput platforms for stress tolerance screening. PEG-induced osmotic stress (using PEG-6000; 5-20% w/v) has also been widely used to simulate drought and salt-mediated water deficit conditions. The use of PEG effectively lowers culture medium water potential without direct ionic interference while separating osmotic from ionic effect components (Hernández-Pérez et al., 2021; 68).

Nonetheless, in vitro systems are limited in that cellular tolerances do not equate to field level agronomic performance. The dynamic complexity of interactions between soil, roots and the microbiome; heterogeneous salinity gradients; and stage-specific responses to stress cannot be adequately simulated in in vitro culture systems (47). This translational limitation highlights the necessity for multi-step validation pipelines that transition systematically from in vitro screening through evaluation in greenhouses, onto multiple locations of field trials, and include standardized protocols for imposition of stress and measurement of phenotypes.

Physiological and Biochemical Mechanisms of Salt Tolerance

Osmotic Stress, Ionic Toxicity, and Primary Stress Responses

In sugarcane, salt stress produces two distinct phases of physiological response. During the first phase, the osmotic phase, the accumulation of solutes will produce an immediate vapour pressure gradient, resulting in stomatal closure through the action of abscisic acid (ABA), which will reduce transpirational water loss but at the same time restrict CO_2 influx and inhibit photosynthesis carbon fixation (63). High-intensity and long-term salinity results in the ionic phase of stress, which results in excessive accumulation of both Na^+ and Cl^- to toxic levels in the plant's shoot with harmful physiological effects, including inhibition of enzyme activity, disruption of protein structure, and the production of reactive oxygen species (ROS) (41; 33).

There have been significant decreases in plant height and other measurable morphological/physiological impacts on sugarcane as a result of the presence of salt stress; these include reduced leaf area index, stem diameter, and internode count (all of which together will ultimately determine the final cane yield) (64). Measurements of chlorophyll content by using SPAD (Soil Plant Analysis Development) value measurements has proven to be a reliable and effective method to measure photosynthetic ability and also indicate the level of saturation (or lack thereof) determining the amount of salt stress experienced by plant (43). Stress responses are further compounded by the existence of both soil salinity and drought conditions on sugarcane plants as evidenced by the multiple stress response responses exhibited by the Japanese cultivar NiF8 (90). The combined effects of saline irrigation resulted in multiple stress response interactions between the two stressors that would be expected, leading to further negative impacts on growth and yield (44)

Adaptive Mechanisms: Osmotic Adjustment, Ion Homeostasis, and Antioxidant Defense

To mitigate damage from salinity, different genotypes of sugarcane that can handle salty soil

utilize multiple mechanisms that are completely independent yet overlap considerably. Through the synthesis and accumulation of compatible solutes (mainly proline, glycine betaine and soluble sugars), osmotic adjustment allows for lower cellular osmotic potential without inhibiting metabolic activities and keeps a suitable water potential gradient between the cellular and sub-state of the water table for ongoing root water absorption (33). Osmoprotectants also stabilize the structure of enzymes and maintain the integrity of membranes during ionic stress conditions.

The second major section of adaptive ability describes ion homeostasis. Having favorable K^+ to Na^+ ratios in the cytoplasm is essential to preserve enzyme function and the electrochemical potential at membranes (16; 88). In plants, the SOS (Salt Overly Sensitive) signaling pathway, which consists of the calcium sensor SOS3, the protein kinase SOS2, and the Na^+/H^+ antiporter SOS1 located at the plasma membrane, forms the main active mechanism for Na^+ extrusion out of the cell (32; 55). The vacuolar sequestration of Na^+ by NHX-type Na^+/H^+ antiporters provides an additional ionic compartment in the cell, since vacuolar Na^+ can be used as a low-cost osmoticum while protecting the cytoplasm from ionic toxicity (33). Long-term salinity stress on subtropical sugarcane varieties has shown substantial variability in ionic homeostasis mechanisms and will lead to a better understanding of physiological mechanisms that will allow for improvement through breeding (16).

The third crucial adaptive mechanism through which organisms manage oxidative stress is through the use of inducible antioxidant defense systems. In response to salt stress, ROS are produced in excess amounts, and as a result, SOD (superoxide dismutase), CAT (catalase), APX (ascorbate peroxidase), and glyoxalase III are all upregulated in a coordinated manner. Glyoxalase III is responsible for detoxifying the cytotoxic compound methylglyoxal, which is produced under stress conditions (77; 51; 57). Additionally, nitric oxide (NO) acts as a multi-faceted signaling molecule that regulates the expression of antioxidant genes and works in conjunction with phytohormones to provide enhanced protection

from stressors (39; 76). Priming of seeds with salicylic acid (SA) has been shown to effectively pre-activate these defence mechanisms during early stages of crop development, allowing for improved establishment in saline environments (5). Furthermore, exogenous application of abscisic acid (ABA) to mature seedlings has been demonstrated to regulate the activity of antioxidant enzymes as well as the dynamics of phytohormones, thereby enhancing water stress tolerance (63).

Molecular Omics and Marker-Assisted Selection

Genetic Architecture Dissection: QTL Mapping and GWAS

The genetic area responsible for salt tolerance is the first step of the molecular breeding program. Traditionally used to determine QTL sites from biparental populations QLTs are typically located using chromosomal regions associated with some salt tolerance characteristic, such as the Na⁺/K⁺ ratio, relative water content (RWC) and cane yield under saline conditions. The statistical significance of these chromosomal regions may be determined using Logarithm of Odds (LOD) scores which are = 3.0 (61; Kumar et al., 2022). The integration of bulk segregant RNA-Seq analysis with high-density genetic mapping has provided substantial improvements in the resolution of QTLs associated with biotic stress traits in sugarcane and can be used as a methodological basis for identifying QTLs associated with abiotic stress (21).

Genome-wide association studies (GWAS) are a powerful tool for mapping the genetic basis of complex traits because they take advantage of historical recombination events that have happened across multiple germplasm panels and result in greater mapping resolution than biparental QTL studies. GWAS has also been successfully used to identify marker-trait associations in traits directly related to sugarcane juice quality and yield, which can provide us with the methodological templates to perform GWAS for salt tolerance (73; 87). It is important to note that the genetic basis of salt tolerance can be mechanistically divided into three

partially-independent but complementary components. The three components of salt tolerance are: i) Ion exclusion—the removal of Na⁺ from the xylem transpiration stream by HKT1;5 mediated transport; ii) tissue tolerance—the sequestration of vacuolar Na⁺ by NHX1-type antiporters; iii) osmotic adjustment—the accumulation of compatible solutes (26; 55). The effective dissection of the genetic basis of any trait must be achieved through trait-specific phenotyping protocols that are capable of distinguishing between the individual mechanistic subcomponents that contribute to the trait rather than treating salt tolerance as one large trait.

Multi-Omics Integration: Transcriptomics, Proteomics, and Metabolomics

Multi-omics techniques can take the static Nature of hereditary coordinates observed in QTL mapping & GWAS and allow scientists to create living & changing representations of these genetic coordinates as they impact biological processes and activity. This was shown by transcriptomic analyses comparing the salt-tolerant (M4209) mutant to its parental line (salt sensitive) which showed a coordinated up-regulation of genes associated with multiple processes necessary for the plants' survival in saline conditions, including photosynthesis, ROS scavenging (peroxidases, SODs), and ion homeostasis. The up-regulation of transcriptional plasticity is a major contributor to the higher cane yield produced by the M4209 mutant when compared to the parental line of sugarcane when grown in saline conditions (32% greater yield; 61). Comparative transcriptome analysis of sugarcane and its wild relative (*Erianthus arundinaceus*) identified candidate genes and metabolic pathways that have been previously identified in salt-responsive transcripts and represent a major opportunity for introgression into cultivated germplasm of sugarcane (e.g. biosynthesis of compatible solutes and ion transporters) (84).

Proteomics is used to discover different isoforms of proteins that are formed as a result of stress with other post-translational modifications that can't be seen by simply observing mRNA levels, as shown by (45). The results from both transcriptome and proteome data have shed light on how there are stress response factors that affect

the synthesis of cell wall components, the energy metabolism process, and chaperone production (46; 52). Through the use of metabolomics, scientists have been able to quantify biomolecules such as osmolytes and antioxidants (which are produced to combat cellular stress) as well as to analyse primary metabolites that are created as a consequence of cellular function, thus providing a direct correlation to cellular stress (46; 52). Small RNA analyses (including miRNA) have also provided information regarding the post-transcriptional regulation of gene expression in response to oxidative stress in sugarcane (51). The calcium sensor CBL/CIPK network has been identified as a key regulatory pathway for controlling ion transport and maintaining ionic balance and homeostasis during abiotic stress conditions (88).

The most effective method of analysis is the combination of a genome-wide association study (GWAS) with expression quantitative trait loci (eQTL) analysis. GWAS identifies single nucleotide polymorphisms (SNPs) that associate with traits and determine if those SNPs affect gene expression in distal or proximal regions. An example of working with distal eQTLs is to show that SNPs that impact transcription factors regulate gene expression at distal locations. This systems genetics approach has been successful for salt tolerance research in maize and sorghum (11; Chakma et al., 2021), and is being used with increasing frequency in sugarcane using an integrated transcriptomic/metabolomic approach (39; 46).

Genomic Selection, Machine Learning, and Predictive Breeding Models

Genomic selection (GS) has emerged as an innovative technology that can provide rapid genetic gain in sugarcane breeding programs with complex genetics and polyploidy. GS models exploit genome-wide SNP marker data to estimate Genomic Breeding Values (GBV) of individual sugarcane clones before excessive financial investment has been made through multi-site phenotypic evaluation. Therefore, GS allows for the reduction of breeding cycles (49; Aswini et al., 2023; 71). GS models using both Bayesian and rrBLUP methodologies have been validated for agronomic traits in the Louisiana sugarcane

breeding program (72) and have enabled tremendous reductions in generation intervals. Additionally, genomic predictions of sugar content and cane yield using 10,435 SNPs across 432 sugarcane clones established the feasibility of GS in very complex, polyploid phenotypes (36).

Machine learning algorithms consistently outperform linear statistical models when it comes to describing non-additive genetics epistasis and complex marker-trait associations. Multi-layer perceptrons, deep learning neural networks have provided more accurate predictions compared to traditional best linear unbiased prediction (BLUP) models for predicting genomic traits in sugarcane (12). Hybrid Bayesian-machine learning approaches that utilize both pedigree and molecular data have been used successfully to improve prediction accuracy of disease-resistance traits for example sugarcane smut resistance (34). These techniques can also be applied directly to genomic predictions of salt tolerance. Brown rust-resistant sugarcane genomic regions have been identified using random forests and materials that utilize support vector machines (SVMs) to detect non-linear epistatic interactions that would typically go undetected by the standard linear methods (4).

Field Evaluation and Breeding Integration

Multi-Environment Field Trials and Statistical Models

Field evaluations under real-life saline environmental conditions are still the best measure of breeding progress towards some salt tolerant crop species. Multi-environmental trials will allow for the separation of true genetic tolerance from phenotypic plasticity and will help to quantify the genotype-by-environment (G x E) interaction that will determine cultivar stability across various saline agricultural systems (32). Using controlled saline irrigation with various electrical conductivity (EC) thresholds allows for the systematic exposure of the germplasm to defined salinity stress levels while providing standardized, field-relevant phenotyping conditions (41).

The new advanced biometric systems have been used to identify genotypes that are stable and salt tolerant. The AMMI model (Additive Main Effects and Multiplicative Interactions) and GGE

(Genotype + Genotype $\times$ Environment) biplot analysis have been used to evaluate 24 different sugarcane genotypes over 2 years of testing under a range of levels of salinity stress and to successfully identify stable high yielding cultivars, such as CoG 7 (41). CoG 7 was commercially released being a stable salt tolerant crop that had better cane and sugar yield than any of the standard check line varieties evaluated in multiple Preliminary and Advanced Yield Trials (82). The radiation induced mutant M4209 is another remarkable success as it produced cane yields that were 32% higher than that of its salt sensitive parent under field saline conditions, which was due to transcriptional reprogramming and improved photosynthesis, with the mechanistic understanding also confirmed with regard to combined heat and salt stress tolerance (61; 62).

Evaluating the various phenotypes of a crop includes looking at morpho-physiological features (such as plant height, leaf area, stem diameter, internode number, and SPAD measured chlorophyll content), biochemical attributes (such as proline levels and levels of activities of antioxidant enzymes), ionic analyses of the Na⁺, K⁺, and K⁺/Na⁺ ratios of roots and shoots, and also agronomic parameters such as yield of cane, brix, pol, and CCS. It is important to conduct a full multi-trait evaluation of plants, since the level of tolerance to adverse environmental factors may differ over the life cycle of a plant; this factor should be taken into consideration when developing screening protocols (66; 16).

Breeding Strategies: Conventional, Mutation, and Molecular Approaches

Through systematic screening of cultivated and wild sugarcane germplasm, conventional breeding programmes have identified sugarcane clones that tolerate salt stress. The G2005047 sugarcane clone was identified as having moderate tolerance to several types of stress (red rot, shoot borers, and salinity) while exhibiting high yielding potential; therefore, this clone will serve as a valuable genetic resource that is a multi-trait tolerant clone. (82) Radiation-induced mutagenesis (in particular) has resulted in many new genetically variable sugarcane lines, including the M4209 mutant line; this

demonstrates that induced mutagenesis is an effective method for producing sugarcane genotypes with different stress tolerated phenotypes relatively quickly. (61; 9).

The implementation of transgene-free genome editing using CRISPR/Cas9 has created transformative opportunities for precisely modifying SOS, NHX and HKT ion transport genes to improve salt tolerance (83; 22). By targeting the MATRILINEAL gene to induce maternal haploids in addition to providing a novel method for developing rapid inbred lines in this naturally outcrossing crop (27). Utilizing plant growth promoting rhizobacteria (PGPR) and nitric oxide will also provide biotechnological methods of enhancing rootzone conditions and resilience against abiotic stress when combined with genetic improvement (76).

Artificial Intelligence and Digital Decision Support Systems

Farmers and breeders alike are seeing a new level of transformation in the precision cultivation of sugarcane as a result of the adoption of artificial intelligence and digital decision support systems (DSS), which are based on data-driven methodologies for monitoring, predicting, and optimising sugarcane production under saline conditions (85; 7). Modern DSS platforms that incorporate multi-sensor remote sensing technology, machine learning analytics and real time decision algorithms provide farmers and breeders with actionable operational recommendations.

Non-destructive, high-throughput monitoring of canopy-level responses to stress using vegetation indices—including NDVI, SAVI, NDRE, and GNDVI—that indicates physiological indicators of both plant health and severity of stress can occur through remote sensing technologies that utilize UAVs outfitted with RGB, multispectral, and hyperspectral sensors (Vinayaka et al. 2024; 79). Additionally, automated detection and classification of disease symptoms and pest damage using convolutional neural networks (CNNs) applied to field imagery can detect symptoms and classifying plants exposed to salt stress (38). AI-based genomic prediction pipelines that utilize pedigree, genomic

markers, and historical multi-environment performance data will facilitate the accurate prediction of progeny performance in target saline environments, greatly reducing the amount of time required for field evaluations (34; 19).

Utilizing IoT sensor networks combined with machine learning algorithms to monitor soil salinity levels, schedule irrigation, and deliver nutrients optimizes agronomic inputs to decrease the intensity of salt stress in real-time (24). Generative artificial intelligence (GAI) and digital twin technologies—virtual computational models predicting an individual's physiological response based on process dynamics over time—are rapidly becoming efficient modalities for optimizing sugarcane production and processing decisions (88; 67). Edge artificial intelligence (AI) computing, which places processing capabilities at the field level via sensors, will likely broaden the reach of these technologies into areas where resources are limited while providing quicker decision-making capabilities (18).

Discussion

Critical Analysis of the In Vitro-to-Field Translation Gap

In addition to the many advantages of in vitro screening platforms, one challenge that is often overlooked is that there is often a large difference in the cellular response that has been demonstrated in an artificial culture system and the performance shown in the in the field where plants are grown. This challenge occurs for a variety of reasons, such as (1) there is a constant, uniform level of stress in an in vitro system, whereas plants experience variable salt stress levels in a field; (2) There is a huge difference between levels of salt that are in an in vitro system and levels of salt that occur naturally in the environment; and (3) there is a lack of understanding about how environmental factors (temporal and spatial stress distributions) and the microbiome, the soil and plant/root interact can influence the response of plants to salinity (47; 32).

In addition, callus culture tolerance, which is usually calculated using parameters like surviving, growth rates and the expression of biochemical markers at supraoptimal salt levels,

may not be measured using the same mechanisms as field level tolerance of the complete plant under saline conditions. Therefore, osmotic compensatory mechanisms that are efficiently activated at the cellular level may not be sufficient to support overall-plant level mechanisms where long distance movement of ions, regulation of stomata and architecture of roots provide additional important dimensions to tolerance. There is a need to standardize in vitro procedures; such as specifying the electrical conductivity threshold values at which stress is experienced and how long the stress will last; the stage in the growth of the plant at which to inflict the stress; and which phenotypic characters will be evaluated when compiling the data from various studies and improving comparisons across studies and increasing translational validity (32, 47).

Genomic Complexity as the Primary Bottleneck in Molecular Breeding

The vast amount of available data searchable on online resources shows that the sheer size and complexity of sugarcane will eventually create barriers to the successful use of molecular breeding for generating salt tolerance in sugarcane. Because there is no way to create high-quality QTLs to effectively breed for salt tolerant sugarcane, because of the great difficulty in creating genetically linked, high-density, and well-anchored genetic maps for sugarcane. The recent release of a near-complete reference genome sequence for the species of *Saccharum* marks an important step towards removing the “genomic black box” (Healey et al., 2023) and will continue to create a barrier to identifying impactful salt tolerance genes and functionally validating these salt tolerance genes in sugarcane.

The functional validation of candidate genes, including SOS1, NHX1, HKT1 homologues, and P5CS, with the aid of reverse genetics methodologies, such as applications of CRISPR/Cas9 for knockouts or overexpression, can be challenging from a technical perspective because of the presence of multiple (homeolog) gene copies within wheat, which could potentially result in functional redundancy and mask the phenotypic effects of manipulating any one of these genes (2; 22). Rapid advancements are being made in the development of cost effective, high

throughput SNP genotyping platforms appropriate for large and heterogeneous breeding population (54), however, there have been no comprehensive validations of polymorphic diagnostic markers that maintain predictive ability across a range of genetic backgrounds for salt tolerance traits.

Research Gaps and Priority Areas

There are several major research gaps that hinder rapid advancement on developing salt-tolerant sugarcane varieties. The first major gap is that there is no comprehensive database for phenomics containing field-relevant physiological characteristics, ionic characteristics and agronomic traits measured across a multitude of genotypes and environments. Without these databases, it will be impossible to develop accurate models for genetic selection (GS) and machine learning to predict salt tolerance. The second major research gap is that we do not know the genetic basis of G×E interactions for salt tolerance, which is essential for predicting how a particular genotype will respond to various salinity levels in diverse environments.

Third, while wild relatives of sugarcane, particularly *Erianthus/Tripidium arundinaceum* and *Saccharum spontaneum*, harbor substantial genetic variation for abiotic stress tolerance, the systematic mining and introgression of wild species alleles into cultivated backgrounds—combined with molecular characterization of introgressed segments—requires greater investment (84; 50). Fourth, the potential of PGPR consortia and rhizosphere engineering for enhancing salt tolerance in the field has been demonstrated in principle but requires large-scale, multi-location validation before practical recommendations can be made (76). Fifth, ethical, regulatory, and socioeconomic dimensions of adopting CRISPR-edited sugarcane varieties require comprehensive stakeholder engagement and policy frameworks, particularly in developing countries where smallholder farmers are primary sugarcane producers.

Future Directions

A variety of transformative technologies will converge to provide the basis for future

salt-tolerant sugarcane development. Long-read sequencing technology (such as PacBio HiFi and Oxford Nanopore Technologies) is providing us with high-quality haplotype-resolved reference genomes for multiple species within the *Saccharum* genus and their wild relatives. The resulting resources will dramatically increase allele mining (the isolation and identification of gene variations) and provide the structure(s) needed to identify structural variation(s) associated with salt tolerance (Healey et al., 2024). They will also provide the genomic scaffolds required to use CRISPR/Cas9 technology to precisely target ion transport (e.g., SOS1, HKT1) and osmolyte biosynthesis (e.g., P5CS) genes for functional improvement (83; 22).

Automated, high-speed evaluation of how much stress corn plants are under based on the information from multiple remote sensors will be possible when researchers combine UAV infrared and hyperspectral reflectance with ground-truth physiological indicators in order to develop real-time, non-destructive measurements of physiological responses to stress in large-scale breeding populations. In conjunction with deep learning-based image analysis and predictive genomic modelling, plant breeders will be able to quickly identify high-stability (high-yielding), salt-tolerant (high-stability) corn genotypes in each of the target production environments (Amaresh et al., 2023; Vinayaka et al., 2023). Also, by using AI models trained on multi-omic and field performance datasets, the breeding cycle can be further compressed through the ability to in silico predict new genotype-environment combinations.

The potential to identify new ways that regulation occurs in plants through the integration of data from genome analyses (DNA), transcriptome (RNA), metabolome (small molecules), and the phenome (observational characteristics) has been greatly increased by the use of single-cell RNA sequencing in combination with spatial transcriptomics. By integrating different types of data (genomic, transcriptomic, proteomic, metabolomic) within a systems biology framework to model how various networks interact together, we will better understand the mechanistic basis of plants' ability to tolerate high levels of salt in the environment and be able to

create rationally designed multi-gene breeding targets to improve tolerance. Another important area for future research in this area is the development of new synthetic biology methodologies to modify the cellular environment for sugarcane plants by engineering optimal combinations of ion transporters, networks of osmolyte production, and cascades of stress-signaling in order to increase sugarcane yield on salt-affected soils.

The integration of Circular Bioeconomy is one of the major "strategic priorities", if you will. Growing salt tolerant types of sugarcane varieties that produce well on saline marginal lands, when these lands are too poor to even think about producing food, offers an avenue of expanding the production of feedstocks for bioenergy, without taking the productive use of food-producing land. To accomplish this goal, it will require a complete integration of all the components of producing and providing for next generation sugarcane, i.e., Molecular Breeding technologies, Advanced Phenotyping Tools, AI-based Management Systems, as well as Policy Environments that promote and foster Equitable Development and Distribution (86; 25).

Conclusion

This paper summarizes findings from five integrated areas of sugarcane salt tolerance research: in vitro selections, physiological and biochemical mechanisms, molecular omics and marker-assisted breeding, multi-environmental field trials, and AI-based precision management. The major conclusions of this review are as follows:

While providing fast and high-throughput initial identification of tolerant genotypes, in vitro screening systems will not replace rigorous physiological and field validation. More should be done to develop standardized protocols for stress and phenotypic measures in order to enhance the translational predictive capacity of in vitro assessments. An overall understanding of osmotic adjustment, ion homeostasis, and antioxidant mechanisms will lead to viable targets for both conventional and molecular breeding. Functional characterization of the components of the SOS pathway, the NHX antiporters, HKT transporters, and the genes for biosynthesis of compatible

solutes must be expedited for sugarcane-specific environments.

The third major advancement is in the ability to integrate transcriptomics, proteomics, metabolomics, and GWAS into systems biology for the purpose of advancing sugarcane salt tolerance research from purely descriptive to mechanistic and predictive. The fourth major advancement is to exploit genomic selection combined with machine learning algorithms to achieve measurable genetic advances in complex polyploid sugarcane; thus representing the greatest immediate opportunity to accelerate precision breeding for salt tolerance phenotypes. The development of stable, widely adapted salt-tolerant cultivars will be achieved through multi-environment field trials employing AMMI and GGE statistical methodologies; the successful development and commercialization of CoG 7 is representative of the translational pathway.

Finally, AI, remote sensing, and digital decision-support systems have radically altered the management of sugarcane in saline environments, offering farmers real-time, evidence-driven strategies for optimizing inputs, thus alleviating stress impact. Sustainable sugarcane production on salt-cursed lands in the impending climate change era would be beholden to the integration of these digital utilities with innovations in genomic breeding. Providing both pathways for this requires ongoing multi-disciplinary collaborations, greater investments in research, plus enabling regulation and policy frameworks to facilitate equitably the deployment of advanced technologies for food security and bioenergy sustainability on a global scale.

References

1. Abdelsalam, N. R., Grad, W. E., Ghura, N. S. A., Khalid, A. E., Ghareeb, R. Y., Desoky, E.-S. M., Rady, M. M., Al-Yasi, H. M., & Ali, E. F. (2021). Callus induction and regeneration in sugarcane under drought stress. *Saudi Journal of Biological Sciences*, 28(12), 7432–7442. <https://doi.org/10.1016/j.sjbs.2021.08.047>
2. Ali, A., Ölmez, F., Altaf, M. T., Liaqat, W., Umar, U. U. D., & Iqbal, J. (2023). Advanced and Sustainable Approaches in Sugarcane Crop Improvements with Reference to Environmental

- Stresses. In *Biotechnology and Omics Approaches for Bioenergy Crops* (pp. 155–182).
3. Amaresh, Aswini, N., Krishnappa, G., Durai, A. A., Manimekalai, R., Swamy, H. K. M., ... & Govindaraj, P. (2025). Next-generation molecular breeding tools to harness higher genetic gains in sugarcane. *Planta*, 262(5). <https://doi.org/10.1007/s00425-025-04842-7>
4. Aono, A. H., Costa, E. A., Rody, H. V. S., Nagai, J. S., Pimenta, R. J. G., Mancini, M. C., ... & Kuroshu, R. M. (2020). Machine learning approaches reveal genomic regions associated with sugarcane brown rust resistance. *Scientific Reports*, 10(1). <https://doi.org/10.1038/s41598-020-77063-5>
5. Apon, T. A., Ahmed, S. F., Bony, Z. F., Chowdhury, Md. R., Asha, J. F., & Biswas, A. (2023). Sett priming with salicylic acid improves salinity tolerance of sugarcane (*Saccharum officinarum* L.) during early stages of crop development. *Heliyon*, 9(5), e16030. <https://doi.org/10.1016/j.heliyon.2023.e16030>
6. Ashraf, M., & Munns, R. (2022). Evolution of Approaches to Increase the Salt Tolerance of Crops. *Critical Reviews in Plant Sciences*, 41(2), 128–160. <https://doi.org/10.1080/07352689.2022.2065136>
7. Assimakopoulos, F., Vassilakis, C., Margaritis, D., Kotis, K., & Spiliotopoulos, D. (2024). Artificial Intelligence Tools for the Agriculture Value Chain: Status and Prospects. *Electronics*, 13(22), 4362. <https://doi.org/10.3390/electronics13224362>
8. Aswini, N., Moniusha, J., Keerthana, S., Amaresh, Nandhini, M., Prathima, P. T., Mohanraj, K., Kumari, S., & Manimekalai, R. (2025). Genomic Exploration for the Sucrose Content in Sugarcane. *Tropical Plant Biology*, 18(1). <https://doi.org/10.1007/s12042-025-09413-4>
9. Barrientos-Alfaro, F. C., Echeverría-Beirute, F., Hernández-Soto, A., Gatica-Arias, A., Hernández-García, C. M., Carvajal-Quesada, P., Rodríguez-Morales, A., & Pérez, J. (2025). Mutation breeding strategies and advances for sugarcane (*Saccharum* spp.) improvement. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 162(3). <https://doi.org/10.1007/s11240-025-03188-y>
10. Budeguer, F., Enrique, R., Perera, M. F., Racedo, J., Castagnaro, A. P., Noguera, A. S., & Welin, B. (2021). Genetic Transformation of Sugarcane, Current Status and Future Prospects. *Frontiers in Plant Science*, 12. <https://doi.org/10.3389/fpls.2021.768609>
11. Cao, Y., Zhou, X., Song, H., Zhang, M., & Jiang, C. (2023). Advances in deciphering salt tolerance mechanism in maize. *The Crop Journal*, 11(4), 1001–1010. <https://doi.org/10.1016/j.cj.2022.12.004>
12. Chen, C., Powell, O., Dinglasan, E., Ross, E. M., Yadav, S., Wei, X., Atkin, F., Deomano, E., & Hayes, B. J. (2023). Genomic prediction with machine learning in sugarcane, a complex highly polyploid clonally propagated crop with substantial non-additive variation for key traits. *The Plant Genome*, 16(4). <https://doi.org/10.1002/tpg2.20390>
13. Chen, S., Lu, Y., Luo, Q., Tan, J., Guan, J., Luo, J., Xu, Y., Chen, M., Zhao, R., da Silva, J. A. T., Zhang, Y., & Ma, G. (2024). Adventitious shoot organogenesis from immature leaves of sugarcane (*Saccharum* spp. hybrid) cultivar Yuetang 03-373. *Journal of Crop Science and Biotechnology*, 27(5), 617–629. <https://doi.org/10.1007/s12892-024-00255-2>
14. Dhansu, P., Kaushik, S., Kumar, R., Kumar, A., Mishra, D. S., Kaushik, P., Tunç, Y., & Khadivi, A. (2026). Salinity responses and tolerance mechanisms in sugarcane (*Saccharum officinarum* L.): Recent updates and future perspectives. *Industrial Crops and Products*, 239, 122556. <https://doi.org/10.1016/j.indcrop.2025.122556>
15. Dhansu, P., Kaushik, S., Kumar, R., Krishnapriya, V., Appunu, C., Shariff, A., Kaur, G., Singh, P., Kumari, A., & Kumar, A. (2026). Intricate relationship of physiological, biochemical and molecular mechanisms in deciphering abiotic stress tolerance in sugarcane. *South African Journal of Botany*, 188, 135–150. <https://doi.org/10.1016/j.sajb.2025.10.052>
16. Dhansu, P., Kumar, R., Kumar, A., Vengavasi, K., Raja, A. K., Vasantha, S., Meena, M. R., Kulshreshtha, N., & Pandey, S. K. (2022). Differential Physiological Traits, Ion Homeostasis and Cane Yield of Sub-Tropical Sugarcane Varieties in Response to Long-Term Salinity Stress. *Sustainability*, 14(20), 13246. <https://doi.org/10.3390/su142013246>
17. Dinesh Babu, K. S., Janakiraman, V., Palaniswamy, H., Kasirajan, L., Gomathi, R., & Ramkumar, T. R. (2022). A short review on sugarcane: its domestication, molecular manipulations and future perspectives. *Genetic Resources and Crop Evolution*, 69(8), 2623–2643. <https://doi.org/10.1007/s10722-022-01430-6>
18. El Jarroudi, M., Kouadio, L., Delfosse, P., Bock, C. H., Mahlein, A.-K., Fettweis, X., Mercatoris, B., Adams, F., Lenné, J. M., & Hamdioui, S. (2024).

- Leveraging edge artificial intelligence for sustainable agriculture. *Nature Sustainability*, 7(7), 846–854. <https://doi.org/10.1038/s41893-024-01352-4>
19. Fan, H., Chao, J., Liu, X., Ma, P., Zhu, G., Li, M., Yan, R., Li, X., Jing, Y., Li, F., Luo, T., & Lakshmanan, P. (2026). Accelerating Sugarcane Breeding with Machine Learning: A Concurrent Multi-trait Predictive Approach. *Sugar Tech*. <https://doi.org/10.1007/s12355-025-01709-9>
 20. Gajjar, K., Mali, S., & Udutha, J. (2022). In vitro response of promising sugarcane varieties for salinity tolerance through shoot tip culture. *The Pharma Innovation*, 11(4S), 2096–2100. <https://doi.org/10.22271/tpi.2022.v11.i4saa.12325>
 21. Gao, Y., Zhou, S., Huang, Y., Zhang, B., Xu, Y., Zhang, G., Lakshmanan, P., Yang, R., Zhou, H., Huang, D., Liu, J., Tan, H., He, W., Yang, C., & Duan, W. (2022). Quantitative Trait Loci Mapping and Development of KASP Marker Smut Screening Assay Using High-Density Genetic Map and Bulk Segregant RNA Sequencing in Sugarcane (*Saccharum* spp.). *Frontiers in Plant Science*, 12. <https://doi.org/10.3389/fpls.2021.796189>
 22. Ghane, A., Malhotra, P., Sanghera, G., Verma, S., Jamwal, N., Kashyap, L., & Wani, S. (2024). CRISPR/Cas technology: fueling the future of Biofuel production with sugarcane. *Functional & Integrative Genomics*, 24(6). <https://doi.org/10.1007/s10142-024-01487-9>
 23. Gómez, D., Hajari, E., & Lorenzo, J. C. (2020). Polynomial regressions reveal that levels of chlorophyll a, carotenoids, soluble phenolics and aldehydes modify mathematically sugarcane shoot multiplication rate in temporary immersion bioreactors under salt stress. *Biologia*, 75(11), 2025–2030. <https://doi.org/10.2478/s11756-020-00505-y>
 24. Gopikrishnan, S., Srivastava, G., & Priakanth, P. (2022). Improving sugarcane production in saline soils with Machine Learning and the Internet of Things. *Sustainable Computing: Informatics and Systems*, 35, 100743. <https://doi.org/10.1016/j.suscom.2022.100743>
 25. Grandis, A., Fortirer, J. S., Navarro, B. V., de Oliveira, L. P., & Buckeridge, M. S. (2023). Biotechnologies to Improve Sugarcane Productivity in a Climate Change Scenario. *BioEnergy Research*, 17(1), 1–26. <https://doi.org/10.1007/s12155-023-10649-9>
 26. Guan, R.-X., Guo, X.-Y., Qu, Y., Zhang, Z.-W., Bao, L.-G., Ye, R.-Y., Chang, R.-Z., & Qiu, L.-J. (2023). Salt Tolerance in Soybeans: Focus on Screening Methods and Genetics. *Plants*, 13(1), 97. <https://doi.org/10.3390/plants13010097>
 27. Guo, Y., Sun, S., Chen, S., Zhang, Y., Wang, W., Deng, Z., ... & Wang, Q. (2024). In vivo induction of sugarcane (*Saccharum* spp.) haploids by genome editing. *Plant Physiology*, 196(2), 731–734. <https://doi.org/10.1093/plphys/kiac418>
 28. Gupta, S., Gupta, K., Nehra, C., Gaur, R. K., & Yadav, D. (2022). Biotechnological Intervention for Sugarcane Improvement Under Salinity. *Sugar Tech*, 25(1), 15–31. <https://doi.org/10.1007/s12355-022-01174-8>
 29. Hartati, R. S., Suhesti, S., Wulandari, S., Ardana, I. K., & Yunita, R. (2021). In-vitro selection of sugarcane (*Saccharum officinarum* L.) putative mutant for drought stress. *IOP Conference Series: Earth and Environmental Science*, 653(1), 012135. <https://doi.org/10.1088/1755-1315/653/1/012135>
 30. Healey, A. L., Garsmeur, O., Lovell, J. T., Shengquiang, S., Sreedasyam, A., Jenkins, J., ... & D'Hont, A. (2024). The complex polyploid genome architecture of sugarcane. *Nature*, 628(8009), 804–810. <https://doi.org/10.1038/s41586-024-07231-4>
 31. Hernández-Pérez, C. A., Gómez-Merino, F. C., Spinoso-Castillo, J. L., & Bello-Bello, J. J. (2021). In Vitro Screening of Sugarcane Cultivars (*Saccharum* spp. Hybrids) for Tolerance to Polyethylene Glycol-Induced Water Stress. *Agronomy*, 11(3), 598. <https://doi.org/10.3390/agronomy11030598>
 32. Hu, Y., & Schmidhalter, U. (2023). Opportunity and challenges of phenotyping plant salt tolerance. *Trends in Plant Science*, 28(5), 552–566. <https://doi.org/10.1016/j.tplants.2022.12.010>
 33. Ýbrahimova, U., Kumari, P., Yadav, S., Rastogi, A., Antala, M., Suleymanova, Z., Zivcak, M., Tahjib-Ul-Arif, Md., Hussain, S., Abdelhamid, M., Hajihashemi, S., Yang, X., & Brestic, M. (2021). Progress in understanding salt stress response in plants using biotechnological tools. *Journal of Biotechnology*, 329, 180–191. <https://doi.org/10.1016/j.jbiotec.2021.02.007>
 34. Inamori, M., Kimura, T., Mori, M., Tarumoto, Y., Hattori, T., Hayano, M., Umeda, M., & Iwata, H. (2024). Machine learning for genomic and pedigree prediction in sugarcane. *The Plant Genome*, 17(3). <https://doi.org/10.1002/tpg2.20486>
 35. Iqbal, A., Khan, R. S., Khan, M. A., Gul, K., Aizaz, M., Usman, M., & Arif, M. (2022). Efficient Regeneration in Sugarcane Using Thin Cell Layer

- (TCL) Culture System. *Sugar Tech*, 25(1), 168–176. <https://doi.org/10.1007/s12355-022-01162-y>
36. Islam, Md. S., McCord, P., Read, Q. D., Qin, L., Lipka, A. E., Sood, S., Todd, J., & Olatoye, M. (2022). Accuracy of Genomic Prediction of Yield and Sugar Traits in *Saccharum* spp. Hybrids. *Agriculture*, 12(9), 1436. <https://doi.org/10.3390/agriculture12091436>
 37. Junaid, M. B., El-Hendawy, S., Al-ashkar, I., Al-Suhaibani, N., & Alotaibi, M. (2023). Integrating Agro-Morpho-Physiological Traits and SSR Markers for Detecting the Salt Tolerance of Advanced Spring Wheat Lines under Field Conditions. *Agriculture*, 13(11), 2135. <https://doi.org/10.3390/agriculture13112135>
 38. Kanade, A., & Paygude, P. (2025). Precision Agriculture in Sugarcane: Review of Disease Detection and Severity Classification Methods. 2025 12th International Conference on Computing for Sustainable Global Development (INDIACom), 1–7. <https://doi.org/10.23919/indiacom66777.2025.11115554>
 39. Khan, M., Ali, S., Al Azzawi, T. N. I., & Yun, B.-W. (2023). Nitric Oxide Acts as a Key Signaling Molecule in Plant Development under Stressful Conditions. *International Journal of Molecular Sciences*, 24(5), 4782. <https://doi.org/10.3390/ijms24054782>
 40. Khan, Q., Qin, Y., Guo, D.-J., Yang, L.-T., Song, X.-P., Xing, Y.-X., & Li, Y.-R. (2023). A Review of the Diverse Genes and Molecules Involved in Sucrose Metabolism and Innovative Approaches to Improve Sucrose Content in Sugarcane. *Agronomy*, 13(12), 2957. <https://doi.org/10.3390/agronomy13122957>
 41. Kumar, R., Dhansu, P., Kulshreshtha, N., Meena, M. R., Kumaraswamy, M. H., Appunu, C., Chhabra, M. L., & Pandey, S. K. (2023). Identification of Salinity Tolerant Stable Sugarcane Cultivars Using AMMI, GGE and Some Other Stability Parameters under Multi Environments of Salinity Stress. *Sustainability*, 15(2), 1119. <https://doi.org/10.3390/su15021119>
 42. Kumar, R., Sagar, V., Verma, V. C., Kumari, M., Gujjar, R. S., Goswami, S. K., Kumar Jha, S., Pandey, H., Dubey, A. K., Srivastava, S., Singh, S. P., Mall, A. K., Pathak, A. D., Singh, H., Jha, P. K., & Prasad, P. V. V. (2023). Drought and salinity stresses induced physio-biochemical changes in sugarcane: an overview of tolerance mechanism and mitigating approaches. *Frontiers in Plant Science*, 14. <https://doi.org/10.3389/fpls.2023.1225234>
 43. Laksana, C., Sophiphun, O., & Chanprame, S. (2023). In vitro and in vivo screening for the identification of salt-tolerant sugarcane (*Saccharum officinarum* L.) clones: molecular, biochemical, and physiological responses to salt stress. *Saudi Journal of Biological Sciences*, 30(6), 103655. <https://doi.org/10.1016/j.sjbs.2023.103655>
 44. Le, T. T. C., Vu, K. L., & Vu, T. T. H. (2024). Growth Characterization of Sugarcane (*Saccharum* spp.) under Salinity and Drought Stresses at the Seedling Stage. *Vietnam Journal of Agricultural Sciences*, 7(3), 2185–2194. <https://doi.org/10.31817/vjas.2024.7.3.01>
 45. Li, A.-M., Liao, F., Wang, M., Chen, Z.-L., Qin, C.-X., Huang, R.-Q., Verma, K. K., Li, Y.-R., Que, Y.-X., Pan, Y.-Q., & Huang, D.-L. (2023). Transcriptomic and Proteomic Landscape of Sugarcane Response to Biotic and Abiotic Stressors. *International Journal of Molecular Sciences*, 24(10), 8913. <https://doi.org/10.3390/ijms24108913>
 46. Li, A., Wu, Q., Yang, S., Liu, J., Zhao, Y., Zhao, P., Wang, L., Lu, W., Huang, D., Zhang, Y., & Que, Y. (2024). Dissection of genetic architecture for desirable traits in sugarcane by integrated transcriptomics and metabolomics. *International Journal of Biological Macromolecules*, 280, 136009. <https://doi.org/10.1016/j.ijbiomac.2024.136009>
 47. Lozada, A., Gómez, D., Acosta, Y., Quintana-Zaez, J. C., Companioni, B., Zevallos-Bravo, B. E., de Lourdes Tapia y Figueroa, M., Serksen, & Lorenzo, J. C. (2025). Impact of strong correlations observed in sugarcane responses to sodium chloride and mannitol within temporary immersion bioreactors. *Vegetos*. <https://doi.org/10.1007/s42535-025-01351-6>
 48. Ma, X., Zeng, X., Huang, Y., Liu, S. H., Yin, J., & Yang, G.-F. (2024). Visualizing plant salt stress with a NaCl-responsive fluorescent probe. *Nature Protocols*, 20(4), 902–933. <https://doi.org/10.1038/s41596-024-01068-x>
 49. Mahadevaiah, C., Appunu, C., Aitken, K., Suresha, G. S., Vignesh, P., Mahadeva Swamy, H. K., Valarmathi, R., Hemaprabha, G., Alagarasan, G., & Ram, B. (2021). Genomic Selection in Sugarcane: Current Status and Future Prospects. *Frontiers in Plant Science*, 12. <https://doi.org/10.3389/fpls.2021.708233>
 50. Mahadevaiah, C., Vignesh, P., Appunu, C., Valarmathi, R., Dhansu, P., Kumar, A., Dharshini, S., Padmanabhan, T. S. S., Narayan, J. A., Selvamuthu, K., Sreenivasa, V., Mahadeva Swamy, H. K., Vinu, V., Raja, A. K., Suresha, G. S.,

- Hemaprabha, G., & Kumar, R. (2023). Physiological Characterization of *Triplidium arundinaceum* and Sugarcane (*Saccharum spp.*) Germplasm for Salinity Stress Tolerance at the Formative Stage. *Sustainability*, 15(8), 6962. <https://doi.org/10.3390/su15086962>
51. Manimekalai, R., Saranya, J., Saravanakumar, K., Rajagopal, S., Jini, N., Selvi, A., Rabisa, V. P., Nandhini, M., Vannish, R., Prathima, P. T., & Gomathi, R. (2024). Small RNA profiling and co-networking analysis identifies stress-responsive miRNAs involved in sugarcane and its wild species. *Plant Physiology Reports*, 30(2), 333–351. <https://doi.org/10.1007/s40502-024-00841-0>
 52. Mancini, M. C., Sforça, D. A., Pimenta, R. J. G., Cardoso-Silva, C. B., Aono, A. H., da Silva, C. C., & de Souza, A. P. (2022). Omics in sugarcane. In *Omics in Horticultural Crops* (pp. 449–465). Elsevier. <https://doi.org/10.1016/b978-0-323-89905-5.00017-3>
 53. Maruprolu, S., S, G., R, G., R, V., B, A., & R, S. (2022). An efficient protocol optimization for callus induction, regeneration and acclimatization in sugarcane cv. CO 94012. *The Pharma Innovation*, 11(4), 989–996. <https://doi.org/10.22271/tpi.2022.v11.i4n.11969>
 54. McNeil, M., Piperidis, G., Bhuiyan, S., Li, J., Wei, X., Collard, B., & Aitken, K. (2022). Development of a high-throughput, low-cost SNP genotyping panel for sugarcane breeding.
 55. Melino, V., & Tester, M. (2023). Salt-Tolerant Crops: Time to Deliver. *Annual Review of Plant Biology*, 74(1), 671–696. <https://doi.org/10.1146/annurev-arplant-061422-104322>
 56. Misra, V., Gosavi, B., Rao, G. P., Viswanathan, R., & Solomon, S. (2026). AI-Driven Innovations in Sugarcane Farming and Sugar Industry in India: Enhancing Productivity, Efficiency, and Sustainability. *Sugar Tech*. <https://doi.org/10.1007/s12355-025-01715-x>
 57. Mohanan, M. V., Thelakat Sasikumar, S. P., Jayanarayanan, A. N., Selvarajan, D., Ramanathan, V., Shivalingamurthy, S. G., Raju, G., Govind, H., & Chinnaswamy, A. (2024). Transgenic sugarcane overexpressing Glyoxalase III improved germination and biomass production at formative stage under salinity and water-deficit stress conditions. *3 Biotech*, 14(2). <https://doi.org/10.1007/s13205-023-03856-w>
 58. Molin, J. P., Wei, M. C. F., & da Silva, E. R. O. (2024). Challenges of Digital Solutions in Sugarcane Crop Production: A Review. *AgriEngineering*, 6(2), 925–946. <https://doi.org/10.3390/agriengineering6020053>
 59. Mubushar, M., El-Hendawy, S., Tahir, M. U., Alotaibi, M., Mohammed, N., Refay, Y., & Tola, E. (2022). Assessing the Suitability of Multivariate Analysis for Stress Tolerance Indices, Biomass, and Grain Yield for Detecting Salt Tolerance in Advanced Spring Wheat Lines Irrigated with Saline Water under Field Conditions. *Agronomy*, 12(12), 3084. <https://doi.org/10.3390/agronomy12123084>
 60. Murugavelu, G. S., Harish Chandar, S. R., Sakthivel, S. K., Ramaswamy, M., Swaminathan, A., & Chinnaswamy, A. (2024). Progress and Updates of CRISPR/Cas9-Mediated Genome Editing on Abiotic Stress Tolerance in Agriculture: A Review. *Sugar Tech*, 27(1), 29–43. <https://doi.org/10.1007/s12355-024-01448-3>
 61. Negi, P., Pandey, M., Dorn, K. M., Nikam, A. A., Devarumath, R. M., Srivastava, A. K., & Suprasanna, P. (2020). Transcriptional reprogramming and enhanced photosynthesis drive inducible salt tolerance in sugarcane mutant line M4209. *Journal of Experimental Botany*, 71(19), 6159–6173. <https://doi.org/10.1093/jxb/eraa339>
 62. Negi, P., Pandey, M., Paladi, R. K., Majumdar, A., Pandey, S. P., Barvkar, V. T., Devarumath, R., & Srivastava, A. K. (2025). Stomata-Photosynthesis Synergy Mediates Combined Heat and Salt Stress Tolerance in Sugarcane Mutant M4209. *Plant, Cell & Environment*, 48(6), 4668–4684. <https://doi.org/10.1111/pce.15424>
 63. Nong, Q., Malviya, M. K., Lin, L., Xie, J., Mo, Z., Solanki, M. K., Solanki, A. C., Wang, Z., Song, X., Li, Y., & Li, C. (2024). Enhancing Sugarcane Seedling Resilience to Water Stress through Exogenous Abscissic Acid: A Study on Antioxidant Enzymes and Phytohormone Dynamics. *ACS Omega*, 9(29), 31684–31693. <https://doi.org/10.1021/acsomega.4c02341>
 64. Preet, K., Dhansu, P., Sehrawat, N., Kumar, R., Appunu, C., Vengavasi, K., Arunkumar, R., Rohit Rana, Kumar, S., & Joon, V. (2024). Morpho-physiological analysis of salinity tolerance in sugarcane genotypes. *Plant Physiology Reports*, 29(2), 356–366. <https://doi.org/10.1007/s40502-024-00782-8>
 65. Qin, X., Shan, B., Liu, J., Zhang, C., Wang, W., Wang, C., & Huo, Z. (2024). A Dynamic Process-Based Method for Screening Salt-Tolerant and High-Yielding Crop Varieties. *Agronomy*, 14(8), 1649. <https://doi.org/10.3390/agronomy14081649>

66. Rao, V. P., Sengar, R. S., & Singh, R. B. (2021). Identification of salt tolerant sugarcane cultivars through phenotypic, physiological and biochemical studies under abiotic stress. *Plant Physiology Reports*, 26(2), 256–283. <https://doi.org/10.1007/s40502-021-00581-5>
67. Ray, P. P. (2024). Generative AI and Its Impact on Sugarcane Industry: An Insight into Modern Agricultural Practices. *Sugar Tech*, 26(2), 325–332. <https://doi.org/10.1007/s12355-023-01358-w>
68. Reyes, J. A. O., Casas, D. E., Gandia, J. L., Parducho, M. J. L., Renovalles, E. M., Quilloy, E. P., & Delfin, E. F. (2023). Polyethylene glycol-induced drought stress screening of selected Philippine high-yielding sugarcane varieties. *Journal of Agriculture and Food Research*, 14, 100676. <https://doi.org/10.1016/j.jafr.2023.100676>
69. Sahu, M., Maurya, S., & Jha, Z. (2023). In vitro selection for drought and salt stress tolerance in rice: an overview. *Plant Physiology Reports*, 28(1), 8–33. <https://doi.org/10.1007/s40502-023-00713-z>
70. Saminadane, T., Geddam, S., Krishnaswamy, P., Jothiganapathy, K., Tamilselvan, A., Ramadoss, B. R., Sri Hari Reddy, P., Singh, U. S., Singh, R. K., Platten, J. D., ... & Guntupalli, P. (2024). Development of early maturing salt-tolerant rice variety KKL(R) 3 using a combination of conventional and molecular breeding approaches. *Frontiers in Genetics*, 14. <https://doi.org/10.3389/fgene.2023.1332691>
71. Sandhu, K. S., Shiv, A., Kaur, G., Meena, M. R., Raja, A. K., Vengavasi, K., Mall, A. K., Kumar, S., Singh, P. K., Singh, J., Hemaprabha, G., Pathak, A. D., Krishnappa, G., & Kumar, S. (2022). Integrated Approach in Genomic Selection to Accelerate Genetic Gain in Sugarcane. *Plants*, 11(16), 2139. <https://doi.org/10.3390/plants11162139>
72. Satpathy, S., Shahi, D., Blanchard, B., Pontif, M., Gravois, K., Kimbeng, C., Hale, A., Todd, J., Rao, A., & Baisakh, N. (2022). Evaluation of Models for Utilization in Genomic Prediction of Agronomic Traits in the Louisiana Sugarcane Breeding Program. *Agriculture*, 12(9), 1330. <https://doi.org/10.3390/agriculture12091330>
73. Senthilkumar, S., Vinod, K. K., Parthiban, S., Thirugnanasambandam, P., Lakshmi Pathy, T., Banerjee, N., Sarath Padmanabhan, T. S., & Govindaraj, P. (2022). Identification of potential MTAs and candidate genes for juice quality- and yield-related traits in *Saccharum* clones. *Molecular Genetics and Genomics*, 297(3), 635–654. <https://doi.org/10.1007/s00438-022-01870-w>
74. Shabbir, R., Javed, T., Afzal, I., Sabagh, A. E., Ali, A., Vicente, O., & Chen, P. (2021). Modern Biotechnologies: Innovative and Sustainable Approaches for the Improvement of Sugarcane Tolerance to Environmental Stresses. *Agronomy*, 11(6), 1042. <https://doi.org/10.3390/agronomy11061042>
75. Shabbir, R., Javed, T., Sun, S.-R., Wang, Z.-Q., Zhang, W., Gao, S.-J., & Wang, Q.-N. (2026). Functional genomics in sugarcane breeding: key challenges and strategies. *Critical Reviews in Biotechnology*, 1–21. <https://doi.org/10.1080/07388551.2026.2614075>
76. Sharma, A., Singh, R. K., Singh, P., Vaishnav, A., Guo, D.-J., Verma, K. K., Li, D.-P., Song, X.-P., Malviya, M. K., Khan, N., Lakshmanan, P., & Li, Y.-R. (2021). Insights into the Bacterial and Nitric Oxide-Induced Salt Tolerance in Sugarcane and Their Growth-Promoting Abilities. *Microorganisms*, 9(11), 2203. <https://doi.org/10.3390/microorganisms9112203>
77. Singh, R. B., Rao, V. P., & Sengar, R. S. (2023). Study of salinity induced oxidative stress and antioxidant responses in callus cultures of sugarcane. *Ecological Genetics and Genomics*, 26, 100164. <https://doi.org/10.1016/j.egg.2023.100164>
78. Sinha, D., Maurya, A. K., Abdi, G., Majeed, M., Agarwal, R., Mukherjee, R., Ganguly, S., Aziz, R., Bhatia, M., Majgaonkar, A., Seal, S., Das, M., Banerjee, S., Chowdhury, S., Adeyemi, S. B., & Chen, J.-T. (2023). Integrated Genomic Selection for Accelerating Breeding Programs of Climate-Smart Cereals. *Genes*, 14(7), 1484. <https://doi.org/10.3390/genes14071484>
79. Soares Cardoso, L. A., Silva Farias, P. R., Corrêa Soares, J. A., Tanajura Caldeira, C. R., & de Oliveira, F. J. (2024). Spatial interpolators for Delineating management zones to mitigate *Mucuna pruriens* in sugarcane plantations in the Eastern Amazon. *Computers and Electronics in Agriculture*, 227, 109615. <https://doi.org/10.1016/j.compag.2024.109615>
80. Somasekar, T., Tamilzharasi, M., Singh, N. K., Andrew Peter Leon, M. T., & Thirumeni, S. (2025). Confirmation and validation of saline-tolerant QTLs for seedling stage tolerance in rice. *Cereal Research Communications*, 53(3), 1249–1269. <https://doi.org/10.1007/s42976-024-00602-7>
81. Srithawong, S., Fang, W., Jing, Y., Pholtaisong, J., Li, D., Khumla, N., Sakuanrungrasirikul, S., & Li, M. (2026). Sugarcane Breeding in the Genomic

- Era: Integrative Strategies and Emerging Technologies. *Plants*, 15(2), 286. <https://doi.org/10.3390/plants15020286>
82. Sudhagar, R., Saravanan, N. A., Kanchanarani, R., Shanmuganathan, M., Ganapathy, S., Babu, C., Thirumurugan, A., Ravichandran, V., Appunu, C., & Anna Durai, A. (2024). Evolution, identification, evaluation, and characterization of a stable salinity tolerant sugarcane variety CoG 7. *Scientific Reports*, 14(1). <https://doi.org/10.1038/s41598-024-70756-1>
 83. Surya Krishna, S., Harish Chandar, S. R., Ravi, M., Valarmathi, R., Lakshmi, K., Prathima, P. T., Manimekalai, R., Viswanathan, R., Hemaprabha, G., & Appunu, C. (2023). Transgene-Free Genome Editing for Biotic and Abiotic Stress Resistance in Sugarcane: Prospects and Challenges. *Agronomy*, 13(4), 1000. <https://doi.org/10.3390/agronomy13041000>
 84. Vignesh, P., Mahadevaiah, C., Parimalan, R., Valarmathi, R., Dharshini, S., Nisha, S., Suresha, G. S., Swathi, S., Mahadeva Swamy, H. K., Sreenivasa, V., Mohanraj, K., Hemaprabha, G., Bakshi, R., & Appunu, C. (2021). Comparative de novo transcriptome analysis identifies salinity stress responsive genes and metabolic pathways in sugarcane and its wild relative *Erianthus arundinaceus*. *Scientific Reports*, 11(1). <https://doi.org/10.1038/s41598-021-03735-5>
 85. Vinayaka, Prasad, P. R. C., Avinash, G., Amaresh, Arun Kumar, R., Murali, P., Palaniswami, C., & Govindaraj, P. (2025). Harnessing AI and Remote sensing for precision sugarcane farming: tackling water stress, salinity, and nitrogen challenges. *Frontiers in Agronomy*, 7. <https://doi.org/10.3389/fagro.2025.1681294>
 86. Wang, D., Zheng, J., Xu, N., Sarsaiya, S., & Zhang, J. (2025). Unlocking the potential of sugarcane: Advances in genomic innovation, biorefinery technologies, and stress resilience, and circular bioeconomy. *Industrial Crops and Products*, 236, 122080. <https://doi.org/10.1016/j.indcrop.2025.122080>
 87. Wang, L., Yeo, S., Lee, M., Endah, S., Alhuda, N. A., & Yue, G. H. (2023). Combination of GWAS and FST-based approaches identified loci associated with economic traits in sugarcane. *Molecular Genetics and Genomics*, 298(5), 1107–1120. <https://doi.org/10.1007/s00438-023-02040-2>
 88. Wang, S., Pan, K., Liao, M., Li, X., & Zhang, M. (2024). Characterization of CBL-CIPK signaling networks and their response to abiotic stress in sugarcane. *International Journal of Biological Macromolecules*, 278, 134836. <https://doi.org/10.1016/j.ijbiomac.2024.134836>
 89. Wang, X., Wu, Q., Zeng, H., Yang, X., Yang, X., Yi, X., Khalil, I., & Que, Y. (2024). Digital evolution and twin miracle of sugarcane breeding. *Field Crops Research*, 318, 109588. <https://doi.org/10.1016/j.fcr.2024.109588>
 90. Watanabe, K., Takaragawa, H., Ueno, M., & Kawamitsu, Y. (2020). Changes in Agronomic and Physiological Traits of Sugarcane Grown with Saline Irrigation Water. *Agronomy*, 10(5), 722. <https://doi.org/10.3390/agronomy10050722>
 91. Wirojsirasak, W., Songsri, P., Jongrunklang, N., Tangphatsornruang, S., Klomsa-ard, P., & Ukoskit, K. (2023). A Large-Scale Candidate-Gene Association Mapping for Drought Tolerance and Agronomic Traits in Sugarcane. *International Journal of Molecular Sciences*, 24(16), 12801. <https://doi.org/10.3390/ijms241612801>

Conflict of Interest Statement

The authors declare no conflicts of interest.

Author Contributions

P.C.: Literature search, data synthesis, manuscript writing, and revision. A.K.: Conceptualization, manuscript review, supervision, and critical revision. Both authors have read and agreed to the final version of the manuscript.

Received : April 2025; Revised : May 2025; Accepted : June 2025



ENHANCEMENT IN YIELD AND QUALITY ATTRIBUTES OF LITCHI THROUGH THE FOLIAR APPLICATION OF NAA, BORON AND ZINC SULPHATE

Shivani¹, V.K. Tripathi^{1*} and Akhilesh Mishra²

¹Department of Fruit Science

²Legume Section

C.S. Azad University of Agriculture and Technology, Kanpur-208002 (U.P.)

*Corresponding Author Email-drvkttripathicsa@gmail.com

Abstract

An experiment was conducted during 2025 at the Department of Fruit Science, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur (U.P.), to assess in the enhancement of yield and quality attributes of litchi through the foliar application of NAA, Boron, and Zinc Sulphate. Nine treatments comprising two concentrations of NAA (40 and 60 ppm), three concentrations of boron (0.4, 0.6, and 0.8%), three concentrations of ZnSO₄ (0.6, 0.8, and 1.0%), and one control were evaluated in a Randomised Block Design with three replications. Foliar sprays were done twice, once before flowering and again at the pea stage of fruit development. Significant differences were observed among treatments. NAA @ 40 ppm recorded highest fruit set (57.46%), lowest fruit drop (61.28%), and highest fruit retention (33.82%). However, Boron @ 0.6% proved superior in improving fruit quality and yield by recording minimum fruit cracking (7.95%), maximum fruit length (4.62 cm), fruit diameter (3.90 cm), fruit weight (20.00 g), pulp weight (16.42 g), TSS (22.55 °Brix), total sugars (18.52%), vitamin C (25.36 mg/100 g), pulp: stone ratio (5.97) and fruit yield (25.31 kg plant⁻¹), with minimum seed weight (2.75 g) and titratable acidity (0.35%). Therefore, a foliar application of boron at 0.6% is recommended to improve yield and quality of litchi cv. Dehradun under the plains of northern India.

Keywords: Litchi, NAA, Boron, ZnSO₄, fruit cracking, yield, quality.

Introduction

Litchi (*Litchi chinensis* Sonn.) is a highly valued subtropical evergreen fruit tree belonging to the family Sapindaceae, originating in South China and now widely cultivated across tropical and subtropical regions worldwide. The fruit is prized for its attractive appearance, sweet taste, pleasant aroma, and translucent white aril, which is rich in carbohydrates, vitamins, organic acids, and phytochemicals, including flavonoids, sterols, and phenolic compounds. India and China together account for nearly 90% of global litchi production, with India ranking second. Among fruit crops in India, litchi ranks seventh in cultivated area and ninth in total production, but sixth in economic value, with a national average

productivity of about 6.1 t/ha (NHB, 2018-19).

Litchi cultivar Dehradun is a late-season cultivar widely grown in Uttar Pradesh and Punjab, yielding approximately 80–90 kg of fruit per plant. Fruit cracking, a common physiological disorder in litchi and other crops such as citrus, grapes, and pomegranate, can cause yield losses of up to 50%, reduce marketability, and make fruits more prone to insect and fungal attacks. Poor pollination, excessive fruit drop, and irregular flowering further limit productivity under field conditions.

Plant bioregulators play a crucial role in improving fruit set, reducing pre-harvest fruit drop, and enhancing overall fruit quality. Naphthalene acetic acid (NAA), a synthetic auxin,

delays abscission by inhibiting the physiological breakdown of calcium pectate in the middle lamella, thereby promoting fruit retention. Application of NAA has also been reported to reduce fruit acidity, increase ascorbic acid concentration, and lower seed number (18). Micronutrients such as zinc and boron are equally important; zinc acts as a cofactor for key metabolic enzymes and serves as a precursor for indole-3-acetic acid (IAA) synthesis, while boron deficiency leads to malformed flowers, reduced fruit set, and poor fruit quality.

Materials and Methods

The experiment was conducted in 2025 in a 66-year-old litchi orchard of cv. Dehradun at the Department of Fruit Science, C.S. Azad University of Agriculture and Technology, Kanpur, Uttar Pradesh. The experiment was laid out in Randomized Block Design with nine treatments and three replications. The nine treatments viz., level of NAA(40, 60ppm), boron (0.4, 0.6 and 0.8%) and ZnSO₄(0.6, 0.8 and 1%) along with control. The recommended doses for all treatments, including the control, were applied using a sprayer with a very fine nozzle. The first spray was done prior to blooming on 02/02/2025, and the second spray was applied on 18/03/2025, when the fruit was at the pea stage. The data collected for various parameters during the experimental period were subjected to statistical analysis. Observations on fruit drop, fruit retention, fruit set, and fruit cracking percentages were recorded throughout the fruiting season. At each harvest, fruit weight and yield per plant were measured. Fruit length and width were measured in five randomly selected fruits using a vernier calliper and expressed in centimetres (cm). Data regarding pulp weight, seed weight, and pulp-to-seed ratio were also documented. Total soluble solids (TSS) of the fruits were determined using a hand refractometer, while titratable acidity and total sugar content were estimated according to the **AOAC (1980)** standard procedures.

Results and Discussion

Fruit set

The average number of fruits per panicle was calculated by counting all tagged panicles in each treatment to estimate fruit set. The foliar treatment with NAA, Boron, and ZnSO₄ significantly increased the fruit set % compared to the control (Table 1). Plants treated with T₁-NAA @ 40 ppm had the highest fruit set (57.46%), closely followed by Boron @ 0.6% (56.97%), ZnSO₄ @ 0.6% (56.95%), and Boron @ 0.8% (56.36%). The control treatment (T₉, water spray) showed the lowest fruit set (52.96%). Higher fruit set with the application of NAA might be due to the formation of sugars from polysaccharides and a decrease in acid content during the maturity and ripening period, which are mostly affected by auxin; these metabolic changes in the present investigation helped improve fruit set. These results are consistent with (12) in Ber, (4) in Guava, (17) in litchi, and (20) in mango.

Fruit cracking

The percentage of cracked fruits was computed after counting the total number of cracked fruits. Foliar treatments with NAA, Boron, and ZnSO₄ significantly reduced the percentage of fruit cracking compared with the control (Table 1). Plants treated with T₄-Boron @ 0.6% showed the least fruit cracking (7.95%), closely followed by ZnSO₄ @ 0.6% (9.55%), NAA @ 40 ppm (10.31%), and Boron @ 0.8% (11.21%). The control group (T₉) had the highest fruit cracking rate (18.84%). Boron helps in strengthen the cell wall and maintain membrane stability, improving peel elasticity and reducing cracking. Zinc enhances enzymatic activity, photosynthesis and tissue strength, thereby improving fruit development and minimizing pericarp rupture. NAA regulates cell enlargement and water balance in fruits, thereby helping to maintain fruit skin elasticity and reducing the incidence of cracking. These results are in conformity with the findings of (10) and (7) in litchi

Fruit drop and fruit retention

Fruit drop and retention were significantly influenced by NAA application; the minimum fruit drop (69.43%) and maximum fruit retention (30.57%) were recorded with 40 ppm of NAA during the investigation period (Table 1). The maximum fruit drop (78.95%) and minimum fruit retention (21.05%) were achieved in plants kept under control (T_9). It is commonly known that when auxin concentration rises, low fruit production is caused by low auxin levels and a lack of ultimate cell fracturing in the middle lamella or near the abscission layer. By inhibiting the enzymes that make pectin soluble, auxin treatment of the fruits prevents these alterations in the middle lamella, resulting in reduced fruit drop and improved fruit retention. These findings align with the results of (21), (22) in aonla, (14) in Kinnow, (12) in ber, (11) in litchi, (4) in guava and (16) in litchi.

Fruit Size (Length and width)

Fruit size, measured as length and width using vernier callipers, was significantly influenced by foliar applications of NAA, Boron, and $ZnSO_4$ (Table 1). Plants treated with Boron @ 0.6% (T_4) recorded the maximum fruit length (4.72 cm) and width (3.98 cm), followed by $ZnSO_4$ @ 0.6% (T_6) with fruit length (4.43 cm) and width (3.74 cm), and NAA @ 40 ppm (T_1) with fruit length (4.31 cm) and width (3.62 cm). The minimum fruit length (3.10 cm) and width (2.85 cm) were recorded in the untreated control (T_9). The improvement in fruit size can be attributed to boron's role in nitrogen metabolism, cellular differentiation, and the translocation of food materials from sources to storage organs. Boron plays an important role in nitrogen metabolism and brings about other changes in fruits that may increase fruit size. These results are in conformity with the findings of (19) and (13) in aonla, and of (10) in litchi.

Fruit Weight

Foliar application of plant bio-regulators and micro-nutrients significantly influenced fruit weight. The maximum fruit weight was recorded in plants treated with T_4 -Boron @ 0.6% (20.00 g), followed by T_1 -NAA @ 40 ppm (19.65 g) and T_5 -Boron @ 0.8% (18.41 g). The control treatment

produced the lowest fruit weight (16.68 g) in Table 1. Application of zinc at a moderate concentration increased fruit weight considerably, with higher sugar accumulation and juice percentage in zinc-treated fruits. The present findings corroborate the results reported by (17), (18), (9), (5), and (10) in litchi.

Pulp Weight and Seed Weight

Significantly improved fruit pulp and reduced seed weight were observed with foliar application of plant bioregulators and micronutrients. Plants treated with T_4 -Boron @ 0.6% recorded the maximum pulp weight (20.73 g) and reduced seed weight (2.75 g), followed by T_6 - $ZnSO_4$ @ 0.6% with pulp weight (17.14 g) and seed weight (3.37 g), and T_1 -NAA @ 40 ppm with pulp weight (18.14 g) and seed weight (3.13 g). The minimum pulp weight (11.36 g) and maximum seed weight (3.96 g) were produced from the untreated control plants (Table 2). Reduced seed weight with increased pulp weight indicates improved edible quality. The present findings corroborate the results reported by Dhua et al. (2005), (9), S, (10) in litchi, and (17) and (20).

Rind Weight

All treatments significantly reduced rind weight compared to the control. Plants treated with Boron @ 0.6% (T_4) recorded the lowest rind weight (1.08 g), followed by Boron @ 0.8% (1.12 g), NAA @ 40 ppm (1.12 g), and Boron @ 0.4% (1.50 g). The highest rind weight (1.93 g) was recorded in the control treatment (T_9) in Table 2. Consumers and traders prefer a thinner rind with a higher pulp proportion, and the present investigation confirms that NAA, Boron, and $ZnSO_4$ can favourably alter the partitioning of assimilates between pericarp and pulp. These results are consistent with those of (17) in litchi, (20) in mango, and (10) in litchi.

Total Soluble Solids

Total soluble solid (TSS) is one of the most important quality parameters in litchi fruits and is directly associated with sweetness and consumer acceptability. The results revealed that foliar application of NAA, Boron, and $ZnSO_4$ significantly increased TSS content compared to the control. Plants treated with T_4 -Boron @ 0.6% recorded the

highest TSS (19.97°Brix), followed by ZnSO₄ @ 0.6% (19.41°Brix), NAA @ 40 ppm (18.15°Brix), and Boron @ 0.8% (17.86°Brix). The lowest TSS (17.61°Brix) was recorded under the control treatment (T₉) in Table 2. Boron facilitates carbohydrate metabolism and translocation of sugars from source to sink, while zinc plays a vital role in photosynthesis and enzyme activation, thereby improving carbohydrate synthesis and transport. The present findings corroborate the results reported by (9), (5), (13), (7), Gupta *et al.* (2022) in litchi, and (3).

Total Sugar

Total sugar content is a key factor of taste and commercial acceptance in litchi. The results revealed that foliar application of NAA, Boron, and ZnSO₄ significantly increased total sugar content compared to the control. Plants treated with T₄-Boron @ 0.6% recorded the highest total sugar (18.52%), followed by ZnSO₄ @ 0.6% (17.93%), NAA @ 40 ppm (16.63%), and Boron @ 0.8% (16.31%). The lowest total sugar content (15.98%) was recorded under the control treatment (T₉) in Table 2. The present findings corroborate the results reported by (18), (6), and (8) in litchi.

Titrateable Acidity

Titrateable acidity is an important quality attribute that indicates the number of organic acids present in the fruit pulp. Lower titrateable acidity in treated fruits generally reflects improved ripening and better palatability. Compared with the control, all treatments significantly decreased titrateable acidity when applied foliarly. Plants treated with T₄-Boron @ 0.6% had the lowest titrateable acidity (0.35%), followed by ZnSO₄ @ 0.6% (0.38%), NAA @ 40 ppm (0.42%), and Boron @ 0.8% (0.45%). The control treatment (T₉) had the highest titrateable acidity (0.59%) among the treatments in Table 2. The decline in acidity with increasing TSS and total sugar reflects enhanced fruit maturity and quality. The present findings corroborate the results reported by (18), (5), (7), and (8) in litchi.

Vitamin C

Vitamin C content is an important nutritional quality attribute in litchi fruits. All treatments

considerably increased ascorbic acid content compared to the control. Plants treated with T₄-Boron @ 0.6% had the highest ascorbic acid content (25.36 mg/100g), followed by ZnSO₄ @ 0.6% (24.77 mg/100g), NAA @ 40 ppm (24.27 mg/100g), and Boron @ 0.8% (23.81 mg/100g). The control treatment had the lowest ascorbic acid concentration (20.42 mg/100g) in Table 2. The application of NAA, along with micronutrients, influences ascorbic acid content by altering metabolic activities and antioxidant enzyme functions in fruit tissues. Boron and zinc act as essential cofactors for enzymatic reactions and can promote synthesis and retention of ascorbic acid in the fruit pulp. The present findings corroborate the results of (18), (10), and (8) in litchi.

Fruit Yield

Fruit yield per plant is the final indicator of the effectiveness and productivity of any orchard management practice. Plants treated with T₄-Boron @ 0.6% had the highest fruit yield (25.31 kg/plant), followed by ZnSO₄ @ 0.6% (24.74 kg/plant), NAA @ 40 ppm (23.73 kg/plant), and Boron @ 0.8% (23.39 kg/plant). The control treatment produced the least amount of fruit (19.85 kg/plant; Table 2). Boron favourably affects photosynthesis, carbohydrate translocation, and cellular division, while zinc promotes enzyme activity, improves pollen fertility, and increases fruit set. NAA helps reduce fruit drop and increase fruit retention, thereby significantly improving overall yield. The present findings corroborate the results reported by (18), (5), (7), and (8) in litchi.

Pulp: Stone Ratio

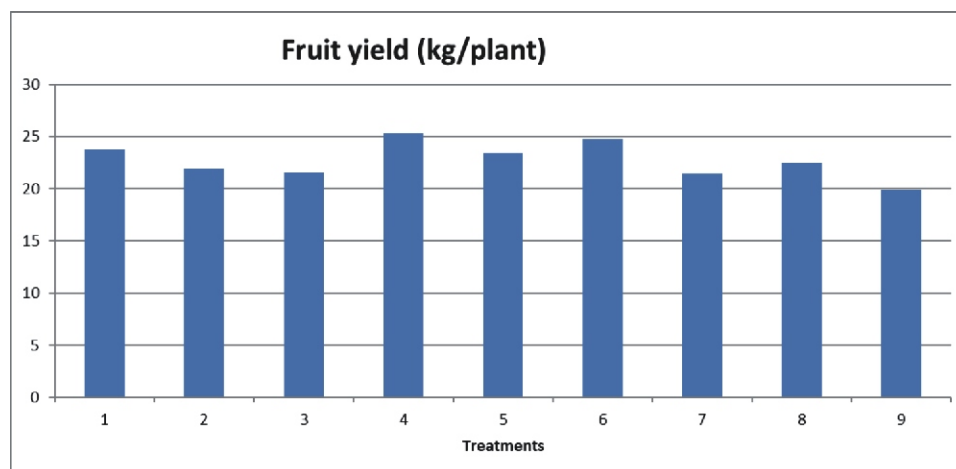
The pulp-to-stone ratio is an important parameter of edible quality in litchi. Plants treated with T₄-Boron @ 0.6% had the highest pulp: stone ratio (6.89), followed by Boron @ 0.8% (5.38), NAA @ 40 ppm (5.08), and ZnSO₄ @ 0.6% (5.17). The control treatment had the lowest pulp: stone ratio (2.91) in Table 2. The enhancement in the pulp: stone ratio may be attributed to the combined effects of greater pulp development and reduced seed growth, both influenced by boron and zinc in assimilate distribution and by auxin-induced parthenocarpic activity. The present findings corroborate the results reported by (5) and (10).

Table 1: Effect of foliar application of NAA, Boron and ZnSO₄ on fruit set, drop, retention, cracking and physical fruit parameters in litchi cv. Dehradun.

Treatments	Fruit set (%)	Fruit drop (%)	Fruit retention (%)	Fruit length (cm)	Fruit diameter (cm)	Fruit weight (g)	Pulp weight (g)	Fruit cracking (%)
T1-NAA @ 40 ppm	57.46	69.43	30.57	4.42	3.79	21.08	16.68	10.31
T2-NAA @ 60 ppm	54.09	73.31	26.69	4.29	3.67	20.34	15.87	12.57
T3-Boron @ 0.4%	53.95	74.37	25.63	4.26	3.65	19.61	15.01	13.61
T3-Boron @ 0.4%	53.95	74.37	25.63	4.26	3.65	19.61	15.01	13.61
T5-Boron @ 0.8%	56.36	72.48	27.52	4.38	3.74	20.97	16.04	11.21
T6-ZnSO ₄ @ 0.6%	56.95	71.45	28.55	4.44	3.84	21.92	17.45	9.55
T7-ZnSO ₄ @ 0.8%	53.41	75.82	24.18	4.18	3.60	18.98	14.91	14.39
T8-ZnSO ₄ @ 1%	55.97	76.41	23.59	4.32	3.72	18.42	14.58	11.63
T9-Control (water spray)	52.96	78.95	21.05	4.09	3.52	17.97	11.94	18.84
C.D. at 5% level	1.92	3.48	0.82	0.028	0.179	0.71	0.75	0.566
S.E. (m)	0.64	1.15	0.27	0.069	0.059	0.23	0.24	0.187

Table 2: Effect of foliar application of NAA, Boron and ZnSO₄ on physico-chemical parameters of litchi cv. Dehradun

Treatments	Seed weight (g)	Rind weight (g)	TSS (°Brix)	Total sugar (%)	Titrateable acidity (%)	Vitamin C (mg/100)	Fruit yield (kg/plant)	Pulp: stone ratio (%)
T1-NAA @ 40 ppm	3.28	1.12	18.15	16.63	0.42	24.27	23.73	5.08
T2-NAA @ 60 ppm	3.33	1.14	17.81	16.20	0.51	22.04	21.90	4.80
T3-Boron @ 0.4%	3.10	1.50	17.61	15.98	0.53	21.97	21.51	4.84
T4-Boron @ 0.6%	2.75	1.08	19.97	18.52	0.35	25.36	25.31	6.89
T5-Boron @ 0.8%	2.98	1.95	17.86	16.31	0.45	23.81	23.39	5.38
T6-ZnSO ₄ @ 0.6%	3.37	1.10	19.41	17.93	0.38	24.77	24.74	5.17
T7-ZnSO ₄ @ 0.8%	2.85	1.22	16.94	15.26	0.55	21.06	21.43	5.23
T8-ZnSO ₄ @ 1%	2.73	1.11	17.80	16.23	0.47	22.41	22.50	5.34
T9-Control (water spray)	4.10	1.93	17.67	15.98	0.59	20.42	19.85	2.91
C.D. at 5% level	0.16	0.06	0.99	0.73	0.03	1.25	1.06	0.14
S.E. (m)	0.05	0.02	0.32	0.24	0.009	0.41	0.35	0.04



Conclusion

From this experiment, it can be concluded that foliar application of Boron significantly improved fruiting, yield and quality attributes in litchi. Spraying of Boron at 0.6% resulted in higher fruit set (56.97%), increased fruit retention (29.82%), along with maximum fruit length (4.62 cm) and width (3.90 cm), fruit weight (22.78 g), pulp weight (18.95 g), pulp: seed ratio (6.89), total soluble solids (19.97°Brix), total sugars (18.52%) and Vitamin C content (25.36 mg/100 g), with minimum fruit cracking (7.95%), fruit drop (70.18%), seed weight (2.75 g), rind weight (1.08 g) and titratable acidity (0.35%) under plains of northern India. These findings are in accordance with the results, where Boron application at comparable concentrations was found to be beneficial for improving fruit retention and quality constituents in litchi cv. Dehradun.

References

1. Anonymous, 2018-19. Indian Horticulture Database. National Horticulture Board, Gurgaon.
2. A.O.A.C. (1980). Association of Official Agricultural Chemists. Washington, D.C., USA. Official Methods of Analysis. 14th eds.
3. Babu, Ram and Tripathi, V.K. (2022). Impact of foliar application of NAA, Zinc and Boron on growth, yield and quality parameters of guava (*Psidium guajava* L.). *Progressive Agriculture*, **22** (2): 190-194
4. Badal, D. S. and Tripathi, V. K. (2021). Influence of foliar feeding of NAA and boron on growth, flowering, fruiting, and yield of winter-season guava (*Psidium guajava* L.) cv. L-49. *Biological Forum-An International Journal*, **13**(3): 387-391.
5. Banyal, A.K. and Rangra, N.S. (2011). Response of yield and quality attributes of litchi cv. Dehradun to soil and foliar application of boron. *Journal of Hill Agriculture*, **2**(1): 33-57.
6. Chaturvedi, O. P., Singh, A. K., Tripathi, V. K. and Dixit, A. K. (2005). Effect of zinc and iron on growth, yield and quality of strawberry cv. Chandler. *Acta Horticultureae*, **696**: 237-240.
7. Chauhan, A. S., Kumar, K., Saini, P. K., Singh, V. and Singh, J. P. (2019). Effect of NAA and zinc sulphate on fruiting, yield of Litchi (*Litchi chinensis* Sonn.) cv. Calcuttia. *International Journal Current Microbiology and Applied Sciences*, **8**(3): 836-843.
8. Gupta, Ajeet, Tripathi, V. K. and Shukla, J. K. (2022). Influence of GA₃, Zinc and Boron on fruit drop, yield and quality of litchi (*Litchi chinensis* Sonn.). *Biological Forum-An International Journal*, **4**(3): 1079-1083
9. Katiyar, P.N., Prakash, O., Singh, J.P. and Singh, P.C. (2008). Effect of zinc sulphate, borax and calcium nitrate on flowering, fruiting, yield and quality of Litchi cv. Dehradun. *Journal of Rural Agriculture Research*, **8**(2): 49-51.
10. Kaur, Sukhjit (2017). Effect of micronutrients and plant growth regulators on fruit set, fruit retention, yield and quality attributes in Litchi cv. Dehradun. *Chemical Science Review and Letters*, **6**(22): 982-986.
11. Kumar, D., Dwivedi, A.K., Tripathi, V.K., Pandey, S., 2023b. Influence of different levels of NAA and zinc sulphate on fruiting, yield and quality attributes of litchi cv. Dehradun. *Progressive Agriculture*, **23**(1), 150–158.
12. Kumar, M., Dwivedi, A.K., Tripathi, V.K., Shukla, A., 2023a. Influence of different levels of NAA and 2, 4, 5-T on fruit drop, fruiting, fruit retention, growth and yield of Indian ber (*Ziziphus mauritiana* Lamk.). *International Journal of Plant and Soil Science*, **35**(9), 16–29.
13. Kumar, A., Tripathi, V. K., Dubey, V., Katiyar, N. K. and Tiwari, P. (2017). Influence of foliar application of calcium, zinc and boron on fruit drop, yield and quality attributes of Aonla (*Embllica officinalis*) cv. NA-7. *Research on Crops*, **18** (1): 91-97.
14. Lal, D., Tripathi, V. K., Kumar, S. and Nayyar, M.A.(2015). Effect of pre-harvest application of gibberellic acid, NAA and calcium nitrate on fruit drop, maturity and storage quality of Kinnow Mandarin. *Research on Environment and Life Sciences*, **8**(4), 561–564.
15. Mohit, Tripathi, V. K., Pandey, S. and Mishra, A. (2023). Impact of foliar application of NAA, Boron and Zinc on fruit drop, yield and quality attributes on aonla (*Embllica officinalis* Gaertn.) cv. NA-7. *Progressive Horticulture*, **55**(1): 30-35
16. Radha, Tripathi, V. K., Verma, S. and Mishra, A. (2023). Influence of different levels of Boron and NAA on fruiting, yield and quality attributes of Litchi (*Litchi chinensis* Sonn.). *Annals of Horticulture* **16** (2): 130-140.
17. Saraswat, N.K., Pandey, U. N. and Tripathi, V. K. (2006). Influence of NAA and Zinc sulphate on fruit drop, cracking, fruit size, yield and quality of litchi cv. Calcuttia. *Journal of Asian Horticulture*, **2**(4): 255- 259.

18. Sharma, P., Singh, A.K. and Sharma, R.M. (2005). Effect of plant bio-regulators (PBRs) and micro-nutrients on fruit set and quality of litchi cv. Dehradun. *Indian Journal of Horticulture*, **62**(1): 24-26.
19. Shukla, H.S., V. Kumar and Tripathi, V. K. (2011). Effect of Gibberellic Acid and Boron on development and quality of aonla fruits 'Banarasi'. *Acta Horticulture*, **890**: 375-380.
20. Singh, T., Tripathi, V. K. and Tiwari, P. (2017). Influence of pre-harvest application of plant bio-regulators and micronutrients on fruit retention, yield and quality attributes of Mango. *Progressive Research-An International Journal*, **12**(Special-IV): 2640-2644
21. Tripathi, V. K., Pandey, S. S., Kumar, A., Dubey, V. and Tiwari, P. (2018). Influence of foliar application of Gibberellic acid, Calcium and Boron on fruit drop, yield and quality attributes of aonla cv. NA-7. *Indian Journal of Agricultural Sciences* **88** (11): 1784-1788.
22. Tripathi, V.K. and Kumar, Y. (2022). Influence of pre-harvest application of GA₃ and ZnSO₄ alone and in combination on fruit drop, yield and quality attributes of mango. *Progressive Horticulture* **54**(1), 62-67.
23. Tripathi, V.K. and Viveka Nand (2022). Effect of foliar application of Boron, Zinc and NAA on fruit retention, yield and quality attributes of aonla. *Progressive Horticulture*, **54**(1): 76-81

Received : April 2025; Revised : May 2025; Accepted : July 2025



GENETIC TRANSFORMATION OF GRAPEVINE USING AGROBACTERIUM- MEDIATED GENE TRANSFER

Aakansha Goswami¹, Bijendra Singh² and R. P. Singh³

¹Professor, Vikrant University, Gwalior

²Dean, College of Horticulture, SVBPUAT, Meerut

³ Former Vice Chancellor, Bikaner Agriculture University, Rajasthan

***Corresponding Author Email-**email@gmail.com

Abstract

By introducing desirable traits into an existing genome while maintaining the plant's genetic integrity, plant genetic engineering is an effective method for enhancing plants. The introduction of valuable genes encoding traits like disease/insect resistance, herbicide tolerance, enhanced nutritional/medical/commercial properties, improved uptake and utilization of growth agents, and many more traits has been accomplished through the genetic modification of a wide variety of plant species. For reasons discussed later in this chapter, the public's perception of genetically modified organisms (GMOs) is currently negative. However, it remains to be seen whether the advantages of reduced chemical use and improved nutritional aspects lead to a world with food security that is environmentally sustainable. Genetic engineering is especially important because grape is very heterozygous and standard breeding methods fail to produce true-to-type offspring. Grapes are also a commodity driven by consumers, and novel varieties, particularly wine cultivars, have little market acceptance. In order to produce indigenous genotypes with novel characteristics, technological breakthroughs like gene transfer must be utilized because vegetative propagation leaves little room for development.

Introduction

The grape (*Vitis* sp.) is one of the most widely grown commercial fruit crops worldwide in temperate, subtropical, and tropical climates. Grapes fall under the Vitaceae family, which Einset and Pratt (1975) further subdivides into the *Euvitis* Planch. (2n=38) and *Muscadinia* Planch. (2n=40) subgenera. Over 60 species of commercial grapes belong to the genus *Vitis*, which is mostly found in temperate regions of the Northern Hemisphere and is nearly evenly distributed between Asia and America. The majority of grape varieties grown today are members of the species *Vitis vinifera* L., which originated in Eurasia and was introduced to the rest of the world by humans. The grapevine liana is a climbing shrub with a genus of deciduous and occasionally evergreen shrubs. The trunk, a permanent stem, is made up

of the canes, which are woody, dormant shoots with buds or "eyes" from which new growth emerges after trimming. *Euvitis* has loose, peeling bark, whereas *Muscadinia* has tight, non-shedding bark. The leaves are irregularly toothed, glabrous or tomentose, non-lobed, or three to seven-lobed. The leaf arrangement on the stalk is distichous, whereas tendrils can be continuous (opposite each leaf) or intermittent (opposite two adjacent leaves). In *Euvitis*, modified branches and tendrils are bifid, whereas in *Muscadinia*, they are unifid. The grapevine's inflorescence is the cyme, a panicle with numerous branches. The tiny, greenish blooms are carried by dense, elongated or short panicles. The dioecious *Vitis* plant became a hermaphrodite through spontaneous mutations during evolution. Hermaphrodite flowers have five sepals that are partially fused (calyx), five petals that are joined at the top (cap, calyptra, or corolla),

five stamens, and a two-located pistil with a small style and stigma. In many wild grapes, there are still male and female blooms on separate vines. The grapevine is classified as self-fertile, self-sterile, and partially self-sterile based on the reproductive organs' functionality. Berries, pedicles, rachis, and peduncles make up the clusters of fruit known as panicles. The fruit is a berry that ranges in size from ovoid to globose. They can be greenish, red, purple, blue, or bluish black in color and have thick or thin skin. The edible portion of the placenta and pericarp frequently tastes good. Wild grapes have a sweeter flavor than modern cultivars.

India has developed and made available a number of commercially viable cultivars in addition to numerous introduced varieties. These cultivars include Tas-e-Ganesh and Pusa Seedless, which are clonal selections of Thompson Seedless, an introduced variety from the United States. Grapes can be dried and turned into raisins, juices, liquors like wine and champagne, and eaten fresh, or they can be canned. Wine accounts for approximately 70% of grape crop production, while table grapes account for less than 30%. Other significant industrial applications for grape byproducts include the production of ethanol, anthocyanin colors, and grape seed oil. In their medical treatises titled, renowned Indian medical philosophers Sasruta and Charaka discuss the therapeutic benefits of grapes. The "Sasruta Samhita" and "Charaka Samhita" were written between 1356 and 1220 BC. Grapes and grape plants were valued by Greek philosophers for their therapeutic properties. European folklore used grapevine leaves to treat hemorrhoid inflammation and bleeding, while grapevine sap was used to treat skin and eye conditions. Rough grapes and raisins were used to treat TB, smallpox, cancer, illness, and problems with the liver and kidney. Resveratrol, a polyphenol found in red wines and grapes, has recently been shown to prevent cancer by causing human melanoma cells to undergo apoptosis (Niles et al., 2003; Yang et al., 2001). This is probably due to the antioxidant qualities it possesses (Chanvitayapongs et al., 1997). Grape seed extracts are now used in cosmetics due to their anti-aging and anti-wrinkle properties. Worldwide, 65,853,393 metric tons of grapes were

produced in 2005. Italy, France, and Spain control the world's grape and wine industries, respectively, with 13.91, 10.92, and 8.84 percent of production. These nations, each with more than a million hectares of grapevines, produce more than half of the world's wine. The United States is the third-largest producer, accounting for 9.64 percent of global production. According to FAO STAT, 2005, China (8.56%) and Iran (4.21%) are the two largest Asian producers.

According to FAO STAT, 2005, 60,000 hectares of land in India produce approximately 1.6 million tonnes of grapes annually, or 1.83 percent of global production. According to Adsule et al. (2006), 78% of this is used for food, 17%-20% is used to make raisins, and 0.5 percent is used to make wine. Fig. 1.1, along with their production, productivity, and cultivation area. India has the highest productivity in the world, with 20 tonnes per ha. Despite the fact that the initial high cost of vineyard development and ongoing costs are a barrier to expanding the area under grape cultivation, India has already entered the profitable industry of wine production due to its high net returns. Advanced production technologies, forward-thinking entrepreneurship, and easy access to institutional financing for the crop from organizations like the National Horticulture Board (NHB), the Agriculture and Processed Food Products Export Development Authority (APEDA), and the National Bank for Agriculture and Rural Development (NABARD) have helped establish the Pune, Nashik, and Sangli districts in Maharashtra as major wine and grape-growing regions. 90% of India's production is exported, with the remainder going to South East Asian nations, 8% to Europe, and 90% to the Gulf. India has a lot of room to expand its export potential, primarily as a result of its harvest season and by concentrating on nearby South East Asian nations. Cultivars that are suitable for both North and South India are being grown due to the differences in climate. Thompson Seedless is grown for commercial purposes (Fig. 1.1. A) is practiced in Maharashtra and Tamil Nadu. In recent years, wine varieties like Sonaka, Manik Chaman, and Sharad Seedless, as well as recently introduced varieties like Flame Seedless, have gained popularity in Maharashtra (Fig. 1.1. B) and the Red

Globe The best places to grow Bangalore Blue and Anab-e-Shahi, two enormous green-seeded table grapes, are in Karnataka and Hyderabad, respectively. All three, Perlette, Beauty Seedless, and Pusa Seedless, have done well in North India. Other widely grown varieties include Bhokri, Cheema Sahebi, Gulabi, Tas-e-Ganesh, Arkavati, Arka Kanchan, Arka Han, and Arka Shyam. Nevertheless, market demand has prompted cultivar diversity in these regions. Grape yield is significantly influenced by both biotic and abiotic stressors. Viticulture is a monoculture system with a small number of genotypes that makes it very easy for pests to get in.

Research Methodology

Collection and preparation of plant materials

The collection and surface sterilization of plant materials utilized in somatic embryogenesis and embryo rescue research, respectively.

Inoculation

Cultures were inoculated in a Microfilter, India, laminar airflow cabinet. The explants were removed on sterile filter paper with sterile scalpels and forceps that had been immersed in 96% rectified spirit and flame sterilized prior to inoculation. Similarly sterilized surgical blades (No. 11 and No. 12) were used to remove the explants. The number of explants used in each replication, the number of replicates, and the number of experimental repetitions is all covered in the material and methods.

Culture conditions

Culture conditions differ for ovulation culture, embryo culture, and somatic embryogenesis. Ovule and rescued embryo cultures were typically incubated at 25 ± 2 °C, while somatic embryo cultures were typically incubated at 28 ± 0.5 °C, as detailed in the materials and methods sections of the corresponding chapters. Depending on the type of tissue being cultured, the illumination varied.

Statistical analysis

Statistics were used to compare the results of optimizing the parameters. The "F" test or arcsine transformation and factorial analysis were

utilized to determine the significance of the treatments, and the completely randomized block design method was used for the analysis of variance. The means and standard deviations of the data were calculated and evaluated using Microsoft Excel.

Hardening of the plantlets

The in vitro plantlets were carefully removed from the test tubes and gently cleaned with tap water to get rid of the agar and media that were sticking to the roots. They were rinsed with tap water after being submerged for ten to fifteen minutes in an aqueous solution containing 1% of the systemic fungicide Bavistin® (BASF, India). After that, the treated plantlets were placed in plastic cups containing a soil-sand ratio of one to one. These cups were first exposed to constant, intense light at 25 ± 2 °C in thin, transparent polythene bags to harden. After two weeks, the polythene bags were cut at the tops to gradually expose the plants to the outside world. The plants were moved to a greenhouse after four weeks, and the plastic bags were completely removed.

Genetic Transformation

The materials and techniques utilized for transformation mediated by *Agrobacterium tumefaciens*.

Results and Discussion

Genetic engineering is a powerful method for the development of plants because it preserves the genetic identity of plants while simultaneously introducing desired traits into an existing genome. This field has made groundbreaking advancements by introducing beneficial genes coding for traits like disease/insect resistance, herbicide tolerance, improved nutritional, medicinal, and commercial properties, improved growth factor uptake, and many more through the genetic modification of a variety of plant species. Even though the general public has a negative opinion of genetically modified organisms (GMOs) for reasons discussed later in the chapter, it remains to be seen whether the advantages of fewer chemical uses and improved nutritional aspects lead to a world that is sustainable in terms of the environment and provides food security. Genetic engineering is especially crucial for grapes

because of their extreme heterozygosity and the inability of conventional breeding methods to produce true-to-type offspring. Additionally, new cultivars, particularly wine cultivars, are not well received by the market because grapes are a commodity heavily influenced by consumers. Technical advancements like gene transfer must be utilized to produce native genotypes with distinct characteristics because vegetative propagation leaves little room for improvement. Utilizing *Agrobacterium tumefaciens* and GFP as the reporter gene, the current study sought to develop a Thompson Seedless transformation strategy that was simple, quick, and efficient. The experiment used new media (MPM) created by Perrin et al. (2001, 2004), which had never previously been utilized for grape transformation. The objectives of the investigation were as follows:

1. Standardization of *Agrobacterium*-mediated transformation in Thompson Seedless by examining variables influencing temporary transformation with GFP as a reporter gene.
2. Research on the elements influencing stable transformation:
 - a. Acetosyringone (AS) and b. polyvinylpyrrolidone (PVP)
 - b. Acetosyringone addition and the use of plant cells in place of acetosyringone on *Agrobacterium* growth and steady transformation, d. Callus kinds
3. Gene expression in altered cells and GFP-assisted or kanamycin-assisted transformed tissue selection (antibiotic-free selection).
4. Calli regeneration after transformation.
5. Using PCR and RT-PCR to characterize transformants molecularly.

Plant material

Embryogenic cultures (friable) of one callus line of Riesling and two callus lines of Thompson Seedless (1A and 2E) maintained on MPM1 media were used in transformation studies. The incubation conditions were 28 ± 0.5 °C, complete darkness, and $60 \pm 5\%$ relative humidity for three weeks. The calli were sub cultured on MPM1 medium for two days prior to co-cultivation with *Agrobacterium*.

Constructs and Agrobacterium strains

The strains of *Agrobacterium tumefaciens* that were utilized for transformation studies were C58pMP90, which contained the binary vector pBINm-gfp5-ER, and C58pGV2260, which contained the binary vector pBI121. Dr. kindly provided these strains. Francis Karst, Colmar, INRA The BamHI-SstI fragment containing the m-gfp5-ER gene has been replaced by the BamHI-SstI fragment containing the GUS gene in the variant of the plasmid pBINm-gfp5-ER (Jefferson et al., 1987) By ligating the CaMV-35S promoter to the HindIII and BamHI sites of pBI101, the coding region of GUS is fused to the 5' end of the nopaline synthase polyadenylation site, resulting in the pBI121 plasmid (Jefferson et al., 1987). The m-gfp5-ER gene was cloned into pBI121 between the SacI and BamHI sites for plant transformation (Haselhoff et al., 1997). Plants acquire resistance to kanamycin from either vector. C58pMP90 and C58pGV2260/pBI121 are referred to as bacteria "A" and "B" in the study for ease of use.

General transformation procedure followed

For C58pGV2260, the bacteria were maintained on YEB (Yeast Extract Broth) solid medium with 50 mgL⁻¹ of rifampicin and kanamycin, and for C58pMP90, gentamycin (20 mgL⁻¹). On an orbital shaker, the bacteria were grown for an entire night in antibiotic-containing YEB+MgSO₄ (10 M) at 28 °C and 200 rpm. After measuring the optical density (OD), the leftover culture was centrifuged for ten minutes at 5000 rpm before being resuspended in fresh YEB containing MgSO₄ and antibiotics. Acetosyringone (3',5'-Dimethoxy-4'-hydroxy-acetophenone; Fluka, Switzerland) (AS) was added to the media, and it was shaken for two more hours at 28 °C and 200 rpm (extended culture). The culture was resuspended in MPM1 liquid medium after being centrifuged for ten minutes at 5000 rpm for two hours to achieve the required OD₆₀₀ of 0.3 to 2.0 for inoculating calli. The process is presented in tabular form for simplicity.

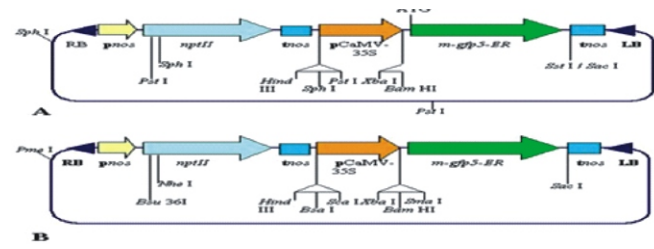


Fig 1. Plasmid maps

Table -1. General protocol used for transformation

Steps	Culture conditions
Pre-culture	YEB + RK + G, 28 oC, 48 hours
Culture	YEB + RK + G, 28 oC, 24 hours
Extended culture with AS	YEB + RK + G + AS 100 μ M, 28 oC, 2 hours
Co-cultivation	MPM1 solid + AS 100 μ M + 0.5 % PVP, 20 oC, 4 days, dark, high humidity
Washing and sub culturing	MPM1 liquid + Cefotaxime 600 mgL ⁻¹ + 0.5 % PVP
Selection	MPM1 solid + Cefotaxime 600-100 mgL ⁻¹ + Kanamycin 25 mgL ⁻¹
Regeneration	MPM-based or WPM + Cefotaxime 100-200 mgL ⁻¹ + Kanamycin 25 mgL ⁻¹

Table -2. Treatments imposed to study the influence of Acetosyringone and plant cells added to liquid culture of Agrobacteria on changes in OD

Treatments		Treatment Code Number		
Acetosyringone	Plant cells	C58pMP9	C58pGV2	YEB (Control)
-	-	0	260	
-	-	1	5	9
+	-	2	6	10
-	+	3	7	11
+	+	4	8	12

Factors affecting transient transformation

The effects of co-cultivation on MPM1 or MPM1+AS medium, bacterial strains, co-cultivation techniques like inoculating calli with a drop of bacterial suspension or creating a suspension of calli with bacterial cells, and co-cultivation at OD600 0.3, 0.6, and 2.0 were evaluated in a variety of experiments to determine the factors that influence transitory transformation. At the two transformation temperatures, only bacteria B and MPM1 media were used in the pretreatment of calli. The remaining tests utilized both microorganisms. In the drop approach, a 6 μ L drop of bacterial solution was used to inoculate the calli (50 μ L PCV each). The calli were homogenized with suspension using the alternative approach (the soup method for

convenience), which involved putting them on a gyratory shaker for 10 minutes, letting them settle, blotting them on filter paper, and then depositing them on MPM1 or MPM1+AS (100 μ M) medium. The best transient expression research treatments were then used in stable transformation experiments. At least twice were carried out on each experiment.

Factors affecting stable transformation

A number of parameters' effects on stable transformation were examined. During and after co-cultivation, these included: (1) decreasing necrosis by adding PVP to MPM1 liquid/solid medium; (2) extending the culture period by adding or removing AS from YEB; (3) substituting AS by adding grape plant cells to YEB; and (4) callus types influencing transformation efficiency. In the beginning, a microbiology reader known as Bioscreen C was used to examine agrobacterium growth curves in order to ascertain whether acetosyringone or plant cells had an effect on the bacteria. 1.05 of Research Express At least twice were carried out on each experiment.

Experiments with microbiology reader Bioscreen C to study Agrobacterium growth

In order to investigate the effect that AS and plant cells have on the growth curves of the Agrobacteria that were used in the study, optical densities of the bacteria were measured using Bioscreen C, a microbiology incubator, and a culture growth monitoring device (OD reader) controlled by Research Express v.1.05 software (Transgalactic Ltd., Finland). The Microsoft Excel package performed the analysis automatically. C58pMP90 and C58pGV2260 were grown overnight for transformation experiments. YEB was given a 10 mL suspension of antibiotics and AS 100 mM if necessary the following day.

A 400 L volume of the suspension was pipetted into a sterile, covered 100-well honeycomb plate. Wells received 10 L PCV of TS line 1A's EC as needed. Each time, the experiment was repeated three times. In the Bioscreen C workstation, the ODs were set to be measured every hour at 600 nm for 40 hours while the plates were immediately incubated at 28 oC. The program Microsoft Excel was used to analyze the data.

Co-cultivation and subculture

The treated calli on MPM1 or MPM1+AS were infiltrated by vacuum at 700 mbar for ten minutes. Untreated calli and tobacco cv served as the negative controls in all tests. As the positive control, properly treated SR1 seedlings were used. Similar to the treated calli, these seedlings were vacuum-sealed. Co-cultivation was incubated for four days at 20 °C with high humidity in the dark.

Tissue necrosis, bacterial growth, and GFP expression were observed four days later. Cefotaxime (Cef), 600 mgL⁻¹, was added following a wash with MPM1 liquid medium. In accordance with the requirements of the experiment, 0.5 percent PVP40 was added to the wash solution. Most of the time, calli were only cleaned once, but if more bacteria were growing, it was cleaned again. To determine whether transformants could be chosen based alone on GFP fluorescence, the calli were blotted dry and put in MPM1 medium (~100 µL PCV each) supplemented with Cef 600 mgL⁻¹ with or without kanamycin 25 mgL⁻¹. The dose-kill curve for kanamycin was previously calculated in the laboratory. Throughout the three weeks that the calli were cultured at 28 °C in the dark, GFP expression was periodically monitored. The data from each experimental iteration were used to calculate the means and standard deviation.

Selection and regeneration

The entire calli, whether necrotic or growing, were harvested after three weeks and subcultured on MPM1 medium supplemented with either 25 mgL⁻¹ of kanamycin or 400 mgL⁻¹ of cef. On calli, GFP expression was observed on occasion. On MPM1 medium supplemented with either kanamycin or a lower Cef dosage (300 mgL⁻¹) after three weeks, only callus sectors with fluorescence were selected and cultivated. The Cef concentration was decreased to 200 mgL⁻¹ so that additional fluorescent calli subcultures could be supported. For long-term maintenance, the calli were subcultured in MPM1 medium with or without Cef 100 mgL⁻¹, and kanamycin was completely removed from the medium. The incubation conditions, unless otherwise specified, were 28 °C in the dark. The regeneration procedure utilized for transformed calli was

identical to that outlined in Chapter 4, Section 4.2, Materials and Methods, with the exception of the conversion into plantlets. Calli were subcultured for three weeks on MPM2 + Cef 200 mgL⁻¹, either with or without kanamycin 25 mgL⁻¹, depending on the duration of the subculture. After three weeks, Calli were transferred to MPM4 with Cef 100 mgL⁻¹ and kanamycin 25 mgL⁻¹. Calli were spread out on the medium with a spatula. The embryos were placed in antibiotic-free Woody Plant Medium (WPM) + BA 1 mM and incubated for 16 hours at 28 °C during the day and night to develop into plantlets. After germination and the development of the shoot, the plantlets were transferred to tubes containing WPM + BA 1 mM and cefotaxime 100 mgL⁻¹. The plants had hardened, as mentioned earlier.

Fluorescence microscopy

To observe the expression of green fluorescent protein in calli, embryos, and developing plantlets, a Nikon SMZ 1500 microscope was illuminated by a Nikon UV lamp and passed through a Nikon filter set with a 465-495 nm band pass excitation filter, a 505 nm dichroic mirror, and a 515+555 nm band pass barrier filter. Photographs were taken with a microscope-connected Nikon 4.1 mega pixel digital camera.

Molecular Analyses

Polymerase chain reaction (PCR)

DNA from the alleged transformed tissues was extracted with either the DNeasy Plant mini kit or the Sigma GenElute™ Plant Genomic DNA miniprep kit, and it was examined for 30 minutes on 1.2% agarose gels in TAE 0.5% at 100 V. PCR analysis was used for the detection of the GFP and nptII genes. The coding region of the GFP gene was examined with the forward primer 5'-TCC-TAT-CAT-TAT-CCT-CGG-CCG-AAT-TC-3' and the reverse primer 5'-GAT-TGT-GTG-GAC-AGG-TAA-TGG-TTG-TC-3'. On the other hand, the nptII gene was examined with the reverse primer 5'-ATC-GGG-AGC-GGC- Denaturation at 95 °C for 5 min, 40 cycles of 95 °C for 45 sec, 62 °C for 45 sec, annealing and elongation at 72 °C for 1 min, and final elongation at 72 °C for 10 min were used to amplify 50 ng to 100 ng of template DNA in a 25 L reaction. Untransformed calli DNA samples

served as the negative control, while transformed Portan (GFP+) and pNPTII +ve or S49 (transgenic St. George transformed GUS and nptII) were utilized as positive controls for the GFP and nptII genes. On a 1.2% agarose gel, the 638 and 700 bp fragments of GFP and NPTII were amplified using a Mupid® electrophoresis apparatus (Eurogentech, Liege, Belgium). After that, they were examined in a Geldoc that was controlled by Molecular Analyst® software and stained with ethidium bromide.

Reverse-transcription polymerase chain reaction (RT-PCR)

Total RNA was extracted using the Qiagen RNeasy® mini kit, and a Nanodrop® ND 1000 spectrophotometer was used to measure the amount of RNA using the Nanodrop 3.0.1 software. DNase was used to digest approximately 1 g of RNA on a 1.2% agarose gel. In a 10 l reaction, the primers 5'-GAT-TGT-GTG-GAC-AGG-TAA-TGG-TTG-TC-3' (reverse) were used to generate cDNA for the first strand from 1 g of RNA. Each reaction received one microliter of Superscript II® reverse transcriptase from Invitrogen. The positive control was run with the code for the housekeeping gene 18S-rna, the primer 5'-TCT-AAG-GGC-ATC-ACA-GAC-CTG-TTA-TT G-3'. A Techne PHC3 thermocycler was used in an early transcription process. The final amplification was carried out in a 25-L reaction with 0.5 or 1 L of cDNA, 10 mM of each dNTP, 0.25 mM of each specific primer, 0.25 units of Taq polymerase, 1X buffer, and MgCl₂. The identical conditions as for PCR applied to the amplification. The amplified products were displayed on a Geldoc using Molecular Analyst® software following electrophoresis on a 1.2% gel and ethidium bromide staining.

Conclusion

We investigated a wide range of transformation-influencing factors. Calli were incubated at 42 °C for 30 minutes prior to co-cultivation, resulting in tissue browning rather than transformation in MPM1 media. For GFP analysis, creating a suspension of plant cells using *Agrobacterium* was more beneficial and successful than inoculating the calli with a drop of bacterial

suspension. The optical densities (OD₆₀₀) of the bacterial suspensions of 0.3, 0.6, and 2.0 did not impede transformation. Calli showed less or no browning at OD₆₀₀ 0.3, as opposed to higher browning levels at 0.6 and 2.0. OD₆₀₀ 0.3 was utilized for the investigation of additional factors because all three ODs produced identical transformation efficacies. During and after co-cultivation, PVP addition reduced stable transformation efficiency and transient GFP expression, but it had no effect on necrosis. The expression of GFP in modified calli was also reduced when they were subculturing on PVP-containing media. However, the stable transformation numbers increased when AS and PVP were incorporated into the culturing medium. The bacterial growth was slowed down and the final transformation efficiency was lower when AS was added to the bacterial culture medium (YEB) for two hours of extended culture. This is evident from the bacterial growth curves and variations in ODs obtained with Bioscreen C. However, the addition of AS was absolutely necessary during the co-cultivation process. Plant cells were added to the bacterial culture medium in place of AS to boost *Agrobacterium* growth. Plant cell growth was also improved when AS was introduced. Stable transformation efficiencies were increased when plant cells were added to *Agrobacterium* culture medium alone or in conjunction with AS. Kanamycin caused tissue necrosis, especially in Riesling and Thompson Seedless callus line 2E. Additionally, it prevented the regrowth of Thompson Seedless callus line 1A embryos. Tissue necrosis was significantly reduced as a result of an antibiotic-free selection strategy. Transgenic calli can be selected and tracked using GFP expression. Regeneration also improved on a medium devoid of kanamycin. We were able to follow transgenic expression from the callus to the plant stage thanks to GFP expression. In a proembryogenic condition, regular subculturing on MPM1 medium maintained the preferred growth of converted calluses, which frequently differentiated into embryos as required. Molecular studies revealed that there were few escapes or chimeric embryos after the propagation strategy and when transgenic lines were selected visually without antibiotic selection. The two Thompson Seedless callus lines 1A and 2E reacted differently to

transformation and plant regeneration because of their varied cell types.

Using MPM-based media, we gained a deeper understanding of the various aspects of gene transfer. GFP's successful use as the sole reporter gene demonstrated that antibiotic or herbicide markers are no longer required for grapevine genetic transformation. Improved transformation efficiencies were achieved by inducing *Agrobacterium vir* genes using plant cells, which we have not yet observed in any plant genetic engineering literature. Together with a transformation system that is tailored to the requirements of each genotype, these findings have the potential to further enhance transformation and regeneration while also providing a deeper comprehension of the intricate connections that exist between plants and *Agrobacterium*.

References

1. K.J. Oparka. 2002. Functional analysis of a DNA-shuffled movement protein reveals that microtubules are dispensable for the cell-to-cell movement of Tobacco mosaic virus. *Plant Cell*. 14: 1207-1222.
2. Goebel-Tourand, I., M.C. Mauro, L. Sossountzov, E. Migianiac, A. Deloire. 1993. Arrest of somatic embryo development in grapevine: histological characterization and the effect of ABA, BAP and zeatin in stimulating plantlet development. *Plant Cell Tissue Org. Cult.* 33: 91-103
3. Khadatkhar, A.; Sawant, C.P.; Thorat, D.; Gupta, A.; Jadhav, S.; Gawande, D.; Magar, A.P. A comprehensive review on grapes (*Vitis* spp.) cultivation and its crop management. *Discov. Agric.* 2025, 3, 9.
4. Li, J.; Zhang, Y.; Bevacqua, E.; Zscheischler, J.; Keenan, T.F.; Lian, X.; Zhou, S.; Zhang, H.; He, M.; Piao, S. Future increase in compound soil drought-heat extremes exacerbated by vegetation greening. *Nat. Commun.* 2024, 15, 10875.
5. Smith, P.; Poch, R.M.; Lobb, D.A.; Bhattacharyya, R.; Alloush, G.; Eudoxie, G.D.; Anjos, L.H.C.; Castellano, M.; Ndzana, G.M.; Chenu, C.; et al. Status of the world's soils. *Annu. Rev. Environ. Resour.* 2024, 49, 73-104.
6. Vega-Muñoz, A.; Gil-Marín, M.; Contreras-Barraza, N.; Salazar-Sepúlveda, G.; Losada, A.V. How to measure organic fruit consumer behavior: A systematic review. *Horticulturae* 2022, 8, 318.
7. Sufyan, M.; Daraz, U.; Hyder, S.; Zulfikar, U.; Iqbal, R.; Eldin, S.M.; Rafiq, F.; Mahmood, N.; Shahzad, K.; Uzair, M.; et al. An overview of genome engineering in plants, including its scope, technologies, progress and grand challenges. *Funct. Integr. Genom.* 2023, 23, 119.
8. Liu, G.-T.; Wang, B.-B.; Lecourieux, D.; Li, M.-J.; Liu, M.-B.; Liu, R.-Q.; Shang, B.-X.; Yin, X.; Wang, L.-J.; Lecourieux, F.; et al. Proteomic analysis of early-stage incompatible and compatible interactions between grapevine and *P. viticola*. *Hortic. Res.* 2021, 8, 100.
9. Figueiredo, J.; Santos, R.B.; Guerra-Guimaraes, L.; Leclercq, C.C.; Renaut, J.; Sousa, L.; Figueiredo, A. Deep into the Apoplast: Grapevine and *Plasmopara viticola* Proteomes Reveal the Secret Beneath Host and Pathogen Communication at 6 h After Contact. *Phytopathology* 2023, 113, 893-903.
10. Wan, D.Y.; Guo, Y.; Cheng, Y.; Hu, Y.; Xiao, S.; Wang, Y.; Wen, Y.Q. CRISPR/Cas9-mediated mutagenesis of VvMLO3 results in enhanced resistance to powdery mildew in grapevine (*Vitis vinifera*). *Hortic. Res.* 2020, 7, 116.
11. Clemens, M.; Faralli, M.; Lagreze, J.; Bontempo, L.; Piazza, S.; Varotto, C.; Malnoy, M.; Oechel, W.; Rizzoli, A.; Costa, L.D. VvEPFL9-1 Knock-Out via CRISPR/Cas9 Reduces Stomatal Density in Grapevine. *Front. Plant Sci.* 2022, 13, 878001.
12. Villette, J.; Lecourieux, F.; Bastiancig, E.; Héloir, M.-C.; Poinssot, B. New improvements in grapevine genome editing: High efficiency biallelic homozygous knock-out from regenerated plantlets by using an optimized zCas9i. *Plant Methods* 2024, 20, 45.
13. Ren, C.; Li, H.; Liu, Y.; Li, S.; Liang, Z. Highly efficient activation of endogenous gene in grape using CRISPR/dCas9-based transcriptional activators. *Hortic. Res.* 2022, 9, uhab037.
14. Kocsisova, Z.; Coneva, V. Strategies for delivery of CRISPR/Cas-mediated genome editing to obtain edited plants directly without transgene integration. *Front. Genome Ed.* 2023, 5, 1209586.
15. Wang, Y.C.; Wei, Y.; Li, X.Y.; Zhang, H.M.; Meng, X.; Duan, C.Q.; Pan, Q.H. Ethylene-responsive VviERF003 modulates glycosylated monoterpenoid synthesis by upregulating VviGT14 in grapes. *Hortic. Res.* 2024, 11, uhae065.

16. Xie, J.; He, C.; Li, Z.; Li, M.; He, S.; Qian, J.; Tan, B.; Zheng, X.; Cheng, J.; Wang, W.; et al. A rapid and efficient *Agrobacterium*-mediated transient transformation system in grape berries. *Protoplasma* 2024, 261, 819-830.
17. Sun, Y.; Zheng, Y.; Wang, W.; Yao, H.; Ali, Z.; Xiao, M.; Ma, Z.; Li, J.; Zhou, W.; Cui, J.; et al. VvFHY3 links auxin and endoplasmic reticulum stress to regulate grape anthocyanin biosynthesis at high temperatures. *Plant Cell* 2024, 37, koae303.
18. Maletich, G.; Gavrilenko, I.; Pushin, A.; Chelombit, S.; Khmel'nitskaya, T.; Plugatar, Y.; Dolgov, S.; Khvatkov, P. Somatic embryogenesis and *Agrobacterium*-mediated transformation in a number of grape cultivars. *Plant Cell Tiss. Org.* 2025, 160, 73-89.
19. Thorpe, C.; Luo, W.; Ji, Q.; Eggenberger, A.L.; Chicowski, A.S.; Xu, W.; Sandhu, R.; Lee, K.; Whitham, S.A.; Qi, Y.; et al. Enhancing biolistic plant transformation and genome editing with a flow guiding barrel. *Nat. Commun.* 2025, 16, 5624-5637.
20. Wang, Y.W.; Liu, L.Y.; Guo, Y.D.; Fan, X.F.; Yue, Y.S.; Mu, N.; Xiao, G.Z.; Teng, K. Efficient transformation using pollen transfected by DNA-coated magnetic nanoparticles in *Pennisetum alopecuroides*. *Acta Prataculturae Sin.* 2025, 34, 1-12.

Received : May 2025; Revised : June 2025; Accepted : July 2025



PATH COEFFICIENT AND CHARACTER ASSOCIATION ANALYSIS OF YIELD-CONTRIBUTING TRAITS IN CUCUMBER (*CUCUMIS SATIVUS* L.)

Jahnvi Pal^{1*}, Bijendra Singh¹, Satya Prakash¹, LK Gangwar¹, Vipin

Kumar¹, Vaishali¹, Mayuri Gupta¹

¹College of Horticulture, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, (U.P). 250110

²College of Agriculture, Dept. of GPB, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, (U.P). 250110

*Corresponding Author Email-jahnavipal123@gmail.com

Abstract

Fruit yield in cucumber (*Cucumis sativus* L.) is a composite trait shaped jointly by numerous morphological, floral and fruit-related components, and this study was undertaken to resolve how these components act on yield, both directly and through one another, as part of a broader programme characterizing cucumber germplasm through tools. Phenotypic and genotypic correlations of thirteen component characters with fruit yield per hectare were decomposed into direct and indirect path coefficients following the standard path-analysis framework. At the phenotypic level, days taken to first female flower opening emerged as the strongest positive direct determinant of yield, trailed by seed number per fruit, fruit length, plot-level fruit yield, individual fruit weight, vine length and harvest-duration. Fruit length showed the strongest overall phenotypic correlation with yield, with vine length, fruit weight and plot yield following closely behind. At the genotypic level, plot-level fruit yield contributed the largest direct effect, ahead of fruit number per vine, timing of male-flower initiation and days to first harvest. Fruit length again showed the highest genotypic correlation with yield despite carrying a negative direct path coefficient, indicating the influence on productivity is transmitted almost entirely through other traits, chiefly plot yield and fruit number. Residual, unexplained variation stood at 0.311 for the phenotypic model and a comparatively small 0.0184 for the genotypic model. Together, the findings point to fruit number per vine, fruit length, fruit weight, fruit diameter and vine length are the vital traits when selecting high-yielding cucumber lines, to guide the choice of genetically distinct, agronomically superior parents.

Keywords: Cucumber, fruit yield, direct effect, indirect effect, path coefficient.

Introduction

Cucumber (*Cucumis sativus* L.) ranks among the more widely grown cucurbit vegetables, cultivated across tropical, subtropical and temperate zones for its tender, edible fruits. The crop displays wide-ranging variability in growth habit, flowering pattern, and fruit size, shape, colour and yield, and this pool of variability constitutes a valuable genetic resource for breeding programmes aimed at improving productivity and other horticulturally important

traits. Research combining morphological observation with molecular marker analysis has repeatedly confirmed rich genetic diversity within cucumber germplasm collections, and such combined data are useful for pinpointing genetically distinct parental material for crop improvement (7; 8).

Because fruit yield is a quantitative trait shaped jointly by numerous component characters, breeding purely on the basis of yield itself is not always efficient, given that the

underlying component traits are interlinked and can affect productivity both directly and indirectly. Simple correlation coefficients reveal how strongly and in what direction two characters move together, but they cannot on their own distinguish a trait's direct influence on yield from an association arising indirectly through some other trait.

Path coefficient analysis addresses this limitation by mathematically separating a correlation coefficient into its direct and indirect components. The technique, originally formulated by (11) and subsequently adapted for crop-improvement research by (1), remains a standard tool for judging which yield-component traits merit the greatest selection emphasis. Dewey and Lu showed that this decomposition uncovers relationships that plain correlation analysis would leave obscured.

Earlier path-analytic work in has consistently implicated fruit number per plant, fruit weight, fruit length, fruit diameter, vine length and various maturity-related traits carry meaningful direct or indirect weight in determining fruit yield (2; 5; 10).

Against this background, the present study set out to work out phenotypic and genotypic path coefficients linking key morphological traits to fruit yield per hectare in cucumber, with the broader aim of flagging the traits best suited for selecting high-yielding, genetically diverse genotypes.

Materials and Methods

This work formed one strand of a larger thesis project titled "Genetic Diversity Analysis on Cucumber Using Morphological and Molecular Markers." The plant material comprised a set of cucumber genotypes that were evaluated for a range of morphological, floral, fruit-related and yield traits.

Fourteen characters were recorded for the path analysis: days to 50% germination, days to first male flower opening, days to first female flower opening, vine length (cm), branch number per vine, fruit number per vine, seed number per fruit, fruit diameter (cm), fruit length (cm), fruit weight (g), days to first harvest, duration of the harvest period, fruit yield per plot (kg) and fruit

yield per hectare (q).

Fruit yield per hectare was treated as the dependent variable, and the remaining thirteen traits served as independent variables. Phenotypic and genotypic correlation coefficients between these traits were partitioned into direct and indirect components following the pathcoefficient procedure originally described by (11) and later adapted for crop trait analysis by (1).

In the resulting path matrices, diagonal entries represent a character's direct effect on fruit yield per hectare, while off-diagonal entries capture its indirect effects transmitted through the other traits. A residual value was also calculated to reflect the share of variation attributable to factors outside the model. Correlation coefficients were tested for statistical significance at the 5% and 1% probability levels.

This morphological path analysis represented only one part of the overall diversity assessment; molecular marker data were analyzed separately to estimate genetic relatedness among genotypes and were not incorporated into the morphological path model.

Results and Discussion

Substantial differences emerged in both the magnitude and sign of direct and indirect effects contributed by the various traits to fruit yield per hectare, whether examined at the phenotypic or the genotypic level.

At the phenotypic level, days to first female flower appearance produced the largest positive direct effect on yield (0.3576). This was followed, in descending order, by seed number per fruit (0.3283), fruit length (0.2334), plot yield (0.2036), fruit weight (0.1519), vine length (0.1440), harvest duration (0.1372) and fruit number per vine (0.0302). Negative direct effects were recorded for days to 50% germination (-0.1539), branch number per vine (-0.1444), days to first male flower appearance (-0.1196), fruit diameter (-0.0826) and days to first harvest (-0.0236).

Fruit length showed the strongest and most highly significant phenotypic correlation with yield (0.622). Its direct effect (0.2334) was reinforced by positive indirect contributions channeled through seed number per fruit (0.1220), plot yield (0.1093),

vine length (0.0787) and fruit weight (0.0727) underscoring fruit length as a trait closely tied to productivity. Vine length likewise correlated strongly and highly significant with yield (0.574), combining a direct effect of 0.1440 with beneficial indirect pathways through fruit length (0.1276), plot yield (0.1134), fruit weight (0.1057) and seed number per fruit (0.0849). This suggests that more vigorous vegetative growth translated into better yield performance in the genotypes studied.

Fruit weight carried a direct effect of 0.1519 and a highly significant overall correlation with yield of (0.546), with its principal indirect routes running through fruit length (0.1117), plot yield (0.1086), vine length (0.1002) and seed number per

fruit (0.0770).

Fruit yield per plot itself had a direct effect of 0.2036 and a highly significant correlation with per-hectare yield (0.539). Seed number per fruit also contributed a substantial direct effect (0.3283) alongside a significant correlation (0.464**). Fruit diameter presented a notable contrast, despite a negative direct effect (-0.0826), its overall phenotypic correlation with yield was positive and highly significant (0.438**). This apparent discrepancy stemmed from favorable indirect effects transmitted through fruit length (0.1160), seed number per fruit (0.1154), plot yield (0.0960), fruit weight (0.0815) and vine

Table 1. Phenotypic path with fruit yield (q/ha)

Chrs	D50G	DFMFA	DFFFA	VL	NBV	NFV	NSF	FD	FL	FW	DDFH	DH	FYP	FYH
D50G	-0.1539	-0.0332	0.0420	0.0087	-0.0190	0.0018	0.1153	0.0009	-0.0143	0.0019	-0.0016	-0.0073	0.0673	0.009
DFMFA	-0.0426	-0.1196	0.1987	0.0076	-0.0294	0.0004	0.0946	0.0002	-0.0079	0.0023	-0.0117	0.0086	0.0055	0.107
DFFFA	-0.0181	-0.0665	0.3576	0.0114	0.0043	-0.0016	0.0397	0.0101	-0.0002	0.0135	-0.0171	-0.0141	0.0232	0.342**
VL	-0.0093	-0.0063	0.0282	0.1440	-0.0047	0.0080	0.0849	-0.0403	0.1276	0.1057	-0.0011	0.0240	0.1134	0.574**
NBV	-0.0202	-0.0244	-0.0105	0.0047	-0.1444	-0.0045	-0.0536	0.0236	-0.0251	-0.0113	0.0018	0.0293	0.0032	-0.231*
NFV	-0.0090	-0.0016	-0.0193	0.0379	0.0214	0.0302	0.1158	-0.0235	0.1009	0.0187	0.0015	0.0330	0.0491	0.355**
NSF	-0.0540	-0.0345	0.0432	0.0372	0.0236	0.0107	0.3283	-0.0290	0.0867	0.0356	-0.0037	-0.0307	0.0505	0.464**
FD	0.0017	0.0004	-0.0439	0.0703	0.0412	0.0086	0.1154	-0.0826	0.1160	0.0815	0.0004	0.0333	0.0960	0.438**
FL	0.0094	0.0041	-0.0003	0.0787	0.0155	0.0131	0.1220	-0.0411	0.2334	0.0727	0.0011	0.0039	0.1093	0.622**
FW	-0.0019	-0.0018	0.0317	0.1002	0.0107	0.0037	0.0770	-0.0443	0.1117	0.1519	-0.0009	-0.0003	0.1086	0.546**
DDFH	-0.0104	-0.0591	0.2579	0.0069	0.0112	-0.0019	0.0514	0.0013	-0.0103	0.0059	-0.0236	-0.0086	0.0178	0.239*
DH	0.0082	-0.0075	-0.0369	0.0252	-0.0308	0.0073	-0.0734	-0.0201	0.0067	-0.0003	0.0015	0.1372	0.0243	0.041
FYP	-0.0509	-0.0033	0.0408	0.0802	-0.0023	0.0073	0.0814	-0.0389	0.1253	0.0810	-0.0021	0.0164	0.2036	0.539**

Resi-0.311

*, ** significant at 5% and 1% level, respectively

D50G- days to 50% germination, DFMFA-days to first male flower appearance, DFFFA-days to first female flower appearance, VL-vine length(cm), NBV- number of branches per vine, NFV- number of fruits per vine, NSF- number of seeds per fruit, FD-fruit diameter(cm), FL -fruit length (cm), FW-fruit weight (g), DDFH- days to first fruit harvest, DH- duration of harvesting, FYP-fruit yield per plot(kg), FYH-fruit yield per hectare (quintal).

length (0.0703) — a clear illustration of why correlation coefficients alone can be misleading about a trait's real contribution to yield. Fruit number per vine contributed only a modest direct effect (0.0302), yet its total correlation with yield was positive and highly significant (0.355), driven largely by indirect effects via seed number per fruit (0.1158), fruit length (0.1009) and plot yield (0.0491).

Days to first female flower appearance combined the largest direct effect (0.3576) with a highly significant positive correlation with yield (0.342). By contrast, branch number per vine

showed both a negative direct effect (-0.1444) and a significant negative correlation with yield (-0.231).

The phenotypic residual effect stood at 0.311, indicating that the traits included in the model accounted for a considerable share of the phenotypic variation linked to fruit yield. These results broadly agree with (2), who likewise identified fruit weight, maturity duration and related traits as important direct and indirect contributors to cucumber yield. (5) similarly found average fruit weight, fruit number, yield and vine length to be positively linked with productivity, while (10) reported significant positive relationships between yield and vine length, fruit number per plant, fruit length, fruit diameter and average fruit weight.

Genotypic path analysis

At the genotypic level, plot-level fruit yield exerted by far the largest positive direct effect on per-hectare yield (0.9838), followed by fruit number per vine (0.6769), days to first male flower appearance (0.5025), days to first harvest (0.4603), fruit diameter (0.1734) and branch number per vine (0.1043).

Negative direct effects were noted for harvest duration (-0.7711), days to 50% germination (-0.6830), days to first female flower appearance

(-0.3873), vine length (-0.3108), seed number per fruit (-0.2181), fruit length (-0.2109) and fruit weight (-0.1302).

Fruit length again showed the highest genotypic correlation with yield of all traits (0.703) highly significant, even though its direct path coefficient was negative (-0.2109). This positive overall association arose almost entirely from strong indirect contributions via plot yield (0.8670) and fruit number per vine (0.3501), demonstrating that fruit length's genotypic link to yield operates mainly through other traits rather than as an independent direct effect.

Fruit yield per plot combined a very large direct effect (0.9838) with a highly significant correlation with per-hectare yield (0.692). Vine length followed with a significant correlation of yield (0.589) despite a negative direct effect (-0.3108), its positive net association arising principally from a very strong indirect pathway through plot yield (0.9957) together with a further contribution from fruit number per vine (0.1950).

Fruit weight followed a similar pattern a negative direct effect (-0.1302) alongside a positive, highly significant correlation with yield (0.580) with net association being driven predominantly by its strong indirect effect through plot yield (0.9111).

Table 2. Genotypic path with fruit yield (q/ha)

Chrs	D50G	DFMFA	DFFFA	VL	NBV	NFV	NSF	FD	FL	FW	DFFH	DH	FYP	FYH
D50G	-0.6830	0.1769	-0.0584	-0.0066	0.0149	0.0376	-0.0784	0.0002	0.0116	-0.0008	0.0305	0.0741	0.5219	0.041
DFMFA	-0.2404	0.5025	-0.2665	-0.0268	0.0261	-0.0242	-0.0756	-0.0036	0.0041	-0.0039	0.3379	-0.1223	0.0444	0.152
DFFFA	-0.1029	0.3457	-0.3873	-0.0255	-0.0011	-0.0617	-0.0328	-0.0252	-0.0011	-0.0149	0.4899	0.0546	0.1320	0.370**
VL	-0.0144	0.0433	-0.0317	-0.3108	0.0043	0.1950	-0.0562	0.0939	-0.1330	-0.0957	0.0284	-0.1295	0.9957	0.589**
NBV	-0.0976	0.1259	0.0039	-0.0127	0.1043	-0.1264	0.0435	-0.0578	0.0150	0.0107	-0.0348	-0.2062	-0.0082	-0.240*
NFV	-0.0379	-0.0179	0.0353	-0.0895	-0.0195	0.6769	-0.0812	0.0497	-0.1091	-0.0178	-0.0373	-0.2763	0.3205	0.396**
NSF	-0.2454	0.1743	-0.0583	-0.0801	-0.0208	0.2519	-0.2181	0.0649	-0.0911	-0.0331	0.1153	0.1766	0.4595	0.496**
FD	-0.0009	-0.0105	0.0563	-0.1684	-0.0348	0.1939	-0.0816	0.1734	-0.1135	-0.0715	-0.0025	-0.2350	0.7677	0.473**
FL	0.0375	-0.0099	-0.0020	-0.1960	-0.0074	0.3501	-0.0942	0.0933	-0.2109	-0.0661	-0.0277	-0.0302	0.8670	0.703**
FW	-0.0041	0.0151	-0.0442	-0.2285	-0.0086	0.0924	-0.0554	0.0952	-0.1071	-0.1302	0.0374	0.0065	0.9111	0.580**
DFFH	-0.0452	0.3689	-0.4123	-0.0192	-0.0079	-0.0548	-0.0546	-0.0009	0.0127	-0.0106	0.4603	0.0254	0.0911	0.353**
DH	0.0656	0.0797	0.0274	-0.0522	0.0279	0.2425	0.0500	0.0528	-0.0083	0.0011	-0.0152	-0.7711	0.3259	0.026
FYP	-0.2576	0.0161	-0.0370	-0.2236	-0.0006	0.5568	-0.0724	0.0962	-0.1322	-0.0857	0.0303	-0.1816	0.9838	0.692**

0.0184

*, ** significant at 5% and 1% level, respectively

D50G- days to 50% germination, DFMFA-days to first male flower appearance, DFFFA-days to first female flower appearance, VL-vine length(cm), NBV- number of branches per vine, NFV- number of fruits per vine, NSF- number of seeds per fruit, FD-fruit diameter(cm), FL -fruit length (cm), FW-fruit weight (g), DDFH- days to first fruit harvest, DH- duration of harvesting, FYP-fruit yield per plot(kg), FYH-fruit yield per hectare (quintal).

Seed number per fruit showed a negative direct effect (-0.2181) but a positive, highly significant overall correlation (0.496), attributable mainly to indirect effects through plot yield (0.4595) and fruit number per vine (0.2519).

Fruit diameter, in contrast, combined a positive direct effect (0.1734) with a highly significant positive correlation with yield (0.473), reinforced by indirect contributions through plot yield (0.7677) and fruit number per vine (0.1939).

Fruit number per vine registered the second-largest positive direct effect at the genotypic level (0.6769) along with a high significant positive correlation with yield (0.396), marking it as one of the most useful biological yield components for selection purposes in cucumber.

Days to first harvest carried a positive direct effect (0.4603) and a high significant positive correlation (0.353), whereas days to first female flower appearance showed a negative direct effect (-0.3873) but a high significant positive overall correlation (0.370), attributable to indirect effects passing through days to first harvest, days to first male flower appearance and plot yield.

Branch number per vine had a positive direct effect (0.1043), yet its overall correlation with yield was negative but significant (-0.240), showing that negative indirect contributions from other traits outweighed its modest direct benefit.

The genotypic residual effect was only 0.0184, at 0.0184, indicating that the traits included in the model together accounted for almost all of the genotypic association with fruit yield.

The divergence between phenotypic and genotypic direct effects observed here suggests that environmental influences, together with the interdependence among component traits, shaped how these characters were ultimately expressed. Comparable shifts between phenotypic and

genotypic contributions have been documented in other cucumber path studies. (2) similarly concluded that decomposing correlations into direct and indirect effects offers sharper insight than correlation coefficients alone, and (10) stressed the value of prioritizing traits with substantial direct effects during selection. (7) found that clustering Indian cucumber accessions by morphology did not always mirror groupings based on SSR markers, and other studies pairing morphological data with EST-SSR or SSR markers have similarly demonstrated the benefit of combining both approaches when characterizing cucumber germplasm for breeding. These results also bear on the wider diversity investigation of which this study forms a part. On this basis, cucumber genotypes combining favorable fruit number per vine, fruit length, fruit weight, fruit diameter, vine length and yield could first be shortlisted through morphological screening, with molecular marker data then applied to select genetically divergent parents for hybridization.

Conclusion

This study showed that cucumber fruit yield is shaped by a range of morphological and yield-related traits acting through both direct and indirect pathways. At the phenotypic level, days to first female flower appearance, seed number per fruit, fruit length, plot yield, fruit weight and vine length all exerted meaningful positive direct effects on yield per hectare, with fruit length showing the strongest overall phenotypic correlation, followed by vine length, fruit weight and plot yield.

At the genotypic level, plot yield produced the largest direct effect, followed by fruit number per vine, days to first male flower appearance and days to first harvest. Among the genuinely biological yield components, fruit number per vine stood out for its strong positive direct genotypic effect, while fruit length, vine length and fruit weight influenced yield mainly through indirect routes rather than direct ones.

These results suggest that selection emphasizing fruit number per vine, together with favourable fruit length, fruit weight, fruit diameter and vine growth, could usefully improve cucumber productivity. Pairing this morphological

information with molecular-marker-based diversity assessment should further assist breeders in identifying parental lines that are both agronomically superior and genetically distinct for future cucumber improvement programmes.

Reference

1. Dewey, D. R., & Lu, K. H. (1959). A correlation and path-coefficient analysis of components of crested wheatgrass seed production. *Agronomy Journal*, 51(9), 515–518.
2. Keshari, D., Singh, B., Singh, R., & Prakash, S. (2021). Assess the extent of path coefficient analysis for fruit yield and its contributing traits in cucumber (*Cucumis sativus* L.). *Annals of Horticulture*, 14(1), 22–26. <https://doi.org/10.5958/0976-4623.2021.00005.0>
3. Kumar, R., Munshi, A. D., Behera, T. K., Jat, G. S., Choudhary, H., Singh, M., & Talukdar, A. (2020). Genetic diversity of cucumber (*Cucumis sativus*) accessions differing in quantitative traits and microsatellite markers. *The Indian Journal of Agricultural Sciences*, 90(11), 2161–2167. <https://doi.org/10.56093/ijas.v90i11.108583>
4. Lnu, R., Dhall, R. K., Manchanda, P., & Kumari, P. (2025). Study on characters associations and path coefficient analysis for yield and quality traits of parthenocarpic cucumber genotypes in poly-net house conditions. *Discover Plants*.
5. Manisha, Singh, A. K., & Pal, A. K. (2021). Studies on correlation and path-coefficient analysis for yield and its contributing characters in cucumber (*Cucumis sativus* L.). *The Pharma Innovation Journal*, 10(10), 551–556.
6. Pal, S., Sharma, H. R., Das, A., & Pandav, A. K. (2017). Character association and path analysis for fruit yield and its contributing traits in cucumber (*Cucumis sativus* L.). *International Journal of Agriculture, Environment and Biotechnology*, 10, 163–170.
7. Pandey, S., Ansari, W. A., Mishra, V. K., Singh, A. K., & Singh, M. (2013). Genetic diversity in Indian cucumber based on microsatellite and morphological markers. *Biochemical Systematics and Ecology*, 51, 19–27. <https://doi.org/10.1016/j.bse.2013.08.002>
8. Pandey, S., Ansari, W. A., Pandey, M., & Singh, B. (2018). Genetic diversity of cucumber estimated by morpho-physiological and EST-SSR markers. *Physiology and Molecular Biology of Plants*, 24(1), 135–146. <https://doi.org/10.1007/s12298-017-0489-9>
9. Veena, R., Sidhu, A. S., Pitchaimuthu, M., & Souravi, K. (2012). Genetic evaluation of some cucumber (*Cucumis sativus* L.) genotypes for some yield and related traits. *Electronic Journal of Plant Breeding*, 3(3), 945–948.
10. Veera, U. R., Sahu, G. S., Tripathy, P., Das, S., Dash, M., & Padhiary, G. G. (2022). Character association and path coefficient studies in cucumber (*Cucumis sativus* L.): Experimental investigation. *International Journal of Plant & Soil Science*, 34(23).
11. Wright, S. (1921). Correlation and causation. *Journal of Agricultural Research*, 20, 557–5

Received : May 2025; Revised : June 2025; Accepted : July 2025



ECONOMIC FEASIBILITY OF WHEAT CULTIVATION UNDER THE TREATMENT OF PUSA HYDROGEL AND VERMI-COMPOST

Sunil Kumar¹, Avinash Kumar Singh¹, Amit Kumar^{*2},

Anuj Yadav¹, Bijendra Singh³ and R.P.Singh⁴

¹Department of Agriculture, KVSCOS, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh, India

¹Department of Biotechnology, KVSCOS, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh, India

³College of Horticulture, Sardar Vallabhbhai Patel University of Agriculture and Technology,
Meerut, Uttar Pradesh, India

⁴Former Vice Chancellor, Swami Keshwanand Rajasthan Agriculture University, Bikaner

***Corresponding Author Email-**amit.agbiotech1581@gmail.com

Abstract

In the Rabi seasons of 2022-2023 and 2023-2024, experiments were conducted at Swami Vivekanand Subharti University in Meerut, Uttar Pradesh, India. The experimental field's soil had a sandy loam texture, was somewhat alkaline, and had good drainage. It had medium levels of accessible potassium and phosphorus but small levels of organic carbon and available nitrogen. Four moisture conservation techniques (pusa hydrogel @ 5 kg/ha, Vermi-compost @ 1t/ha, pusa hydrogel @ 5 kg/ha+Vermi-compost @ 1t/ha and no application) and three irrigation levels-first (at CRI stage), second (at CRI, booting and milking), and third (at CRI, late tillering, late jointing, flowering and milking stage)-were tested in a split plot design with three replications. The number of effective tillers/spike, spikelets/spike (41.3 and 38.9), and test weight (41.2 and 39.5g) were the wheat growth parameters and yield-attributing characters that were significantly higher under five irrigation levels than under three and one, with the lowest values occurring under first irrigation in 2022-2023 and 2023-2024, respectively. In the first and second years, the wheat that received five irrigations outperformed the wheat that received three and one irrigations by 7.6, 68.8, and 8.1 and 69.4%, respectively. Likewise, during the trial, wheat cultivated with three and five irrigations yields a greater straw yield of 5.98 and 5.84 and 28.88 and 26.65q/ha, respectively. During 2022-23 and 2023-24, the combined application of pusa hydrogel @ 5 kg + vermin-compost @ 1t/ha over no application at harvest produced 33.2 and 31.0% greater dry matter production/m and 41.1 and 39.3% more grains/spike, respectively. The application of pusa hydrogel @ 5 kg+ vermi-compost @ 1 t/ha produced the highest B: C ratios (3.18 and 3.12), while five irrigations followed by three irrigations during the first and second years produced the highest B: C ratios (3.25 and 3.21). In addition, this treatment maintained the soil's moisture and fertility levels while providing the crop with the best nutrient uptake. Thus, in sandy loam soils of the North Western Plain Zones of Western Uttar Pradesh, 5 kg of pusa hydrogel plus 1 t of vermi-compost per hectare with five irrigations (at CRI, late tillering, late jointing, flowering, and milking stage) seemed to work well for wheat.

Key words:- Wheat, Vermi-compost, Pusa Hydrogel, CRI Stage, Economic Feasibility, Moisture Conservation.

Introduction

Wheat (*Triticum aestivum* L.), is the second major staple crop in India. It's said that wheat is the "King of Cereals." Over a billion people in 43 countries around the world rely on wheat as a reliable food source (5). It provides about 20% of the calories that an individual consumes. Approximately half of the world's 224.49 million hectares of wheat are planted in impoverished countries. It is currently one of the important India's cereal crops. After rice, wheat crop is the second-largest acreage and yield. Wheat from the Poaceae family has its origins in South-West Asia; it is the second largest producer in the world, producing over 12 per cent of world wheat, and has the fastest growing consumption as it is the second largest consumer after China. In total, there were 792.40 million total tons of wheat produced and total wheat seed sown was approximately 224.49 million hectares worldwide with an estimated product of approximately 3.52 tons per hectare (ton-building rate). In India, wheat is grown on 31.61 million hectares (approximately 13.43% of total growing acres) and is responsible for producing 113.29 million tons of wheat (an increase of approximately 1.02% from 2022) with a production level of approximately 3464 kg/ha (2). One of the primary causes of the low wheat yields in India and around the world is weed infestation (20). Wheat yield is decreased by weed infestation by 37 to 50%, although it can be further decreased by using appropriate weed management techniques (Amare et al., 2014). Although there are several methods available, nobody is positive how to completely eradicate weeds (16 & 15). Apart from being expensive and labor-intensive, manual weeding cannot be refined until weeds have sufficiently developed their vegetative parts.

Hydrogels are 3-dimensional polymeric materials that can absorb greater amounts of water than soil or most biomaterials and are made from cross-linked polymers (13). Synthetic

polymers known as hydrogels are sold under brand names including PUSA Hydrogel and Super Absorbent. PUSA Hydrogel is a semi-synthetic superabsorbent that has been developed by IARI (Indian Agricultural Research Institute) and has the potential to increase production on a per unit input basis. The swelling capacity of this product is reported to be 350 times or more than its own weight in purified water and can have a swelling capacity of over 500 times (19). Swelling ratio increases with increasing temperature (up to 50 degrees Celsius) and there is no negative impact on the physical matrix. In terms of enhancing yield (per unit of water and nutrients), PUSA Hydrogel is beneficial under conditions of moisture stress (Priya et al., 2022). It also increases the physical improvements of soil, seed germination, seedling emergence, root density, and root growth, which aid plants in surviving long periods of moisture stress (10). Hydrogel can also decrease herbicide and fertilizer leaching and reduce crop irrigation needs (26; 25; 23; 6).

In addition, it promotes early flowering and tillering in dense climates to delay the maximum wilting point (7). Vermicompost, or VC, produced through the decay of organic materials by worms contains nutrients in a readily available form for plants to use (22). There is overwhelming evidence that adding VC to soil allows for greater development in plant growth and helps the growth and yields of grains and legumes (7). The use of vermicompost continues to generate interest as a soil conditioner; incorporating VC into existing soils improves the physical and chemical characteristics of that soil (3; 9).

Material and Methods

This experiment was performed at Swami Vivekanand Subharti University in Meerut, and the germplasm was obtained from Sardar Vallabhbhai Patel University of Agriculture and Technology (Uttar Pradesh, India). The study took place during the rabi season of 2022 to 2023 and 2023 to 2024, as detailed in Table 1.

Table-1 Details of treatment during the experiments

Factor (A) Irrigation Levels - 03	
1. I ₁ , 01 Irrigation	At CRI (Crown Root Initiation) stage
2. I ₂ , 03 Irrigation	At Crown Root Initiation stage, Booting stage and Milking stage
3. I ₃ , 05 Irrigation	At Crown Root Initiation stage, tillering stage, jointing stage, Flowering state and Milking stage
Factor (B) Moisture conservation practices – 04	
1. Treatment - 1	Control
2. Treatment - 2	Pusa hydrogel at a rate of 5 kg per hectare
3. Treatment - 3	Vermi compost at a rate of 1 tons per hectare
4. Treatment - 4	Pusa hydrogel at a rate of 5 kg per hectare with vermi compost at a rate of 1 tons per hectare

A sandy loam soil was utilized for this trial with low access nitrogen, organic carbon, a moderate level of available phosphorus and potassium, as well as an alkaline reaction. Soil samples were obtained using a post auger at the depths of zero to fifteen and fifteen to thirty. These samples were collected both at the start and at the end of each crop harvest, in order to determine whether or not they contained moisture. The gravimetric approach was used to measure moisture content; average moisture values on a per soil depth basis determined total water consumed and total moisture generated during the course of the trial.

Out of the total twelve combinations of two different factors used in the treatments tested, there were three levels of irrigation supplied to the main plot. These irrigation levels consisted of the initial irrigation applied at crown-root initiation (CRI), with the second level providing 3 irrigations applied at CRI, booting, and milking, and finally, the third level with 5 irrigations applied at CRI, late tillering, late jointing, flowering and milking. The second subplot factor evaluated various moisture conservation practices utilizing control grown with no added inputs, and 5 kg/ha of hydrogel (Pusa hydrogel) and 1 ton/ha of vermicompost, or combined applications of Pusa hydrogel and vermicompost. Testing of these practices was completed using a split plot design with three replications. Hydrogel is a manmade polymer that cannot dissolve in water but has high water affinity and can hold large amounts of it (21; 14). Hydrogel shows considerable potential for use in regions with limited irrigation resources, thus increasing the quantity of water available to farmers during the planting of crops. Hydrogel has

the capability to hold water at 80-180 times its volume and has the ability to release water gradually during a growing period (11). The physical characteristics of the soil, including infiltration rates, density, soil structure, and compaction, were affected positively by the incorporation of hydrogels. Pusa Hydrogel was added to soils in a 1:10 (W/W) ratio and added in bands along the seed line during planting at a rate of 5 kg/ha corresponding to each of the treatments. The decomposed vermicompost obtained from Swami Vivekanand Subharti University, Meerut, had a N-P-K composition of 2.3% nitrogen, 1.6% phosphorus, and 1.5% potassium (dry weight basis), which was also used based on the treatments. Vermicompost was incorporated into the soil (1 metric ton/hectare) ten days before planting in both seasons. The recommended cultural practices for conventional wheat cultivation were followed in both years. The wheat variety PBW 590 was sown in rows 20 cm apart, at a depth of 5 cm, with an application rate of 125 kg seed per hectare on December 24 and 22 of 2022 and 2023, respectively. The leaf area index (LAI) was determined using a leaf area meter (LA-3100). The biological yield (grains and straw) was recorded on a plot basis by sun-drying and weighing the food in every sampling net plot after it was harvested (except for roots), which was then converted to q/ha. Once harvested, threshed, washed and dried, the wheat grain yield was measured at 14% moisture content. The Harvest Index, derived from the ratio of economic yield to biological yield, has been calculated using the formula outlined by (8).

$$\text{H.I.} = \frac{\text{EconomicYield(kgha}^{-1}\text{)}}{\text{EconomicYield(kgha}^{-1}\text{)}}$$

The organic carbon content and nutrients available in soil have been calculated according to (17). The analysis of the collected data has been evaluated through statistical analysis using a computer-based software program, namely SPSS for statistical analysis of split plot design.

Economic Analysis

The total expenses of wheat cultivation included the following costs: seeds, fertilizers, herbicides, labor, and machinery to the land to complete the necessary preparation, irrigation, and fertilizer application, protection of the plants during the growing season, harvest, and threshing. The time to fulfill each of these activities was also included. To obtain the irrigation cost of a given plot of land, the total number of hours was multiplied by the number of hours required to irrigate that land. The Indian Government announces the minimum support price for wheat as gross income. The net income was calculated as gross income minus total costs.

The cost of cultivation was calculated by considering the fixed and variable expenses associated with different treatments of inputs. The input prices that existed during the experimental period were utilized to calculate input costs. The financial returns derived from marketable produce (grains and straw) were calculated by multiplying the output amount by their unit price as follows:

Gross returns (₹ha⁻¹) = Return from grains + Return from straw

Monetary return from grains (₹ha⁻¹) = Grain yield (q ha⁻¹) x minimum support price (₹q⁻¹)

Monetary return from straw (₹ha⁻¹) = Straw yield (₹q⁻¹) x local price (₹q⁻¹)

Net Return (₹ha⁻¹) = Gross Return (₹ha⁻¹) - Cost of cultivation (₹ha⁻¹)

$$\text{B : C Ratio} = \frac{\text{Grossreturn(₹ha}^{-1}\text{)}}{\text{Costofcultivation(₹ha}^{-1}\text{)}}$$

Result and discussion

Growth Characteristics

The irrigation levels significantly influenced yield parameters such as spike length, number of

spikelet's per spike, number of grains per spike, and test weight, whereas the number of spikelet's per spike was not significantly different, as presented in Table 1. The highest effective tillers (345 and 322)/m², resulting from a combined rate of Pusa Hydrogel at the rate of 5 kg/ha and vermicompost at the rate 1 tons per hectare were the next highest effective tillers (317 and 293)/m² with a low vermicompost at the rate 1tons per hectare. The lowest effective tillers were recorded with vermicompost at the rate 1tons per hectare (317 and 293)/m², and the highest spike lengths of 10.22 and 9.93 cm were recorded with the same treatments, while the lowest spike lengths of 7.45 and 7.25 cm were observed in the same treatments, and the highest number of spikelets per spike were 13.52 and 12.23, while the lowest number of spikelets were 10.56 and 10.44 when compared to crops that received one irrigation. The maximum number of grains per spike was 41.25 (2022-23) and 38.94 (2023-24), whereas the lowest number of grains per spike (CRI irrigation only) was significantly less than all other treatments. The maximum test weight was obtained after five irrigations, while the minimum test weight was recorded with one irrigation.

The irrigation levels and moisture conservation methods had a significant effect on the yields (grain yield, straw yield, biological yield, and harvest index) during both years of the experiment, but there was no significant effect of the moisture conservation method on the harvest index in both years (Table 1). The degree of relationship between the level of irrigation and the method of moisture conservation was not significant. The crops that were not fertilised produced 34.6% and 38.4% less grain per hectare than the combined application of Pusa hydrogel at 5 kg/ha and vermicompost at 1 ton/ha in 2022-23 and 2023-24, respectively. Wheat crops grown using the combined application of Pusa hydrogel at 5 kg ha⁻¹ and vermicompost at 1 t ha⁻¹ produced the highest total grain output of all treatment combinations, proving to be the most effective combination in both years. The application of Pusa hydrogel at 5 kg/ha is superior to the control for increasing grain yield but is not as successful when that isolated amendment is compared to vermicompost alone in both years (Table-2).

Straw yields for crops fertilized with Pusa Hydrogel at 5 kg per hectare and Vermicompost at 1 ton per hectare in both growing seasons (2022-23 and 2023-24) were significantly higher than for any other treatments except when utilizing Vermicompost alone (1 ton per hectare) during the same years. Regarding the maximum straw yield, applying Pusa Hydrogel at 5 kg per hectare produced equivalent yields to using Vermicompost at 1 ton per hectare and had a maximum yield much greater than that of the Control treatment. The Control treatment also yielded significantly less than the treatment combinations during both years (Table-2).

The biological yield of crops grown under conditions without Pusa hydrogel applied at 5 kg ha⁻¹ and without vermicompost applied at 1 t ha⁻¹ (control) was significantly less than that of those grown with either of the treatments alone and was significantly less than all combinations of the two treatments. The highest biological yield of crops was from a combination of both Pusa hydrogel applied at 5 kg ha⁻¹ and vermicompost applied at 1 t ha⁻¹, which produced the same biological yield as vermicompost applied alone at 1 t ha⁻¹. Both vermicompost applied at 1 t ha⁻¹ and Pusa hydrogel applied at 5 kg ha⁻¹ produced a significant amount of grain yield when compared to the control in all three years of the study.

Crops applying a combination of Pusa hydrogel (5 kg ha⁻¹) and vermicompost (1 t ha⁻¹) produced the highest harvest index, while the next highest was observed for crops applying vermicompost (1 t ha⁻¹). In comparison, the Pusa hydrogel treatment (5 kg ha⁻¹) had a higher harvest index than that of the control group. However, the highest harvest index was recorded for crops treated with both Pusa hydrogel (5 kg ha⁻¹) plus vermicompost (1 t

ha⁻¹) and crops treated with vermicompost (1 t ha⁻¹) alone. Economic feasibility

The economic analysis of wheat cultivation influenced by various moisture conservation techniques and irrigation scheduling is illustrated in Table 3. The expense of cultivating wheat rises with enhanced water regimes, albeit the increase was minimal across both years. The cost of cultivation was highest in the 05 irrigation levels (Rs 36304 and Rs 35938), followed by the 03 irrigation levels, while the lowest cost was observed in the 01 irrigation level plots (Rs 33248 and Rs 32856) during the years 2022-23 and 2023-24, respectively. Among the various irrigation schedules, the maximum net profit and benefit-cost ratio were seen at five irrigation levels; nevertheless, gross income was also reported at these five irrigation levels. This may be attributed to superior water use efficiency compared to alternative irrigation schedules, as well as a relatively greater rise in grain output than in other treatments. The lowest net profit, gross income, and B: C ratio (2.25 and 2.21) were recorded in the first irrigation level for both years, respectively.

Among moisture conservation strategies, the greatest values of gross and net returns, as well as the benefit-to-cost ratio, were observed with the application of 5 kg Pusa hydrogel combined with 1 ton of vermicompost per hectare in both years. This method yielded net returns exceeding control by Rs 28,105 and Rs 29,558 in the fiscal years 2022-23 and 2023-24, respectively. The respective increase in gross return was 38.6% and 42.9%. The cultivation costs ranged from Rs 32,263 to Rs 36,096 in the first year and from Rs 31,856 to Rs 35,746 in the second year across various treatments.

Table 1: Yield attributes of wheat as influenced by irrigation levels and moisture conservation practices at various stages of crop growth

Treatment	Yield Attributes									
	Effective tillers (m ²)		Spike length (cm)		No of spikelets/spike		No. of grains/spike		Test weight (g)	
	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24
Irrigation levels										
I1 (at CRI stage)	281	265	7.6	7.5	10.6	10.4	26.4	25.7	36.32	35.27
I2 (at CRI, Booting and Milking)	326	299	9.4	9.0	13.4	12.6	38.8	37.7	39.73	38.22

I3 (at CRI, Late tillering, Late jointing, Flowering and Milking stage)	365	342	9.6	9.1	13.5	12.2	41.3	38.9	41.20	39.46
S Em $\pm$	1.5	1.4	0.17	0.16	0.14	0.16	0.20	0.12	0.26	0.13
C.D. (P=0.05)	4.1	3.8	NS	NS	0.57	NS	0.87	0.57	1.10	0.55
Moisture conservation practices										
Control	317	293	7.5	7.3	9.7	10.4	30.2	29.0	35.15	33.65
Pusa hydro gel @5 kg ha ⁻¹	323	301	9.4	9.1	13.1	12.2	38.3	36.6	38.87	36.85
Vermi compost @ 1 t ha ⁻¹	335	312	9.8	9.4	13.3	13.0	40.3	38.7	41.10	39.20
Pusa hydro gel @5 kg ha ⁻¹ + Vermi compost @1 t ha ⁻¹	345	322	10.2	9.9	14.9	13.7	42.6	40.4	42.44	40.57
S Em $\pm$ 1	1.1	1.0	0.05	0.08	0.11	0.22	0.25	0.21	0.11	0.18
C.D. (P=0.05)	3.2	3.0	0.19	0.26	0.36	0.67	0.74	0.61	0.33	0.57

Table 2: Effect of different irrigation levels and moisture conservation practices at various stages of crop growth on grains, straw and biological yield (q ha⁻¹) and harvest index of wheat

Treatment	Yields (q ha-1)						Harvest index (%)	
	Grain		Straw		Biological			
	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24
Irrigation levels								
I1 (at CRI stage)	27.25	26.58	51.74	50.37	78.99	76.95	38.46	38.48
I2 (at CRI, Booting and Milking)	42.75	41.63	57.72	56.21	100.48	97.84	42.54	42.55
I3 (at CRI, Late tillering, Late jointing, Flowering and Milking stage)	46.01	45.02	73.62	72.02	119.62	117.03	44.43	44.44
S Em ±	0.30	0.47	0.59	0.68	0.87	1.12	0.12	0.18
C.D. (P=0.05)	1.28	1.82	2.32	2.67	3.62	4.58	0.45	0.59
Moisture conservation practices								
Control	30.71	28.12	56.11	55.01	86.82	83.13	41.50	41.43
Pusa hydro gel @5 kg ha-1	42.30	41.42	59.53	58.35	101.83	99.77	41.54	41.51
Vermi compost @ 1 t ha-1	44.46	43.17	62.47	60.68	106.93	103.85	41.57	41.56
Pusa hydro gel @5 kg ha-1+ Vermi compost @1 t ha-1	46.97	45.62	65.98	64.09	112.95	109.71	41.58	41.57
S Em ±	0.88	0.68	1.47	1.52	2.48	2.52	0.27	0.12
C.D. (P=0.05)	2.97	3.00	4.38	4.50	7.46	7.60	NS	NS

Table 3: Effect of irrigation levels and moisture conservation practices on economic feasibility of wheat

Treatment	Cost of cultivation (₹ ha ⁻¹)		Gross income (₹ ha ⁻¹)		Net Return (₹ ha ⁻¹)		B : C Ratio (₹ ha ⁻¹)	
	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24
Irrigation levels								
I1 (at CRI stage)	33248	32856	74644	72750	41396	39894	2.25	2.21
I2 (at CRI, Booting and Milking)	34436	34148	103032	100334	68596	66186	2.99	2.94
I3 (at CRI, Late tillering, Late jointing, Flowering and Milking stage)	36304	35938	117932	115244	81628	79306	3.25	3.21
S Em $\pm$	-	-	-	-	-	-	-	-
C.D. (P=0.05)	-	-	-	-	-	-	-	-
Moisture conservation practices								
Control	32263	31856	82802	77998	50539	46142	2.57	2.45
Pusa hydro gel @5 kg ha ⁻¹	34911	34358	103398	101282	68487	66924	2.96	2.94
Vermi compost @ 1 t ha ⁻¹	35243	35146	108618	105480	73375	70334	3.08	3.00
Pusa hydro gel @5 kg ha ⁻¹ + Vermi compost @1 t ha ⁻¹	36096	35746	114740	111446	78644	75700	3.18	3.12
S Em $\pm$	-	-	-	-	-	-	-	-
C.D. (P=0.05)	-	-	-	-	-	-	-	-

Conclusion

The results of a two-year experimentation indicate that irrigation at higher levels promotes the growth, yield attributes, yield, and productivity of wheat crops. Five irrigations (CRI, late tillering, late jointing, flowering, and milking stages) resulted in maximum growth, yield attributes, yield, productivity, and net and gross income for wheat production. Pusa hydrogel (5 kg/ha) and vermiculite compost (1 ton/ha) provided the best moisture retention and produced maximum yield and quality wheat. This combination provided maximum uptake of nutrients by the crop, gross and net income per hectare, and maintained soil fertility and moisture levels. The combination of 5 kg of pusa hydrogel per hectare of wheat with five irrigations (CRI, late tillering, late jointing, flowering, and milking stages) is recommended for growing wheat in sandy loam soils in the northwestern plain zones of western Uttar Pradesh.

Reference

1. Amare T, Sharma JJ and Zewdie K 2014. Effect of Weed Control Methods on Weeds and Wheat (*Triticum aestivum* L.) Yield. *World J Agric Res.* 2(3):124-128.
2. Anonymous 2023. Agriculture Statistics at a Glance, Ministry of Agriculture and Farmers Welfare, New Delhi.
3. Arancon N Q, Edwards C A, Lee S, and Byrne R 2006. Effects of humic acids from vermicomposts on plant growth. *European Journal of Soil Biology* 46: 65-69.
4. Chakraborty D, J K Ladha D S Rana et al. 2017. A Global Analysis of Alternative Tillage and Crop Establishment Practices for Economically and Environmentally Efficient Rice Production. *Scientific Reports.* 7: 1-11
5. Choudhary AK, Yadav DS, Sood P, Rahi S, Arya K, Thakur SK, et al. 2021. Post emergence herbicides for effective weeds management enhanced wheat productivity, profitability and quality in northwestern Himalaya, A participatory mode technology development and dissemination. *Sustainability.* 13(10):5425.
6. Dar S B and Ram H 2017. Productivity of wheat (*Triticum aestivum* L.) in relation to hydrogel as influenced by different irrigation regimes and nutrient levels. *Int. J. Chem. Stud.* 5(5), 609- 613.
7. Devendra C 2016 Improvement of value supply chains in sustainable small ruminant production to consumption systems in Asia. *Indian J. Anim. Sci.* 86, 3-10.
8. Donald C M 1962. In search of yield. *Journal of Australian Institute of Agricultural Sciences* 238: 171-178.
9. Edwards C A and Burrows I 1988. The potential of earthworm composts as plant growth media. In *Earthworms in Environmental and Waste Management.* SPB Academic Publishing, The Hague, the Netherlands. pp: 211-220.
10. Ekebafé LO, Ogbeifun DE and Okieimen FE 2011. Polymer applications in agriculture. *Biokemistri* 23(2): 81 - 89.
11. El-Hady O A and Abo-Sedera S A 2006. Conditioning effect of composts and acrylamide hydrogels on a sandy calcareous soil. II physico-biochemical properties of the soil. *Int. J. Agric. Biol.* 8(6), 876-884.
12. El-Hady O A and Abo-Sedera SA 2006. Conditioning effect of composts and acrylamide hydrogels on a sandy calcareous soil. II- Physico-bio-chemical properties of the soil. *International Journal of Agricultural Biology* 8: 876-884.
13. Kalhapure A, Kumar R, Singh V P and Pandey D S. 2016. Hydrogels: a boon for increasing agricultural productivity in water stressed environment. *Curr. Sci.,* 2016, 111(11), 1773-1779.
14. Narjary B and Aggarwal P 2014. Evaluation of soil physical quality under amendments and hydrogel applications in a soybean-wheat cropping system. *Commun. Soil Sci. Plant Anal.,* 45(9), 1167-1180.
15. Pandey IB, Kumar K. 2005. Response of wheat (*Triticum aestivum* L.) to seeding methods and weed management. *Indian J Agron.* 50(1):48-51.
16. Pandey J, Verma AK 2004. Effect of isoproturon tank mix with metsulfuron and 2, 4-D on weeds and yield of wheat. *Indian J Agron.,* 49(2):114-116.
17. Prasad R, Shivay YS, Kumar D and Sharma SN 2006. Learning by doing exercises in soil fertility (A Practical Manual for Soil Fertility), Division of Agronomy, Indian Agricultural Research Institute, New Delhi, pp. 68.
18. Priya S, Niwas R, Yadav AS, Singh R, Kumar A 2022. Effect of integrated weed management practices on growth and yield of (*Triticum aestivum* L.) wheat under timely sown condition. *PharIssuema Innov J.* 11(7):1285-1288.
19. Rehman A, Ahmad R and Safdar M 2011. Effect of hydrogel on the performance of aerobic rice sown under different techniques. *Plant Soil. Environ.,*

- 2011, 57, 321-325.
20. Sahu R, Sharda K, Kumar D, Mandal SK 2018. Weed population, weed biomass and grain yield of wheat as influenced by herbicides application. Indian J Weed Sci. 50(3):302-304.
 21. Schacht E H 2004. Polymer chemistry and hydrogel systems. J. Phys.: Conf. Ser. 3, 22-28.
 22. Singh J S Singh A P Vig et al 2018 "Conventional Farming Reduces the Activity of Earthworms: Assessment of Genotoxicity Test of Soil and Vermicast." Agriculture and Natural Resources (), 52: 366-370.
 23. Singh S P, Singh R K and Kumar S 2017 Effect of irrigation schedule, mulching and hydrogel on wheat (*Triticum aestivum* L.). J. Pharmacog. Phyto., , 6(5), 2659-2973.
 24. Singh S A, Sharma K, Khajuria J, Singh A P 2020. Vig. Soil Properties Changes Earthworm Diversity Indices in Different Agroecosystem." BMC Ecology, (2020)-20: 27.
 25. Tyagi V, Singh R K and Nagargade M 2015. Effect of hydrogel, NPK and irrigation levels on yield, nutrient uptake and water use efficiency of wheat (*Triticum aestivum* L.). Res. Crop.16(4), 653-656.
 26. Verma SK, Singh RP, Kumar S 2017. Effects of irrigation and herbicides on the growth and yield attributes of wheat (*Triticum aestivum* L.). Bangladesh J Bot.;46(3):839-845.

Received : May 2025; Revised : June 2025; Accepted : July 2025



OPTIMIZATION OF PLANT GROWTH REGULATORS FOR IN VITRO CALLUS INDUCTION AND SHOOT PROLIFERATION IN THE NATURAL SWEETENER

STEVIA REBAUDIANA (BERTONI)

Amit Kuma^{*1}, Priyanka Chaudhary¹, Reshu Chaudhary²,

Bijendra Singh² and R. P. Singh³

¹Department of Biotechnology, KVSCOS, Swami Vivekanand Subharti University,
Meerut, Uttar Pradesh, India

²College of Horticulture, Sardar Vallabhbhai Patel University of Agriculture and Technology,
Meerut, Uttar Pradesh, India

³Former Vice Chancellor, Swami Keshwanand Rajasthan Agriculture University, Bikaner

***Corresponding Author Email-**amit.agbiotech1581@gmail.com

Abstract

Stevia rebaudiana (Bertoni), a perennial herb of the Asteraceae family, is the source of zero-calorie steviol glycosides (notably stevioside and rebaudioside A) that are 250–300 times sweeter than sucrose and possess antidiabetic, antihypertensive, anti-inflammatory and antioxidant properties (Salehi et al., 2023). Because seed germination is poor and the species is highly self-incompatible, in-vitro propagation offers the most reliable route to genetically uniform, large-scale planting material (Abouelela et al., 2025). The present study optimised plant growth regulator (PGR) combinations for callus induction and shoot proliferation from leaf explants of *S. rebaudiana* on Murashige and Skoog (MS) medium. For callogenesis, 2,4-dichlorophenoxyacetic acid (2,4-D) was combined with either kinetin (Kn) or α -naphthaleneacetic acid (NAA), and for shoot proliferation, 6-benzylaminopurine (BAP) was combined with NAA at eight concentration ratios. The maximum callus-induction frequency ($86.2 \pm 3.6\%$), yielding green, organogenic callus, was obtained on MS medium supplemented with 2,4-D + NAA at $1.5 + 3.0 \text{ mg L}^{-1}$, which outperformed all 2,4-D + Kn combinations. For shoot proliferation, MS medium with BAP + NAA at $0.5 + 1.0 \text{ mg L}^{-1}$ gave the highest response (88% of explants), the greatest mean shoot length ($3.61 \pm 0.21 \text{ cm}$ after four weeks) and a four-fold multiplication rate, whereas higher cytokinin–auxin ratios were progressively inhibitory. The optimised protocol provides an efficient, reproducible system for rapid clonal multiplication of this commercially important sweetener crop and a platform for downstream steviol-glycoside production and germplasm conservation.

Keywords: *Stevia rebaudiana*; in-vitro propagation; callus induction; shoot proliferation; plant growth regulators; 2,4-D; BAP; NAA; steviol glycosides.

Introduction

Stevia rebaudiana (Bertoni) is a perennial herb of the Asteraceae (Compositae) family, native to Paraguay and Brazil, whose leaves accumulate sweet diterpene steviol glycosides—principally stevioside and rebaudioside A—that are approximately 250–300 times sweeter than sucrose yet non-caloric, non-cariogenic and

non-fermentable (8; 6). These compounds are heat- and pH-stable, making stevia extracts attractive for foods, beverages and pharmaceuticals, and especially valuable for diabetic and weight-conscious consumers (4).

Beyond their sweetening role, steviol glycosides display antidiabetic, antihypertensive, anti-inflammatory, antioxidant, anticancer and

antimicrobial activities, which has driven sharply rising global demand for stevia biomass (8). Conventional propagation, however, is constrained: seed germination is poor and erratic owing to strong self-incompatibility, and the resulting seedlings vary widely in glycoside content, while stem cuttings are slow and damage the donor plant (1). Plant tissue culture therefore provides the most efficient, eco-friendly route to rapid, genetically uniform mass propagation and to the conservation and improvement of elite germplasm (2).

In micropropagation, the type, concentration and ratio of plant growth regulators (PGRs) decisively control callus induction, organogenesis and shoot multiplication; auxins such as 2,4-dichlorophenoxyacetic acid (2,4-D) and α -naphthaleneacetic acid (NAA) drive dedifferentiation and callogenesis, whereas cytokinins such as 6-benzylaminopurine (BAP) and kinetin (Kn) promote shoot induction and proliferation (5; 3). The present study was undertaken to optimise PGR combinations on Murashige and Skoog (MS) medium for efficient callus induction and shoot proliferation from leaf explants of *S. rebaudiana*, and thereby to establish a reliable protocol for its rapid in-vitro multiplication.

Materials and Methods

Plant material and explant sterilisation

Healthy plants of *Stevia rebaudiana* were maintained at the Department of Biotechnology, Keral Verma Subharti College of Science, Swami Vivekanand Subharti University, Meerut. Nodal segments with axillary buds and young leaves were used as explants. Explants were washed under running tap water, treated with 10% Tween-20 for 5–10 min, surface-sterilised with 70% ethanol for 3 min followed by 0.1% mercuric chloride (HgCl_2) for 1–2 min, and rinsed four to five times with sterile double-distilled water.

Culture medium and conditions

Explants were inoculated on Murashige and Skoog (1962) medium supplemented with 3% (w/v) sucrose and the appropriate PGRs. The pH was adjusted to 5.6 and 0.8% (w/v) agar was added before autoclaving. Cultures were incubated at $25 \pm 2^\circ\text{C}$ under a 16-h photoperiod provided by white fluorescent light.

Callus induction

Leaf explants (~1.5 cm) were placed dorsal-side in contact with MS medium supplemented with 2,4-D in combination with either kinetin (Kn) or NAA at five ratios each (0.5 + 1.0, 1.0 + 2.0, 1.5 + 3.0, 2.0 + 4.0 and 2.5 + 5.0 mg L^{-1}). Callus-induction frequency was recorded, and callus intensity was scored on a 1–4 scale (+ to ++++).

Shoot proliferation

Callus and nodal explants showing a good response were transferred to MS medium supplemented with BAP and NAA at eight combinations (0.5 + 0.5, 0.5 + 1.0, 0.5 + 1.5, 0.5 + 2.0, 1.0 + 0.5, 1.0 + 1.0, 1.0 + 1.5 and 1.0 + 2.0 mg L^{-1}). The percentage of explants forming shoots, mean shoot length at 2, 3 and 4 weeks, and the multiplication fold were recorded.

Statistical analysis

Each treatment comprised replicated cultures, and experiments were repeated. Data are presented as mean $\pm$ standard error (SE) of the recorded observations.

Results and Discussion

Explant sterilisation

Among the surface-sterilisation treatments evaluated, sequential treatment with 70% ethanol (3 min) followed by 0.1% HgCl_2 for 1–2 min gave the best balance of contamination control and explant survival, producing the highest proportion of clean, viable explants for subsequent culture.

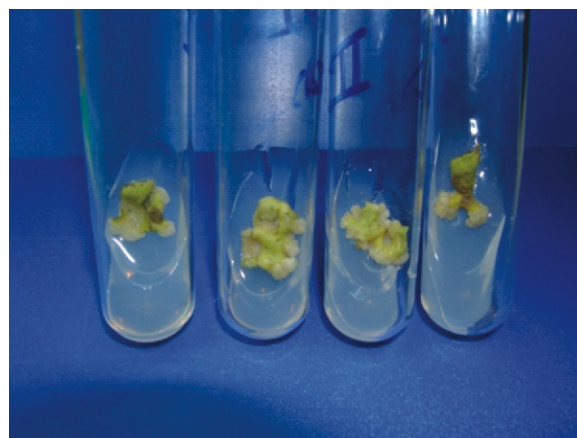


Figure 1. Initiation of Callogenesis from Leaf Explants



Figure 2. Regeneration and Multiplication of Shoots from Callus-Derived Cultures

Callus induction

Callus initiation appeared at the cut surfaces of leaf explants 16-20 days after inoculation, and its frequency and quality depended strongly on the auxin-cytokinin combination (Table 1). Within the 2,4-D + Kn series, callus-induction frequency rose with concentration to a maximum of $68.3 \pm 3.4\%$ at $1.5 + 3.0 \text{ mg L}^{-1}$, producing white-green compact callus, and then declined at higher levels (Figure-1). The 2,4-D + NAA series was consistently superior: callus frequency peaked at $86.2 \pm 3.6\%$ at $1.5 + 3.0 \text{ mg L}^{-1}$, yielding green, organogenic callus, before falling at $2.0 + 4.0$ and $2.5 + 5.0 \text{ mg L}^{-1}$. The combination of 2,4-D with NAA was therefore more effective for callogenesis from leaf explants than 2,4-D with kinetin, consistent with reports that 2,4-D and NAA are potent inducers of dedifferentiation and callus proliferation in *S. rebaudiana* and related medicinal species (3; 7).

Table 1. Effect of plant growth regulator (PGR) combinations on callus induction from leaf explants of *Stevia rebaudiana* on MS medium. Values are mean $\pm$ SE; callus intensity scored 1-4 (+ to ++++). The highest response is highlighted.

PGR combination (mg L^{-1})	Callus induction frequency (%)	Callus intensity	Nature of callus
2,4-D + Kn			
0.5 + 1.0	38.4 ± 2.6	+	Pale, friable

1.0 + 2.0	52.7 ± 3.1	++	White, globular
1.5 + 3.0	68.3 ± 3.4	+++	White-green, compact
2.0 + 4.0	49.6 ± 2.9	++	White, globular
2.5 + 5.0	35.1 ± 2.4	+	Pale, friable
2,4-D + NAA			
0.5 + 1.0	47.5 ± 2.8	++	White, globular
1.0 + 2.0	66.8 ± 3.2	+++	Whitish-yellow, compact
1.5 + 3.0	86.2 ± 3.6	++++	Green, organogenic
2.0 + 4.0	57.3 ± 3.0	+++	Whitish, compact
2.5 + 5.0	44.0 ± 2.5	+	Pale, friable

Shoot proliferation

Leaf-derived explants and callus transferred to MS medium containing BAP and NAA showed marked differences in shoot response across the eight combinations (Table 2). The highest proliferation was obtained at BAP + NAA $0.5 + 1.0 \text{ mg L}^{-1}$, where 88% of explants formed shoots with a mean shoot length increasing from $1.42 \pm 0.16 \text{ cm}$ at two weeks to $3.61 \pm 0.21 \text{ cm}$ at four weeks and a four-fold multiplication rate. Increasing the NAA level at constant BAP (0.5 mg L^{-1}) beyond 1.0 mg L^{-1} progressively reduced the response, with the lowest value (58%) at $0.5 + 2.0 \text{ mg L}^{-1}$. Raising BAP to 1.0 mg L^{-1} did not improve proliferation; the best response in that series was only 63% at $1.0 + 0.5 \text{ mg L}^{-1}$, declining to 43% at $1.0 + 2.0 \text{ mg L}^{-1}$ (Figure-2).

These results indicate that a low cytokinin level combined with a moderate auxin level (BAP + NAA, $0.5 + 1.0 \text{ mg L}^{-1}$) is optimal for shoot proliferation in *S. rebaudiana*, whereas higher auxin or cytokinin concentrations are inhibitory—an outcome in close agreement with recent micropropagation studies reporting that low-to-moderate BAP, alone or with a small amount of NAA, maximises shoot number and length in stevia (1; 5). The balance between cytokinin and auxin, rather than their absolute concentrations, thus governs the switch between callus proliferation and shoot organogenesis (3).

Table 2. Effect of BAP + NAA combinations on shoot proliferation from *Stevia rebaudiana* cultures on MS medium. Values are mean $\pm$ SE. The highest response is highlighted.

BAP + NAA (mg L ⁻¹)	Explants forming shoots (%)	Mean shoot length (cm)			Multiplication fold
		2 weeks	3 weeks	4 weeks	
0.5 + 0.5	68	1.31 $\pm$ 0.18	1.95 $\pm$ 0.20	2.78 $\pm$ 0.22	2
0.5 + 1.0	88	1.42 $\pm$ 0.16	2.74 $\pm$ 0.19	3.61 $\pm$ 0.21	4
0.5 + 1.5	73	1.18 $\pm$ 0.22	1.92 $\pm$ 0.24	3.02 $\pm$ 0.25	3
0.5 + 2.0	58	1.05 $\pm$ 0.20	1.63 $\pm$ 0.21	2.71 $\pm$ 0.23	2
1.0 + 0.5	63	1.22 $\pm$ 0.19	1.81 $\pm$ 0.20	2.52 $\pm$ 0.22	2
1.0 + 1.0	54	1.16 $\pm$ 0.18	1.69 $\pm$ 0.20	2.41 $\pm$ 0.21	2
1.0 + 1.5	48	1.39 $\pm$ 0.19	1.61 $\pm$ 0.20	2.39 $\pm$ 0.21	1
1.0 + 2.0	43	1.41 $\pm$ 0.20	1.52 $\pm$ 0.21	2.31 $\pm$ 0.22	1

Leaves and other vegetative explants are preferred for in-vitro regeneration because they preserve the genetic constitution of the parent genotype, an important consideration for a self-incompatible, heterozygous species such as stevia (1). Although callus-mediated (indirect) regeneration enables in-vitro selection, somaclonal-variation studies and genetic transformation, prolonged subculture of callus can increase the risk of somaclonal variation; controlled subculture intervals and low-to-moderate PGR levels, as optimised here, help to maintain clonal fidelity (2; 7). The optimised callus-induction and shoot-proliferation system established in this study can be coupled with downstream rooting, acclimatisation and elicitation strategies to enhance steviol-glycoside yield and to support large-scale, water-efficient production of this natural sweetener (1).

Conclusion

An efficient in-vitro protocol for callus induction and shoot proliferation in *Stevia rebaudiana* was established using leaf explants on MS medium. The combination of 2,4-D + NAA at 1.5 + 3.0 mg L⁻¹ gave the maximum callus-induction frequency (86.2 $\pm$ 3.6%) with green, organogenic callus, while BAP + NAA at 0.5 + 1.0 mg L⁻¹ produced the highest shoot proliferation (88% of explants), the greatest shoot length (3.61 $\pm$ 0.21 cm at four weeks) and a four-fold multiplication rate. Cytokinin–auxin balance, rather than absolute hormone concentration, was the key determinant of morphogenic response. The protocol offers a rapid,

reproducible route to genetically uniform planting material and a foundation for steviol-glycoside production and germplasm conservation of this commercially valuable sweetener crop.

References

1. Aboulela, M. B., Eid, M., Ali, F. M., & Owis, A. I. (2025). Optimizing a rapid tissue culture method for steviol glycoside production from *Stevia rebaudiana* to address Egypt's sugar deficit. *Scientific Reports*, 15(1), 25495. <https://doi.org/10.1038/s41598-025-10491-3>
2. Ahmad, N., Rab, A., & Ahmad, N. (2022). Light-induced biochemical variations in secondary metabolite production and antioxidant activity in callus cultures of *Stevia rebaudiana* (Bertoni). *Journal of Photochemistry and Photobiology B: Biology*, 226, 112369. <https://doi.org/10.1016/j.jphotobiol.2021.112369>
3. Bayraktar, M. (2021). Micropropagation and production of steviol glycosides from *Stevia rebaudiana*. In *Bioprocessing of plant in vitro systems* (pp. 117–146). Springer. https://doi.org/10.1007/978-3-030-30185-9_19
4. Carrera-Lanestosa, A., Coral-Martínez, T., Ruíz-Ciau, D., & Moguel-Ordóñez, Y. (2025). Recent advancement in medicinal properties and safety aspects of *Stevia rebaudiana*: Zero-calorie sugar plant. *Sugar Tech*, 27(4), 1–20. <https://doi.org/10.1007/s12355-025-01658-3>
5. Idrees, M., Sania, B., Hafsa, B., Kumari, S., Khan, H., Fazal, H., Ahmad, I., Akbar, F., Ahmad, N., Ali, S., & Ahmad, N. (2022). Spectral lights trigger biomass accumulation and production of antioxidant secondary metabolites in adventitious root cultures of *Stevia rebaudiana* (Bert.). *Comptes Rendus Biologies*, 345(1), 13–28. <https://doi.org/10.5802/crbiol.74>

6. Kasti, A. N., Nikolaki, M. D., Synodinou, K. D., Katsas, K. N., Petsis, K., Lambrinou, S., Pyrousis, I. A., & Triantafyllou, K. (2022). The effects of stevia consumption on gut bacteria: Friend or foe? *Microorganisms*, 10(4), 744. <https://doi.org/10.3390/microorganisms10040744>
7. Libik-Konieczny, M., Capecka, E., Tuleja, M., & Konieczny, R. (2020). Synthesis and production of steviol glycosides: Recent research trends and perspectives. *Applied Microbiology and Biotechnology*, 105(10), 3883–3900. <https://doi.org/10.1007/s00253-021-11306-x>
8. Salehi, B., López, M. D., Martínez-López, S., Victoriano, M., Sharifi-Rad, J., Martorell, M., Rodrigues, C. F., & Martins, N. (2023). Steviol glycosides from *Stevia rebaudiana*: An updated overview of their sweetening activity, pharmacological properties, and safety aspects. *Molecules*, 28(3), 1258. <https://doi.org/10.3390/molecules28031258>

Received : May 2025; Revised : June 2025; Accepted : July 2025



SYNERGISTIC EFFECTS OF SELECTED HERBAL PLANT EXTRACTS WITH ANTIBIOTICS AGAINST CARBAPENEM-RESISTANT *ESCHERICHIA COLI*: A SYSTEMATIC REVIEW

Sandhya¹, Amit Kuma^{2*}, Bijendra Singh³ and R.P.Singh⁴

¹Department of Microbiology, Keral Verma Subharti College of Science, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh, India

²Department of Biotechnology, Keral Verma Subharti College of Science, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh, India

³College of Horticulture, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India

⁴Former Vice Chancellor, Swami Keshwanand Rajasthan Agriculture University, Bikaner

*Corresponding Author Email-amit.agbiotech1581@gmail.com

Abstract

Background: The rapid emergence of carbapenem-resistant *Escherichia coli* (CREC) infections is a major threat to global public health, prompting the search for alternative therapeutic options. In this review, the latest research on the antimicrobial activity and synergy of herbal plant extracts on carbapenem-resistant *Escherichia coli* (CREC) and multidrug-resistant (MDR) clinical isolates is discussed. Key findings point to the capacity of equol, naringenin, and tetrandrine to reinstate the effectiveness of conventional antibiotics, including carbapenems and colistin. Furthermore, the mechanisms of action include the disruption of cell wall and membrane integrity, the inhibition of efflux pump activity, and the downregulation of resistance genes, including carbapenemases and *mcr-1*. Synergistic interactions, indicated by a Fractional Inhibitory Concentration (FIC) index of less than 0.5, reveal the potential of plant-derived compounds as effective adjuvants. Although in vivo studies in murine models indicate a positive outcome in terms of reduced bacterial loads, translation to the clinic is hindered by a lack of human studies and standardized extraction methodologies. This report presents a holistic overview of these plant-derived approaches, revealing a glimpse of their potential as next-generation antimicrobials.

Keywords: CREC, Herbal extracts, antimicrobial resistance (AMR), Medicinal plants, multidrug-resistant bacteria, Synergistic antibacterial activity

Introduction

Escherichia coli is a ubiquitous Gram-negative bacterium that serves as both a commensal organism in the human gut and a major opportunistic pathogen responsible for a wide range of community- and healthcare-associated infections. Urinary tract infections (UTIs), bacteremia, sepsis, and meningitis (primarily in newborns) all contribute to significant morbidity and mortality around the world. The emergence and proliferation of carbapenem-resistant

Escherichia coli (CREC) in recent years have caused great concern for public health since carbapenem antibiotics represent the last line of drugs against the treatment of serious infections resulting from multidrug-resistant (MDR) Gram-negative bacteria. The lack of activity of these drugs creates limited treatment options and greater challenges in providing clinical care (1).

Multiple resistance mechanisms co-exist in *Escherichia coli*, causing it to be resistant to carbapenems and other types of antibiotics. One of

the main resistance mechanisms is the creation of carbapenemase enzymes, which break down the carbapenem class of antibiotics. Overexpression of efflux pump systems is an additional resistance mechanism as they actively pump out the antibiotic from the bacterial cell and limit the concentration of the drug to below therapeutic levels within the bacterial cell. Resistance mechanisms also include structural changes to outer membrane porins, thereby decreasing the permeability of the membrane and limiting the ability of an antibiotic to enter the bacterial cell. Together, these mechanisms confer high-level resistance and greatly reduce susceptibility to multiple classes of antimicrobial agents (6).

Due to the reduced effectiveness of traditional antibiotics as well as the slow development of new synthetic drugs, there is an increasing amount of interest in plant-based natural alternatives from plant sources. Many different types of plant extracts are rich in bioactive secondary metabolites that have shown evidence of having antimicrobials, including polyphenols, alkaloids, flavonoids, and terpenoids. Not only do these compounds exhibit antibacterial effects, but they have also been shown to alter the resistance mechanisms of many different bacterial species, allowing for them to be used as a co-treatment with existing antibiotics. This has caused these compounds to become promising alternatives for treating antibiotic resistance (8; 9). Additionally, the known synergistic relationship between plant-based compounds and traditional anti-bacterial medications has been more closely studied in recent years. These combinations can increase the activity of antibiotics by acting on different mechanisms of action, such as: disruption of bacterial cell membranes, inhibition of efflux pumps, suppression of resistance genes, and obstruction of quorum sensing (QS) and biofilm formation. When combining multiple modes of action, the chance that resistance will develop is decreased, and clinical efficacy will be improved.

This aims to comprehensively evaluate the potential of medicinal plant extracts in the management of carbapenem-resistant *Escherichia coli* infections. The focus will be on research conducted between 2021 and 2026, with

special emphasis placed on understanding how certain phytochemicals exert their effects alone and when combined with other commonly used antibiotics. The synthesis of the available evidence in this review will allow for a better understanding of the role plant-based therapies can play in attempting to reduce the burden of CREC infection and address the ever-evolving issue of antimicrobial resistance.

Methods and Approaches in the Sources

The studies reviewed employed various standardised methodologies to evaluate the extracts. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) determinations remain the gold standard for the evaluation of the intrinsic potency of the extracts (3; 5). Synergism of the extracts was mainly assessed by employing the checkerboard assay to determine the Fractional Inhibitory Concentration Index (FICI), whereby extracts exhibiting FICI values of ≤ 0.5 were regarded as synergistic (4; 11).

Advanced imaging and molecular techniques have been utilised to achieve the goal. Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) were used to observe the physical damage to the bacterial cell envelopes (2; 4; 15). Confocal Laser Scanning Microscopy (CLSM) and Fluorescence-based assays, e.g., Propidium Iodide uptake, were utilised to evaluate membrane permeability and biofilm architecture (2; 7). Molecular mechanisms, e.g., the suppression of the transcription of resistance genes, were studied by employing Quantitative Real-Time PCR (qRT-PCR) (1; 13; 17). Phytochemical characterisation of the compounds, as well as the extracts, of the plants of interest, typically involves the use of High-Performance Liquid Chromatography (HPLC) or Gas Chromatography-Mass Spectrometry (GC-MS) (5).

Findings and Comparative Analysis

(a) Restoration of Antibiotic Susceptibility

Recent antimicrobial research has uncovered a

groundbreaking and exciting discovery: various plant-sourced compounds can help restore the antibiotic susceptibility to carbapenem-resistant *Escherichia coli* (CREC) so that antibiotics will work to kill bacteria again. This discovery demonstrates a trend away from conventional methods of killing bacteria (bactericidal) or stopping bacterial growth (bacteriostatic) provided by antibiotics, and toward the use of Resistance-Modifying Agents (RMAs) that reverse the mechanisms of resistance rather than killing the bacteria. Such therapies are important due to the growing number of multidrug-resistant organisms for which few treatment options remain. One example of an RMA is equol, a product of daidzein (an isoflavone found in soybeans), which has been shown to restore CREC susceptibility to meropenem. Research has shown that equol inhibits CREC because it acts on both the molecular and functional pathways of resistance, affecting both the genetic expression of carbapenemase genes and the structural integrity of the bacterial cell (specifically the cellular membrane). By reducing the mechanisms that confer antibiotic resistance, equol increases the intracellular concentration and activity of meropenem, thereby re-sensitising CREC (1). Additionally, the alkaloid tetrandrine, which can be found in *Stephania tetrandra*, has been shown to restore colistin susceptibility in *E. coli* strains containing the mcr-1 gene (a primary cause of plasmid-mediated resistance to colistin). Tetrandrine works by multiple means; it inhibits efflux pumps, alters membrane readiness and interacts with MCR-1 protein to inhibit its function. The result of these actions is a reduction in the phenotypic resistance allowing colistin to again exert strong bacteriocidal effects on strains that were formerly resistant (4). RMAs are a class of compounds that have typically not shown strong antimicrobial activity by themselves. Instead, their primary use is to enhance the efficacy of existing antibiotics by neutralising or bypassing resistance mechanisms. This enhancement of activity in association with the available antibiotics makes RMAs very attractive for use in clinical practice because these types of compounds prolong the useful life of antibiotics currently available, require less use of higher doses of antibiotics and could help to slow or eliminate the

emergence of more resistant organisms.

(b) Synergistic Effects and FIC Indices

Another interesting phenomenon is the synergy of herbal extracts with conventional antibiotics. The combination of the extracts of the herbal compound naringenin with the conventional drug amikacin was found to show significant synergy with both ATCC 25922 and clinical strains of *E. coli* (2). The extracts of the leaves of the plant *Pimenta dioica* showed synergy with the conventional drug ampicillin, which reduced the MBC from more than 10 mg/mL to 300-500 µg/mL against ESBL-producing *E. coli* (15). Some of the other interesting combinations of herbal extracts with conventional antibiotics that showed synergy are as follows: Synergistic effects of herbal products with traditional antibiotics suggest they can work together to kill some bacteria that are resistant to single medications. In one study (17), carvacrol (an option found in essential oils) combined with cefixime (an antibiotic) produced a fractional inhibitory concentration index (FICI) of 0.5, which demonstrates that carvacrol and cefixime worked together to inhibit bacterial growth. The use of guava leaf extract along with the antibiotic ofloxacin has also demonstrated promise against antibiotic-resistant strains in treating urinary tract infections (12). Furthermore, the use of mannan (a polysaccharide from the yeast, *Saccharomyces cerevisiae*) in combination with ciprofloxacin or norfloxacin resulted in greater than a 300% increase in the effectiveness of these antibiotics against antibiotic-resistant strains (14). Polyherbal extracts from *Morinda lucida* and *Pterocarpus santalinoides* demonstrated extremely low FICI values (as low as 0.09 mg/mL) against multidrug-resistant (MDR) isolates, indicating strong synergy and potential as adjunctive therapies to treat resistant bacteria (11).

(c) Phytochemical Profiles of Active Extracts

Active plant extracts may comprise complex mixtures of bioactive compounds that play a role in their antimicrobial and therapeutic activities. Advanced techniques, such as High-Performance Liquid Chromatography (HPLC), Gas chromatography-mass spectrometry (GC-MS),

etc., have been used to identify the bioactive compounds from medicinal plants. Phenolic compounds/flavonoids are the most commonly found bioactive compounds, which have been identified from many plants, including *Origanum vulgare*, where sixteen phenolic compounds were identified, as well as from the leaves of the guava plant and the fruit of the *Pimenta dioica* plant (5; 12; 15). These compounds are well known for their antioxidant and antimicrobial properties. Alkaloids are another significant group of bioactive compounds. Tetrandrine and other alkaloids have been identified in plants like *Morinda lucida* and *Pterocarpus santalinoides* (4; 11). Terpenoids are also significant in the bioactivity of plant extracts. Eugenol has been identified as the major bioactive compound in the chloroform extract of *Pimenta dioica* (15). Besides the above-mentioned major bioactive compounds, several other phytochemicals like saponins, tannins, and glycosides are commonly found in various polyherbal formulations, which are responsible for their combined bioactivity (11; 20).

Mechanisms of Action

(a) Membrane and Cell Wall Disruption

The principal mode of action for these various types of extracts and bioactive compounds that exhibit antimicrobial properties works by destroying the envelope of bacterial cells, thereby making the cells non-functional. This type of action is particularly important for Gram-negative bacteria, in which an outer membrane serves as a protective barrier against many different antimicrobial types. Of those compounds that were tested, naringenin produced significant amounts of damage to the bacterial cell wall. This has been confirmed by observations made using scanning electron microscopy (SEM) and through an increased level of alkaline phosphatase (AKP) activity. The increase in AKP activity represents a change in cell wall permeability and structural damage; therefore, demonstrating that naringenin is capable of causing disruption of the bacterial envelope (2). The combined effect of tetrandrine and colistin also produces a strong disrupting effect on the outer membrane of the bacterial cell. This combination produces a marked alteration in the morphology of the bacterial cell, which can

include rupturing the outer cell membrane and leaking of the intracellular materials, and producing observable “patches and holes” on the exterior surface of the bacterial cell. This type of structural damage results in the loss of vital cytoplasmic materials and complete death of the bacterial cell, demonstrating the enhanced effectiveness of combination therapy (4). Moreover, both extracts from *Origanum vulgare*, and its primary active chemical component, carvacrol, showed considerable membrane-disruptive action. The mechanism by which these compounds disrupt membrane integrity includes; alteration in the permeability barrier of the cell membrane, thereby causing a disturbance in the homeostasis of intracellular pH levels, and an imbalance of inorganic ion concentrations. Disruption of physiological homeostasis is an important factor in affecting bacterial metabolism and survival (5 and 17). In addition, eugenol, a common phenylpropanoid substance, is very effective at killing bacteria by causing cell lysis. Eugenol allows leakage of important intracellular components such as proteins and lipids thus causing the compromise of membrane integrity and resulting in irreparable cell damage. This additional mode of action further substantiates plant-derived compounds as potential antimicrobial agents against resistant strains of bacteria (15).

(b) Inhibition of Efflux Pumps and Proton Pumps

Efflux pumps are a major component in the development of antimicrobial resistance among Gram-negative bacteria due to their ability to remove a wide range of drugs from the organism, resulting in less chemical concentration within the cell and decreasing therapeutic effectiveness. The mechanisms of these pumps, which typically utilize the proton motive force, allow multidrug-resistant bacteria to survive in the presence of a variety of chemically diverse antimicrobial agents. Therefore, one effective way to reverse the mechanisms of efflux pumps is via the use of efflux pump inhibitors (EPIs); given the success of tetrandrine as an EPI, the use of tetrandrine can result in an increase in the intracellular concentration of drugs, enhancing antimicrobial efficacy and thereby improving the

potentiation of standard antibiotics used on multidrug-resistant organisms (4). Also, extracts from plants like *Garcinia lucida* and *Theobroma cacao* have been found to have potent EPI activity. These naturally occurring compounds affect the functionality of bacterial efflux mechanisms, thereby decreasing the capacity of pathogens to get rid of antimicrobial substances. Consequently, they increase the intracellular concentration of antibiotics and enhance bactericidal activity. In addition, extracts from *Origanum vulgare* and *Garcinia lucida* have been demonstrated to inhibit H⁺-ATPase proton pumps. These proton pumps are vital for the maintenance of proton motive force (PMF), which supplies both the energy-generating capacity of cells as well as the processes required for transporting substances across cellular membranes, such as efflux pumping. By impeding the use of energy produced via protons (H⁺) to drive the energy-generating mechanism for transporting effluxes antibiotics out of a cell, the extracts impair the energy source required by bacteria to use their efflux pump mechanism, thereby indirectly inhibiting efflux drug transport. The combined effects of both inhibiting direct efflux pumps and collapsing the PMF produce a synergistic effect that promotes the efficacy of antibiotics against multidrug-resistant bacteria (5; 20).

(c) Molecular Modulation of Resistance Genes

Equol is a compound produced from isoflavone, which comes from plant sources. In part, by acting to inhibit transcription of the genes that encode for the enzyme carbapenemases in *E. coli*, equol effectively reduces synthesis of carbapenemase enzymes that can hydrolyze and inactivate carbapenems. The inhibition of transcription will lead to a restoration of bacterial susceptibility, thus making antibiotics more effective when used to treat bacterial infections (1). Similarly, the polyphenol-rich extracts from green tea (e.g. catechins) have shown the ability to inhibit several antimicrobial resistance genes, including *mcr-1*, conferring to the efficacy of colistin; *bla_{TEM}*, conferring to the efficacy of β -lactam antibiotics; and *floR*, conferring to the efficacy of phenicol antibiotics. There is a high likelihood that green tea extracts may be effective against MDR bacteria at the molecular level due to the simultaneous

inhibitory effects on several different resistance genes (13). Some phytochemicals not only regulate transcription, but they also directly influence resistance-associated proteins. For example, tetrandrine has been shown to directly inhibit the action of the MCR-1 protein via direct molecular interactions. Tetrandrine interacts with three important amino acids located in the active site of the MCR-1 enzyme, Leu419, Ala420, and Tyr476, by forming hydrogen bonds between its structure and these residues. Tetrandrine disrupts the ability of the MCR-1 enzyme to catalyse the modification of lipid A, which is required for colistin resistance. Therefore, while tetrandrine inhibits the expression of resistance genes, it also directly inhibits the function of the resistance protein (4).

(d) Anti-virulence and Biofilm Inhibition

Extracts from plants have been shown to have an effect on the way pathogenic bacteria utilise virulence factors; these infectious agents are vital to establishing, maintaining, and amplifying infections. Conventional antibiotics generally focus on killing and inhibiting the growth of pathogenic bacteria, while anti-virulence strategies are designed to undermine these disease-causing organisms by interfering with their ability to communicate, adhere, and create protective structures (biofilm formation) for themselves. Anti-virulence strategies will also decrease the ability of pathogenic organisms to disseminate and grow, therefore decreasing any selection pressures that would contribute to the emergence of antimicrobial resistance. A key bioactive component of many aromatic plant extracts is carvacrol, which has been shown to dramatically down-regulate the expression of multiple important virulence-associated genes, including *luxS* and *pfs*-both of which are vital components of the quorum-sensing system (a mechanism by which bacteria communicate to coordinate specific behaviours, including biofilm production, movement, and production of virulence factors). By inhibiting the expression of *luxS* and *pfs* genes, carvacrol interferes with quorum-sensing systems, thereby preventing bacteria from communicating and forming stable, long-lasting biofilms (17). Several other compounds, including Equol and *Origanum*

vulgare extract, have been shown to possess significant antibiofilm activity. Both of these agents are effective at both preventing initial biofilm formation and disrupting previously formed biofilms. Biofilms are incredibly complex communities of microorganisms that are surrounded by a thick layer of extracellular polymeric substances (EPS) produced by the microbes themselves. EPS protects the microorganisms from the actions of the host immune system and antimicrobial agents, making biofilms strongly associated with the development of chronic, recurrent, and persistent infections, especially in clinical circumstances involving long-term indwelling medical devices. The ability of Equol and *Origanum vulgare* to penetrate into and destabilise the EPS increases the susceptibility of bacteria to antimicrobial therapy and results in improved outcomes (1; 5).

Clinical Isolate Efficacy and In Vivo Evidence

The effectiveness of these extracts extends to all laboratory-produced organisms that have been developed for research purposes. In addition, there are many studies involving the use of clinical strains, including extended-spectrum beta-lactamase (ESBL) producers, and carbapenem-resistant Enterobacteriaceae (CRE) from urinary tract infections (UTI) and other types of infections to show efficacy/incidence of infection (10). For successful therapeutic use of an antimicrobial agent, the efficacy must be confirmed in an animal model, preferably in vivo; in a neutropenic mouse model, the combination therapy of tetrandrine and colistin significantly reduced (2.76 log₁₀ CFU/g) the amount of mcr-1 bearing *E. coli* than that of colistin (5.60-log₁₀ CFU/g) alone. Additionally, symbiotic (*Bifidobacterium breve* and galacto-oligosaccharides) have demonstrated their protective capacity against multidrug-resistant *Acinetobacter baumannii* in murine models, showcasing the potential of utilising natural products in treating infections (19).

Limitations of the Evidence

There are also many limitations to the present literature regarding the antimicrobial efficacy of plant extracts; one limitation being the lack of standardisation between studies in how plant

extracts are prepared and evaluated. The biological activity and reproducibility from study to study may be affected by the variability in potency and composition of the extracts, which is dependent upon several variables: The plant species used as the source of the extract, the geographic region in which the plant was grown, the conditions of the plant at harvest, and the methods used for extracting the active components of the plant. Quantitatively, the effect of these variables is difficult to determine without the elimination of the variables listed above and/or appropriate reporting of minimum inhibitory concentrations (MICs) (Palma et al., 2023). Many researchers have demonstrated that when plant extracts are tested along with traditional antibiotics, these extracts have a synergistic impact. However, when examining carbapenem-resistant strains of Enterobacteriaceae, one often finds either non-existent baseline MIC values or insufficient data to support their use as effective monotherapy agents (3; 5). Additionally, the majority of phytochemical toxicity profiles published to date show only limited levels of support for the absence of significant toxicity. Some sets of data have shown little evidence of hemolysis in pure extracts of *Origanum vulgare*; unfortunately, however, there remain very few studies documenting the long-term safety, toxicity, and pharmacokinetic profiles for many of the various phytochemicals (5; 8). Further complicating matters is the variability between methodologies used in different publications; different experimental designs, design of synergy testing, use of different clinical isolate panels, etc., make comparisons of results and therefore the validity of conclusions difficult to establish across the literature.

Research Gaps and Future Directions

There is ample existing evidence to indicate a number of areas that demand investigation in future research. A primary limitation of the current evidence is that there are very few adequately designed, large-scale human clinical trials assessing the safety, efficacy, and therapeutic utility of all herbal-antibiotic combinations. There have been a number of in vitro and preclinical studies demonstrating that many plant extracts exhibit substantial

antimicrobial activity against resistant organisms; however, their clinical utility has not been established using comprehensive clinical assessments (18). Formulation science represents another important area of research; the development of more advanced drug delivery systems for phytochemicals may allow for more effective utilisation of these compounds therapeutically. Nanotechnology has been applied to improve the stability, solubility, and bioavailability of active compounds in plants via various methods of putting them on or in different nanotechnology systems (e.g., Plant extracts in silver nanosphere or Plant extracts in other nano formulations) (19). Further research is necessary to provide better insight into how these compounds derived from plants work by understanding the mechanisms by which they are antimicrobial. Specifically, while membrane disruption and increased permeability of the cell have been well studied as basic mechanisms to explain plant-based compounds, much is still unknown about specific molecular targets and pathways that are affected by specific plant extracts. By utilising modern “omics” (e.g., transcriptomics, proteomics), it may be possible to gain further insight into the molecular interactions and biological responses associated with these compounds. As a result, we may be able to clarify how these compounds produce the antimicrobial effects observed with them.

Conclusion

Herbal extracts have been extensively investigated for their antimicrobial potential against carbapenem-resistant *Escherichia coli*, a pathogen of significant clinical concern due to its high level of resistance to last-resort antibiotics. However, recent scientific studies also show that some of these compounds (derived from plants) enhance the effectiveness of currently available antibiotics when combined with them, which is especially important in overcoming mechanisms of resistance and in returning bacterium to its susceptible state. Of the various bioactive compounds identified in plant extracts, the key antimicrobial and resistance-modifying compounds are equol, naringenin, and tetrandrine. These three compounds produce their

antimicrobial effects by at least three unique, but complementary, mechanisms of action: disruption of the integrity or permeability of the bacterial cell membrane, inhibition of the ability of the bacterium to extrude antibiotics from within, and interference with the ability of antibiotics to penetrate into the bacterial cell and decrease susceptibility to traditional antibiotic therapies. Through these various actions, these plant-based compounds alter the structural characteristics of the bacterial membrane allowing the antibiotic to have greater ability to penetrate into the bacterium. They also inhibit efflux pump activity allowing for longer residence time of the antibiotics within the bacterium. In addition, all three compounds have been shown to inhibit the expression of genes involved in antimicrobial resistance, continuing to target the genetic basis of antimicrobial resistance.

The results of these mechanisms are a synergistic increase in the efficacy of antibiotics (especially for important drugs like meropenem and colistin, usually used as a last resort therapy). Herbal compounds may also increase the effectiveness of these antibiotics; therefore, they could help lower the dose needed, reduce the toxic side effects associated with them and delay the development of further resistant organisms. These observations suggest that herbal compounds could be used as adjuncts to antimicrobial therapy, particularly to help manage infections caused by multidrug-resistant and carbapenem-resistant organisms.

Even though promising results were obtained, these results have generally not been tested in patients; most of the data so far have been based on in vitro or early laboratory studies. As such, published results must be confirmed by a large clinical study to be useful to patients. Further, the data must be supported by carefully controlled doses with known pharmacokinetics, bioavailability, and no toxicity; determining these factors before they are included in routine treatment regimens. In conclusion, the use of plant-extracted bioactive compounds as an innovative way to treat *E. coli* that has developed resistance to carbapenems holds great promise. In addition, their ability to act at many different sites of resistance and to enhance the activity of

already-existing antibiotics means that these compounds may provide an alternative pathway to prolong the life of newly discovered antimicrobials. However, further research will be required before we can support their use clinically.

References

- Kim, H. R., & Eom, Y. B. (2021). Synergistic activity of equol and meropenem against carbapenem-resistant Escherichia coli. *Antibiotics*, 10(2), 161.
- Yi, L., Cao, M., Chen, X., Bai, Y., Wang, W., Wei, X., ... & Zhang, J. (2024). In vitro antimicrobial synergistic activity and the mechanism of the combination of naringenin and amikacin against antibiotic-resistant Escherichia coli. *Microorganisms*, 12(9), 1871.
- Jangid, H., Ray, A., & Kumar, G. (2025). BIOSYNTHESIS OF SILVER NANOPARTICLES BY SOIL-DERIVED *Rhizopus arrhizus* HGS2I2: ANTIBACTERIAL POTENTIAL AGAINST CARBAPENEM-RESISTANT Escherichia. Coli AND *Klebsiella pneumoniae*, AND ANTIOXIDANT PROPERTIES. *inflammation*, 18(3), 1717-1724.
- Shafiq, M., Yao, F., Bilal, H., Rahman, S. U., Zeng, M., Ali, I., ... & Jiao, X. (2022). synergistic activity of tetradrine and colistin against mcr-1-Harboring Escherichia coli. *Antibiotics*, 11(10), 1346.
- Saci, S., Msela, A., Saoudi, B., Sebbane, H., Trabelsi, L., Alam, M., ... & Houali, K. (2024). Assessment of antibacterial activity, modes of action, and synergistic effects of *Origanum vulgare* hydroethanolic extract with antibiotics against avian pathogenic Escherichia coli. *Fitoterapia*, 177, 106055.
- Alam, M., Bano, N., Ahmad, T., Sharangi, A. B., Upadhyay, T. K., Alraey, Y., ... & Saeed, M. (2022). Synergistic role of plant extracts and essential oils against multidrug resistance and gram-negative bacterial strains producing extended-spectrum β -lactamases. *Antibiotics*, 11(7), 855.
- Kong, J., Wang, Y., Han, Y., Zhou, H., Huang, Z., Zhang, X., ... & Zhou, T. (2023). Octominin: An antimicrobial peptide with antibacterial and anti-inflammatory activity against carbapenem-resistant Escherichia coli both in vitro and in vivo. *Journal of Global Antimicrobial Resistance*, 35, 172-180.
- S. Khadatare, "Enhancing Antibiotic Efficacy: Synergy Between Medicinal Plant Extracts and Conventional Antibiotics in Combating MDR Infections," International Journal For Multidisciplinary Research, vol. 7, no. 3, 2025. DOI: 10.36948/ijfmr.2025.v07i03.45945.
- "Saliem, A. H., & Abdulridha, R. N. (2022). Synergistic effect of Capparis spinosa fruits extract in comparison with ciprofloxacin against resistant E. Coli O157: H7. *University of Thi-Qar Journal of agricultural research*, 11(2), 242-256.
- "Acharjee, M., Zerin, N., Ishma, T., & Mahmud, M. R. (2023). In-vitro anti-bacterial activity of medicinal plants against Urinary Tract Infection (UTI) causing bacteria along with their synergistic effects with commercially available antibiotics. *New Microbes and New Infections*, 51, 101076.
- Achukwu, N. O., Enweani-Nwokelo, I. B., & Achukwu, P. U. (2024). Synergistic invitro antimicrobial activity of polyherbal combination of *Morinda lucida* fruit and *Pterocarpus santalinoides* seed against multi-drug resistant clinical bacterial isolates. *African Journal of Clinical & Experimental Microbiology*, 25(4).
- Mitra, S., Hodiwala, A. V. B., & Kar, H. (2024). Susceptibility and synergistic effects of guava plant extract and antimicrobial drugs on Escherichia coli. *Cureus*, 16(1).
- AL-siraj, S. S., Badr, J. M., & Abd El-Tawab, A. A. (2024). ANTIBACTERIAL POTENTIALS OF SOME PLANTS EXTRACTS AND THEIR COMBINATIONS AND THEIR SYNERGISTIC ACTIVITIES AGAINST MULTIDRUG RESISTANT BACTERIA. *Bulletin of Pharmaceutical Sciences Assiut University*, 47(2), 1431-1446.
- Al-Helli, N. F. A., & Salman, J. A. S. (2024). Synergistic effect of mannan extracted from *Saccharomyces cerevisiae* with antibiotics against multidrug resistant Escherichia coli. *Journal of the College of Basic Education*, 30(124), 25-42.
- Gore, M., Raut, D., & Aruna, K. (2021). Antimicrobial activity of *Pimenta dioica* (L.) merr. leaves and its synergistic activity with ampicillin against esbl producing clinical isolates. *Journal of microbiology, biotechnology and food sciences*, 11(1), e653-e653.
- Palma, F., Dell'Annunziata, F., Folliero, V., Foglia, F., Marca, R. D., Zannella, C., ... & Galdiero, M. (2023). Cupferron impairs the growth and virulence of Escherichia coli clinical isolates. *Journal of Applied Microbiology*, 134(10), lxad222.

17. Asadi, S., Nayeri-Fasaei, B., Zahraei-Salehi, T., Yahya-Rayat, R., Shams, N., & Sharifi, A. (2023). Antibacterial and anti-biofilm properties of carvacrol alone and in combination with cefixime against *Escherichia coli*. *BMC microbiology*, 23(1), 55.
18. Zahid, Z., Kyyaly, A., & Jubrail, J. (2025, September). Potentiation of Antibacterial Activity of Cefixime in Synergy with *Cirsium arvense* (L.) Scop. Against Resistant Bacterial Isolates. In *Medical Sciences Forum* (Vol. 35, No. 1, p. 9). MDPI.
19. Zeedan, G. S. G., Abdalhamed, A. M., & Ghazy, A. A. (2023). Strategies for prevention and control of multidrug-resistant Bacteria in ruminants. *World's Veterinary Journal*, 13(1), 45-56.
20. Ngaffo, C. M., Tankeo, S. B., Guefack, M. G. F., Wamba, B. E., Nayim, P., Bonsou, I. N., ... & Mbaveng, A. T. (2021). In vitro antibacterial and antibiotic-potentiation activities of five edible plant extracts and mode of action against several MDR Gram-negative phenotypes. *Investigational Medicinal Chemistry and Pharmacology*, 4(1), 00049.

Received : May 2025; Revised : June 2025; Accepted : July 2025



ENHANCEMENT IN FRUIT RETENTION, YIELD AND QUALITY ATTRIBUTES OF AONLA CV. NA-7 THROUGH THE FOLIAR APPLICATION OF BORON AND ZINC

Gopal Gond and V.K. Tripathi*

Department of Fruit Science, C.S. Azad University of Agriculture and Technology, Kanpur-208002 (U.P.)

*Corresponding Author Email-drvktripathicsa@gmail.com

Abstract

An experiment was carried out at the Nursery Farm Kalyanpur, Department of Fruit Science, C. S. Azad University of Agriculture and Technology, Kanpur (UP), during 2025 to enhance fruit retention, yield, and quality attributes of Aonla cv. NA-7 through the foliar application of boron and zinc, using nine treatments viz., T₁-Borax @0.4%, T₂-Borax@ 0.6%, T₃-ZnSO₄ @ 0.6%, T₄-ZnSO₄ @ 0.8%, T₅-Borax @ 0.4% + ZnSO₄ @ 0.6%, T₆-Borax @ 0.4% + ZnSO₄ @ 0.8%, T₇- Borax @ 0.6% + ZnSO₄ @ 0.6%, and T₈-Borax @ 0.6% + ZnSO₄ @ 0.8%, T₉-Control (Water spray) on twenty eight year old plants of aonla cv. NA-7 replicated thrice in Randomized Block Design. From the experiment, it is reported that the higher concentration of Borax @ 0.6% + ZnSO₄ @ 0.8% (T₈) significantly enhance fruit retention (33.88%), fruit yield (86.12kg/plant), fruit length (4.16cm), fruit width (4.98cm), fruit volume (31.24cc), fruit weight (36.55g), specific gravity (1.17g/cc), pulp weight (35.23g), pulp: stone ratio (26.71), T.S.S. (14.86°Brix), ascorbic acid (625.56mg/100g pulp), total sugars (12.90%) and with minimum stone weight (1.32g) reduced fruit drop (66.12%), and titratable acidity (1.65%) followed by Borax @ 0.4 % (T₁) as compared to control (T₉) treatments.

Keywords: Aonla, Zinc, Boron, Fruit retention, Yield and Quality attributes.

Introduction

Aonla (*Emblica officinalis* Gaertn.) belongs to the family Euphorbiaceae and is indigenous to tropical Southeast Asia, particularly in the Central and Southern parts of India (10). The Indian gooseberry fruit pulp is a key component of the powdered triphala and chyawanprash, which treat various disorders. The fruit is often used to make canned food (murabba), pickles, candies, jams, sauces, dried chips, and other products. Fruits contain water (81.2%), protein (0.5%), fat (0.1%), carbohydrates (14.1%), calcium (0.05%), phosphorus (0.02%), and iron (1.2%). It contains phenols, polyphenols, tannins, and alkaloids, and is a rich source of vitamin C (600 mg/100 g of pulp), more than guava and citrus fruits. Aonla is hardy and drought-resistant, with a deep root system and a deciduous appearance, since it sheds its

determinate shoots in February and March. It is successfully cultivated in a wide range of soils (Sodic and Saline), but a well-drained, fertile loamy soil is best. It can also do well even in moderately alkaline soil.

Climatic conditions in arid and semi-arid to dry, hot, and cold arid regions, and in rain-fed to rainfall areas, are suitable for aonla cultivation. Effective management of accessible natural resources is crucial to the success of organic agriculture in dry environments (17). The foliar application of micronutrients is vital in improving fruit set, productivity and quality in fruit plants. It also plays a beneficial role in recovery from nutritional and physiological disorders. Micronutrients play a significant role in regulating many physiological processes and are widely used in the fruit industry. In this context, many scientists have conducted studies on various

fruit crops, including aonla, highlighting a distinct emphasis on enhancing growth, flowering, fruiting, and yield quality. In India, widespread micronutrient deficiencies, especially of zinc and boron, in horticultural crops have been reported. Reduced yield and sometimes reduced quality, due to necrosis and fruit drop, are associated with boron and other micronutrient deficiencies. Indian gooseberry is a minor fruit crop, and no systematic research has been conducted to improve aonla fruit set, yield, and quality using boron (20). Boron also plays a vital role in ovule development, pollen tube growth, and fruit set. The intensity of damage caused by fruit dropping can be minimized by foliar application of nutrients which also help improve aonla yield and fruit quality. Similarly, zinc also plays an important role in the growth and development of fruits. It is one of the essential elements in the formation of chlorophyll and, hence, is helpful for photosynthetic activity. Zinc is a constituent of some enzymes, indole-3-acetic acid in plants, and is necessary for CO₂ evolution, carbohydrate utilization, and phosphorus metabolism, and is effective in retaining the maximum fruit percentage per panicle with increasing fruit size and fruit weight in many fruits (25). The combined application of boron and zinc at higher concentrations resulted in higher yields and better-quality aonla fruits (2). Keeping these in view, the present investigation was carried out to study the influence of boron and Zinc sulphate on aonla fruits in the plains of northern India.

Materials and Methods

The study was conducted with 28-year-old aonla trees planted in the Nursery Farm, Kalyanpur, Department of Fruit Science, C.S. Azad University of Agriculture and Technology, Kanpur (U.P.). Twenty-seven well-established, healthy, and uniform aonla trees of cv. NA-7 were selected for the experimental purpose. The experiment was laid out in a randomized block design with nine treatments viz., T₁-Borax @0.4%, T₂-Borax @ 0.6%, T₃-ZnSO₄ @ 0.6%, T₄-ZnSO₄ @ 0.8%, T₅-Borax @ 0.4% + ZnSO₄ @ 0.6%, T₆-Borax @ 0.4% + ZnSO₄ @ 0.8%, T₇-Borax @ 0.6% + ZnSO₄ @ 0.6%, and T₈-Borax @ 0.6% + ZnSO₄ @ 0.8%,

T₉-Control (Water spray), with three replications. Foliar applications of boron and zinc were made at the fruit-setting stage on August 13, 2025. Standard and uniform cultural operations, plant protection measures, and basal applications of manures and fertilizers were carried out according to the recommended schedule for aonla in all treatments. The observations were recorded for fruit drop, fruit retention, and yield, along with various other physico-chemical parameters of the fruits. Yield parameters comprised fruit weight (g), fruit diameter (cm), fruit length (cm), stone weight (g), pulp weight (g), fruit volume (ml), and yield/plant (kg). Fruit weight, length, and diameter were measured from randomly selected fruits in all four canopy directions. Quality parameters included total soluble solids (°Brix, by hand refractometer), ascorbic acid content (mg/100g pulp) was determined by the procedure detailed (AOAC, 1980), titratable acidity (%) by acid-alkali titration (AOAC, 1980), total sugars (%) estimated by Fehling's solution method and TSS: acid ratio computed as the quotient of TSS and acidity values (AOAC, 1980).

Results and Discussion

Fruit drop and retention per cent: During the present study, fruit drop was significantly reduced, and fruit retention was higher in plants treated with different micronutrient doses (Table 1). Reduced amount of fruit drop (66.12%) and higher fruit retention (33.88%) were recorded with the application of Borax @ 0.6% + ZnSO₄ @ 0.8%. In contrast, the maximum fruit drop (80.85%) and minimum fruit retention (19.15%) were recorded under the control. The reduction in fruit drop and increase in fruit retention percentage by the borax spray may be due to the indirect action of boron in auxin synthesis that delayed the formation of the abscission layer during the early stage of fruit development. Various workers, such as (8) in ber cv. Gola, (22) litchi cv. Purvi, (23), (25), and (2) in aonla cv. NA-7 has also reported reduced fruit drop with the highest fruit retention.

Fruit size (length and width)

In the present investigation, fruit size (length and width) was significantly increased by the foliar application of micronutrients in different

concentrations (Table 1). Plants treated with Borax @ 0.6% + ZnSO₄ @ 0.8% produced significantly higher fruit length (4.16 cm) and width (4.98 cm). Meanwhile, the plants kept under control produced fruits with shorter length (3.65 cm) and width (4.22 cm). This improvement in fruit size might be due to greater mobilization of food materials from their production sites to storage organs under the influence of applied micronutrients. Since boron plays an important role in nitrogen metabolism and other changes in fruits, it might have increased the fruit size. Thus, increases in fruit size with the application of Borax and ZnSO₄ might be due to the significant increase in cell division and cell enlargement induced by NAA treatment. The results obtained are in consistent with the findings of (18), (26), and (25) in Aonla cv. NA-7.

Fruit weight and volume

The weight and volume of fruits were significantly influenced by the foliar application of Borax and ZnSO₄ (Table 1). Significantly higher weight (36.55 g) and volume (31.24 cc) were recorded for fruits produced by plants treated with Borax @ 0.6% + ZnSO₄ @ 0.8%. It is also observed that with a reduction in micronutrient doses (boron and zinc), fruit weight and volume also decrease but remain significantly higher than in the control, which produced the minimum fruit weight (28.02 g) and volume (27.24 cc). This increase in fruit weight and volume might be due to improved nutrient and photosynthate supply in plants treated with foliar applications of Borax and ZnSO₄, which may have facilitated the rapid synthesis of metabolites, mainly carbohydrates, and their translocation to the fruits, resulting in relatively greater pulp content. Boron is an essential nutrient that is involved in several physiological processes in fruit, including the maintenance of cell wall integrity and to some extent, inhibiting the senescence of litchi fruits. These results are in accordance with the findings of (25) and (2) in aonla; (14) and (15) in litchi.

Fruit specific gravity

All treatments with different micronutrient doses showed significant effects on fruit specific gravity (Table 1). Fruits with a higher specific gravity (1.17 g/cc) were produced by plants treated

with Borax @ 0.6% + ZnSO₄ @ 0.8%. In contrast, fruits with the lowest specific gravity (1.03g/cc) were produced from untreated control plants. This increase in specific gravity with higher concentration of Borax and ZnSO₄ might be due to an increased fruit pulp and decreased stone weight. These findings are consistent with those of (18), (19), and (2) in aonla.

Fruit pulp weight

Fruit pulp weight was significantly increased by foliar application of micronutrients at different doses (Table 1). Significantly more weight of pulp (35.23 g) was recorded in fruits, which were harvested from the plants treated with the Borax @ 0.6% + ZnSO₄ @ 0.8%, closely followed by 32.85 g of pulp in fruits produced from with Borax @ 0.6% + ZnSO₄ @ 0.6% treatment, whereas fruits with the minimum pulp weight (26.15 g) was recorded in fruits produced from untreated (control) plants. This increase in fruit pulp weight with zinc application is due to zinc's direct involvement in accelerating biochemical activity in plant parts and in the translocation of mineral nutrients during fruit development. These findings are consistent with those of (3) in strawberry, (27) in aonla, and (9) in ber.

Fruit stone weight and pulp: stone ratio

A reduction in stone weight with an increased pulp-to-stone ratio was observed with the application of different doses of micronutrients compared to the control (Table 2). The minimum stone weight (1.32 g) and higher pulp: stone ratio (26.71) were recorded in the fruits produced from the plants treated with Borax @ 0.6% + ZnSO₄ @ 0.8%, whereas the maximum weight of stone (1.87 g) and minimum pulp: stone ratio (13.98) were recorded in fruits produced from plants kept as untreated control (T₀). This improvement in the pulp-to-stone ratio might be due to greater accumulation of food substances in elongated cells and in the intercellular spaces of the mesocarp. These results have been supported by the findings of (27), (23), (6) and (19) in aonla.

Fruit yield (kg/plant)

The foliar application of different doses of micronutrient treatments significantly influenced the fruit yield per plant as compared to the control (Fig. 1). Foliar application of increased

concentration of borax and ZnSO_4 significantly improved the yield, resulting in 86.12 kg/plant, which was recorded under higher concentration of Borax @ 0.6% + ZnSO_4 @ 0.8%. In contrast, the lowest fruit yield of 71.74 kg/plant was recorded in plants kept under control (T_9). This significant increase in fruit yield in plants treated with the combined application of Borax and ZnSO_4 might be due to their direct role in the synthesis of photosynthates, which increase fruit size, weight, and volume, ultimately increasing in the yield. Results on fruit yield are in agreement with those of (16) for guava and (21) for strawberry cv. Chandler; (26), (28), (5) in phalsa, (2022), and (7) in cape gooseberry.

Total Soluble Solids and Total sugars

The maximum total soluble solids and total sugars content (14.86°Brix and 12.90%, respectively) were recorded in the fruits produced from the plants treated with the foliar application of Borax @ 0.6% + ZnSO_4 @ 0.8%, followed by 14.78 °Brix of T.S.S. and 12.80% of total sugars, respectively were recorded from the plants treated with the application of Borax @ 0.6% + ZnSO_4 @ 0.6% (T_7). In contrast, the minimum T.S.S. (12.87 °Brix) and total sugar content (11.20%) were recorded in the control (untreated) plants (Table 2). This increase in T.S.S. and total sugars with boron and zinc sulphate application might be due to these micronutrients (boron and zinc) being involved in photosynthesis, leading to greater accumulation of oligosaccharides and polysaccharides. Besides this, they also regulate enzymatic activity and metabolize carbohydrates into simple sugars. (28) in phalsa and (4) in litchi also recorded an increase in total sugar and T.S.S. content with higher concentrations of boric acid treatments. These results conform with the findings of (11), (19) in aonla, (7) in cape

gooseberry, (14) and (13) in mango cv. Amrapali.

Titratable acidity

Titratable acidity content in fresh fruits was significantly decreased by the application of different doses of micronutrients. The minimum titratable acidity (1.65%) was found in the fruits produced from the plants treated with Borax @ 0.4%, closely followed by titratable acidity (1.68%) from ZnSO_4 (0.8%) application. In contrast, the maximum content (1.98 %) was recorded in fruits produced from untreated control plants (Table 2). This decrease in the titratable acidity content of fruits might be due to increased T.S.S. and total sugars. Acids under the influence of chemicals might have been converted into sugars and their derivatives by reactions that reverse the glycolytic pathway, used as substrates for respiration, or both. The results support the findings of (18) in aonla cv. Banarasi, (24), (11) and (19) in aonla.

Ascorbic acid

The maximum ascorbic acid content (625.56 mg/100g pulp) was recorded in the fruits produced from the plants treated with Borax @ 0.6% + ZnSO_4 @ 0.8% closely followed by 618.53 mg/100g pulp and 614.87 mg/100g pulp with Borax @ 0.6% + ZnSO_4 @ 0.6% (T_7) and Borax @ 0.4% + ZnSO_4 @ 0.8% (T_6), respectively, which remained higher than rest of the other treatments. In contrast, the lowest ascorbic acid content (583.56 mg/100g pulp) was observed in the control treatment. This increase in ascorbic acid content with the application of borax and zinc is due to uninterrupted synthesis of its precursor, such as glucose-6-phosphate, during the conversion of starch into various sugars, and to a low oxidation rate. The findings are consistent with the results of (18), (14), (6), (23), (12) in guava, and (14) in litchi.

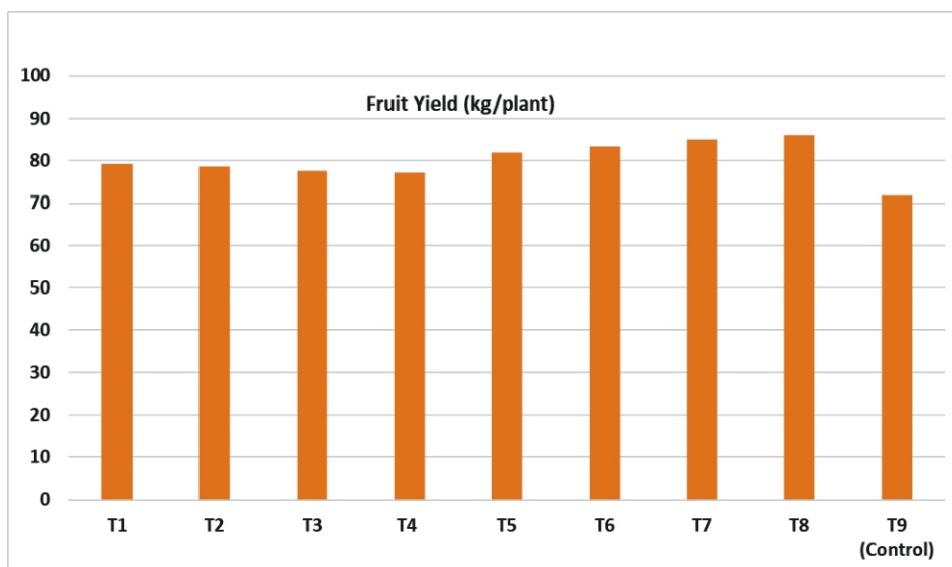
Table 1. Effect of foliar application of Boron and Zinc on fruiting, yield and physical attributes of Aonla cv. NA-7

Treatments	Fruit drop (%)	Fruit retention (%)	Fruit length (cm)	Fruit width (cm)	Fruit weight (g)	Fruit volume (cc)	Pulp weight (g)	Stone weight (g)	Fruit yield (kg/plant)
T1 -Borax @ 0.4%	71.24	28.76	4.01	4.62	32.08	29.65	30.43	1.65	79.24
T2-Borax @ 0.6%	71.65	28.35	4.03	4.65	32.12	29.42	30.53	1.59	78.63
T3-ZnSO ₄ @ 0.6%	72.21	27.79	3.93	4.51	30.32	28.35	28.79	1.54	77.52
T4-ZnSO ₄ @ 0.8%	72.55	27.45	3.98	4.57	31.85	30.12	30.29	1.56	77.14

T5-Borax @ 0.4 % + ZnSO ₄ @ 0.6 %	70.38	29.62	4.06	4.68	32.25	29.27	30.77	1.48	81.87
T6-Borax @ 0.4 % + ZnSO ₄ @ 0.8%	69.54	30.46	4.1	4.79	33.32	29.85	31.91	1.41	83.32
T7-Borax @ 0.6 % + ZnSO ₄ @ 0.6%	68.83	31.17	4.14	4.85	34.21	30.1	32.85	1.36	84.96
T8-Borax @ 0.6 % + ZnSO ₄ @ 0.8 %	66.12	33.88	4.16	4.98	36.55	31.24	35.23	1.32	86.12
T9-Control	80.85	19.15	3.65	4.22	28.02	27.24	26.15	1.87	71.74
S.E. (m.) ±	0.79	0.79	0.05	0.07	0.41	0.36	0.42	0.03	0.95
C.D. at 5% level	2.38	2.38	0.16	0.21	1.24	1.07	1.27	0.08	2.86

Table 2. Effect of foliar application of Boron and Zinc sulphate on fruit quality attributes of aonla cv. NA-7

Treatments	Specific gravity (g/cc)	Pulp: stone ratio (%)	T.S.S. (o Brix)\	Titrateable acidity (%)	Ascorbic acid (mg/100g)	Total sugar (%)
T1 -Borax @ 0.4%	1.08	18.47	13.78	1.65	607.53	12.4
T2-Borax @ 0.6%	1.09	19.21	14.16	1.77	610.28	12.8
T3-ZnSO ₄ @ 0.6%	1.07	18.7	13.75	1.72	602.27	11.8
T4-ZnSO ₄ @ 0.8%	1.06	19.45	13.87	1.68	598.74	11.9
T5-Borax @ 0.4 % + ZnSO ₄ @ 0.6 %	1.1	20.8	14.62	1.79	609.56	12.7
T6-Borax @ 0.4 % + ZnSO ₄ @ 0.8%	1.12	22.66	14.65	1.86	614.87	12.8
T7-Borax @ 0.6 % + ZnSO ₄ @ 0.6%	1.14	24.16	14.78	1.82	618.53	12.8
T8-Borax @ 0.6 % + ZnSO ₄ @ 0.8 %	1.17	26.71	14.86	1.79	625.56	12.9
T9-Control	1.03	13.98	12.87	1.98	583.56	11.2
SE (m) ±	0.02	0.52	0.24	0.03	0.18	0.18
C.D. at 5% level	0.06	1.58	0.72	0.08	0.55	0.54



CONCLUSION

From the results obtained during present investigation, it can safely be concluded that plants treated with Borax @ 0.6% + ZnSO₄ @ 0.8% have significantly reduced fruit drop with increased fruit retention along with increase in physical parameters like fruit size (length and width), fruit weight, fruit volume, fruit specific gravity, pulp weight, fruit yield (kg/plant), as well as chemical attributes like T.S.S., total sugars and ascorbic acid content of fruits with reduced stone weight and titratable acidity. Based on these results, it is recommended that, to obtain higher yields of quality aonla fruits, plants should be sprayed with a combination of Borax @ 0.6% + ZnSO₄ @ 0.8% in the north Indian plains of Uttar Pradesh.

References

1. AOAC (1980). Official Methods of Analysis. Association of Official Analytical Chemists, Washington DC.
2. Arya, K., Tripathi, V.K. and Awasthi, M. (2024). Impact of foliar application of plant bio-regulator and micronutrients on fruit retention, yield and quality attributes of Aonla. *Annals of Horticulture*, **17**(1&2): 51-58.
3. Chaturvedi, O.P., Singh, A.K., Tripathi, V.K. and Dixit, A.K. (2005). Effect of zinc and iron on growth, yield and quality of Strawberry cv. Chandler. *Acta Horticulturae*, **696**, 237-240.
4. Gupta, A., Tripathi, V.K. and Shukla, J.K. (2022). Influence of ga3, zinc and boron on fruit drop, yield and quality of litchi (*Litchi chinensis* Sonn.). *Biological Forum-An International Journal*, **14**(3): 1079-1083.
5. Kashyap, S., Tripathi, V. K., Kumar, V. and Kumar, A. 2024. Effect of gibberellic acid and boron alone and in combination on vegetative growth, fruiting and yield of phalsa. *Annals of Horticulture*, **16**(2): 123–129.
6. Kumar, A., Tripathi, V.K., Dubey, V., Katiyar, N.K. and Tiwari, P. (2017). Influence of foliar application of calcium, zinc and boron on fruit drop, yield and quality attributes of aonla (*Emblca officinalis*) cv. NA-7. *Research on Crops*, **18** (1): 91-97.
7. Kumar, R., and Tripathi, V. K. (2025). Influence on yield and quality attributes of cape gooseberry (*Physalis peruviana* L.) with the application of nitrogen, zinc and boron. *Indian Journal of Horticulture*, **82**(4), 440–447.
8. Kumar, S. and Shukla, A.K. (2010). Improvement of old cv. Gola orchard through budding and micronutrients (Boron and zinc) management. *Indian Journal of Horticulture*, **67**(3): 322-327.
9. Meena, V.S., Yadav, P.K. and Singh, O.P. (2008). Yields attributes of Ber (*Zizyphus mauritiana* Lank.) cv. Gola as influenced by foliar application of ferrous sulphate and borax. *Agricultural Science Digest*, **28**(3): 219-221.
10. Morton, J.F. (1960). Chemical analysis of the emblica (*Phyllanthus emblica* L.) and its potential as a food source. *Economic Botany*, **14**(2): 119-128.
11. Mohit, Tripathi, V.K., Pandey, S. and Mishra, A. 2023. Impact of foliar application of NAA, boron and zinc on fruit drop, yield and quality attributes of Aonla (*Emblca officinalis* Gaertn.) cv. NA-7. *Progressive Horticulture*, **55**(1): 30-35.
12. Raghava, M. and Tiwari, J.P. (1998). Effect of boron on growth, quality, and shelf-life of fruits of guava (*Psidium guajava* L.) cv. Saradar. *Progressive Horticulture*, **30** (1/2): 68-72.
13. Rajak, U., Rani, R., Kumar, R., Mandal, B.K. and Prasad, K.K. (2010). Effect of foliar application of urea, borax and zinc on flowering, fruiting, and fruit quality of mango (*Mangifera indica* L.) cv. Amrapali. *Environment and Ecology*, **28**(3): 1668-1671.
14. Rawat, A. S. and Tripathi, V.K. 2024. Effects of naphthalene acetic acid and zinc sulphate on fruiting behaviour and quality of litchi (*Litchi chinensis* Sonn.) cv. Rose Scented. *Asian J. Soil Sci. Plant Nutr.*, **10**(1): 101–108.
15. Saraswat, N.K., Pandey, U.N. and Tripathi, V. K. (2006). Influence of NAA and Zinc sulphate on fruit drop, cracking, fruit size, yield and quality of litchi cv. Calcuttia. *Journal of Asian Horticulture*, **2**(4): 255- 259.
16. Sharma, R.K., Kumar, R. and Thakur, S. (1993). Effect of foliar feeding of potassium, boron and zinc on yield and quality of guava. *Indian Journal of Horticulture*, **48** (4):312-314.
17. Shukla, H.S. and Verma, G. (2004). Effect of foliar application of NAA, potassium sulphate and zinc sulphate on fruiting and fruit characteristics of ber (*Ziziphus mauritana* Lamk.) cv. Banarasi Karaka. In Proceeding of International Seminar on Rec. Trend in Hi-tech and PHT, Organized by C.S.A.U.A.&T., Kanpur from Feb. 4-6: 167-168.
18. Shukla, H.S., Kumar, V. and Tripathi, V.K. (2011). Effect of gibberellic acid and boron on development and quality of aonla fruits 'Banarasi'. *Acta Horticulture*, **890**: 375-380.

19. Singh, A.K., Singh, P.P., Singh, S., Bhargava, R. and Makwana, P. (2016). Variability in morphological and physico-chemical traits of aonla (*Emblica officinalis*) genotypes collected from north-eastern region of India. *Indian Journal of Agricultural Sciences*, **86** (8): 992–997.
20. Singh, J.N., Singh, S.P. and Bahadur, L. (1989). Determination of maturity standards of aonla (*Emblica officinalis* Gaertn.) cultivars under eastern conditions of Uttar Pradesh. *Haryana Journal of Horticultural Sciences*, **18**: 216–20.
21. Singh, V.K. and Tripathi, V.K. (2010). Efficacy of GA₃, boric acid and zinc sulphate on growth, flowering, yield and quality of strawberry cv. Chandler. *Progressive Agriculture-An International Journal*, **10** (2): 345-348.
22. Sinha, A.K., Singh, C. and Jain, B.P. (1999). Effect of plant growth substance and micronutrients on fruit set, fruit drop, fruit retention and cracking of litchi cv. Purvi. *Indian Journal of Horticulture*, **56**(4):309-311.
23. Tiwari, P., Tripathi, V.K. and Singh, A. (2017). Effect of foliar application of plant bio-regulators and micronutrients on fruit retention, yield and quality attribute of aonla. *Progressive Research-An International Journal*, **12**(4):2565-2568.
24. Tripathi, V.K. and Shukla, H.S. (2011). Influence of calcium chloride and calcium nitrate on physico-chemical composition of aonla 'Banarasi'. *Acta Horticulturae*, **890**: 371-374.
25. Tripathi, V.K. and Viveka Nand (2022). Effect of foliar application of Boron, Zinc and NAA on fruit retention, yield and quality attributes of aonla. *Progressive Horticulture*, **54** (1):76-81.
26. Tripathi, V.K., Pandey, S.S., Kumar, A., Dubey, V. and Tiwari, P. (2018). Influence of foliar application of gibberellic acid, calcium and boron on fruit drop, yield and quality attributes of aonla (*Emblica officinalis*) cv. NA-7. *Indian Journal of Agricultural Sciences*, **88** (11): 1784–88.
27. Verma, R.S., Singh, P.C. and Chaturvedi, O.P. (2008). Effect of foliar spray of zinc and boron on the physical parameters of aonla fruit cv. Banarasi. *The Asian Journal of Horticulture*, **3**(2): 344-345.
28. Yadav, R. K. and Tripathi, V. K. 2025. Effect of pruning intensity and foliar nutrition on growth, yield and quality of Phalsa cv. Sharbati. *Indian Journal of Horticulture*, **82**(1): 49-54.

Received : May 2025; Revised : June 2025; Accepted : July 2025



ECONOMIC ASSESSMENT OF AGROFORESTRY SYSTEMS IN SAHARANPUR DISTRICT OF WESTERN UTTAR PRADESH

Divya Yadav¹, Charan Singh¹ and Harikesh Singh^{2*}

¹Forest Research Institute Deemed University, Dehradun;

²GMV, Rampur Maniharan, Saharanpur,

*Present Address: Principal, Ganna Utapadak (PG) College, Baheri (UP)

*Corresponding Author Email-divyaext@gmail.com

Abstract

Agroforestry has emerged as a sustainable land-use option for enhancing farm income while augmenting domestic wood supply. The present study evaluated the economic viability of poplar-based agroforestry systems in Saharanpur district of Western Uttar Pradesh, focusing on boundary and block plantations integrated with wheat and paddy crops. The study covered five tehsils and involved data collection from 150 agroforestry farms using stratified random sampling. Economic analysis was conducted over a six-year rotation using net present value (NPV) and benefit–cost ratio (BCR) at a 10% discount rate.

Results indicated widespread adoption of agroforestry dominated by *Populus deltoides* (G-48). Poplar-based boundary plantation with wheat and paddy proved more profitable than block plantation, generating a net return of `19.23 lakh ha<sup>-1</sup>, an NPV of `12.45 lakh ha⁻¹, and a BCR of 3.15:1, compared to a BCR of 2.89:1 under block plantation. The study concludes that boundary plantation of poplar with wheat and paddy is the most economically viable system in the district. Besides improving farm profitability, this system contributes significantly to meeting industrial wood demand and can be replicated in regions with similar agro-climatic and topographical conditions across the Indo-Gangetic Plains.

Key words: Agroforestry, *Populus deltoids*, Wheat, Paddy, Benefit–cost ratio, Net present value, Boundary and block plantation

Introduction

India's wood and wood-based products sector continues to face a substantial domestic supply deficit, leading to a growing dependence on imports of both raw materials-such as logs and timber-and processed products, including plywood and medium-density fibreboard (MDF), to meet the rising demand from the furniture and construction industries (13). In this context, agroforestry systems in Western Uttar Pradesh, particularly in Saharanpur district, have emerged as a critical and sustainable source of industrial wood, predominantly through the cultivation of poplar and other fast-growing tree species.

To address raw material shortages and control production costs, wood-based industries have increasingly strengthened market linkages with local farmers through agroforestry partnerships. These collaborations have facilitated the adoption of technology-intensive tree plantations on private agricultural lands. A study by TERI (2000) reported that the adoption level of agroforestry in Saharanpur district exceeded 90 per cent, indicating its widespread acceptance among farmers.

Four major agroforestry systems have been identified in the district, namely agri-silviculture, agri-horticulture, agri-silvi-horticulture, and

silvi-horticulture. Among these, the agri-silviculture system is the most prevalent, with wheat and paddy being the dominant intercrops grown alongside poplar plantations. Agroforestry systems offer a combination of short-term and long-term benefits to farm households by enhancing livelihood security and promoting sustainable resource use. However, the adoption of such systems depends not only on the biological compatibility of agricultural crops and tree species but also on their economic viability and profitability.

In this context, the present study aims to assess the economic performance and sustainability of different agroforestry systems practiced in Saharanpur district of Western Uttar Pradesh. The findings are expected to provide valuable insights for farmers, policymakers, and other stakeholders, enabling informed decision-making and promoting sustainable farming systems where diversified cropping patterns, wood demand, and farmers' livelihoods coexist in a mutually reinforcing manner.

Materials and Methods

Study area and administrative setup

The study was conducted in Saharanpur district, located in the north-western part of Uttar Pradesh, India. The district comprises five tehsils-Behat, Deoband, Nakur, Rampur Maniharan, and Saharanpur-and 11 development blocks. Geographically, it lies between 29°34'-30°23' N latitude and 77°05'-77°58' E longitude, covering an area of 3,689 km² with an average elevation of 284 m above mean sea level. Saharanpur forms the northernmost part of the Doab region between the Ganga and Yamuna rivers and is largely plain, except for the Shiwalik hills in the northern and northeastern parts. The Yamuna is the major river, along with Solani, Hindon, Ratmau, and Nagdev.

Demographic profile

According to the Census of India (2011), Saharanpur district has a population of 3.47 million, of which 69.23% resides in rural areas and 30.77% in urban areas, with an average population density of 940 person/km².

Soil, climate, and rainfall

The district falls within the western Indo-Gangetic Plain and is characterized by alluvial soils, predominantly sandy to sandy loam in texture. These soils are highly permeable with low water-holding capacity and are prone to erosion. The climate is tropical sub-humid, representing a transitional zone between the dry Punjab plains and the humid Middle Ganga plains. Annual rainfall varies from 498 to 1,566 mm, while temperatures range from 6.6°C in winter to 45.0°C in summer, with a mean annual temperature of about 23.3°C.

Land holding and Land use pattern

The average landholding size in the district is 1.27 ha, indicating the dominance of small and marginal farmers (Statistical Abstract, U.P., 2013). More than 75% of the total reporting area is under net sown area. The gross cropped area is 4, 14,297 ha, reflecting intensive agricultural practices in the region.

Irrigation

The irrigation coverage has been much higher in Saharanpur district to the extent that 93.40 % of the net area sown in the district has been brought under irrigation. In this way most of the cultivated land is irrigated in the district. Tube wells and canals are the major source of irrigation in the district (Statistical Abstract, U.P., 2013).

Pilot study

A pilot study was conducted on 250 respondents (50 per tehsil) to adopt the appropriate methodology, selecting sample size & location, cropping pattern, agroforestry practices and other information to carry out research on objectives. Selection of respondents was done by using snow-ball sampling method also known as network, chain or reputational sampling. These respondents were not being included in the final sample.

Sampling procedure

A stratified random sampling technique was adopted for the study, with the district divided into five strata based on tehsils-Behat, Deoband, Nakur, Rampur Maniharan, and Saharanpur-representing different topographic conditions. Three villages were

randomly selected from each stratum using Census 2011 village numbers, resulting in a total of 15 sample villages. From each village, agroforestry farms were randomly selected for both boundary and block poplar plantations, yielding a total of 150 sample farms. Crop yield estimation for wheat and paddy was carried out using five randomly placed $1 \times 1 \text{ m}^2$ quadrats per farm under shaded and unshaded conditions, while tree growth parameters were recorded from 20 randomly selected poplar trees per farm.

Economics of Existing Agroforestry Systems

All inputs and outputs of agroforestry practices were recorded on a per-acre basis and converted to a per-hectare basis, as farmers commonly divided their land into quadrats, with an average quadrat size of one acre.

Farmer Estimates

Crop production data were collected through structured farmer interviews, wherein farmers reported yields at plot, field, or farm level. Costs and returns were estimated on a per-hectare basis for poplar-based agroforestry systems over a six-year rotation, including all tree establishment and management inputs up to poplar harvesting.

Measurement of Cost and Benefit Variables

(I) Input Cost

Input costs for poplar-based boundary and block agroforestry systems with wheat and paddy were estimated for a six-year period (2011-16) across all tehsils of Saharanpur district. Input costs included: Cost A, covering establishment and management of poplar trees; Cost B, comprising operational and management expenses for wheat; Cost C, including all cultivation costs for paddy; and Cost D, representing the rental value of owned land. All cost components were combined and converted to a per-hectare basis to estimate total production costs for the agroforestry systems.

(II) Output / Benefit / Return

Returns from poplar-based agroforestry systems were estimated for a six-year period. Output A included income from poplar in the form of sale of pruned wood and auction value of standing trees. Output B comprised returns from wheat grain and straw yields, while Output C included per-hectare returns from paddy grain and straw yields recorded from agroforestry farms.

Economic Evaluation

Economic evaluation was carried out to assess the financial feasibility of tree-crop based agroforestry systems by analysing the flow of costs and returns over the crop life cycle. The productivity of invested capital was examined using discounted cash flow measures, including the discount factor, discounted rate of interest, net present value (NPV), and benefit-cost ratio (BCR), to evaluate the economic viability of existing agroforestry practices.

- (i) **Discount factor:** A discount factor is a decimal number multiplied by a cash flow value to discount it back to the present value.

The discount factor formula is:

$$\text{Discount Factor} = 1 / (1 + \text{Discount Rate})^{\text{Period Number}}$$

- (ii) **Discounted rate of interest:** The discount rate also refers to the interest rate used in discounted cash flow analysis to determine the present value of future cash flows. For this study, ten percent discounted rate of interest is calculated to obtain discounted cash inflows and outflows

- (iii) **Net present value** Net present value is the difference between the present value of cash inflows and the present value of cash outflows that occur as a result of undertaking an investment. It may be positive, zero or negative. If the NPV is higher for agroforestry than for all feasible alternatives, it is potentially adoptable (10).

$$\text{Net present value} = \sum_{t=0}^n \frac{R_t - C_t}{(1+i)^t}$$

Where, R_t = total return/benefits for a period of t year

C_t = total cost for a period of t years

i = discount rate

t = time period

n = total number of periods

- (iv) **Benefit cost ratio (BCR):** The BCR is calculated by dividing the total discounted value of the benefits by the total discounted value of the costs.

$$\text{Benefit cost ratio} = \frac{\sum_{t=0}^n \frac{R_t}{(1+i)^t}}{\sum_{t=0}^n \frac{C_t}{(1+i)^t}}$$

Where, R_t = total return/benefits for a period of t year

C_t = total cost for a period of t years

i = discount rate

t = time period

n = total number of periods

Statistical analysis

Two independent samples t-test was used to test the null hypothesis by Statistical Programmes version 8 (STPR8). Mean, percentage, frequency and percent difference were calculated by using MS-Excel.

Results and Discussion

From the pilot study, it was observed that agroforestry has been adopted on large scale in the all tehsils of Saharanpur (S. Pur) district. (12) has also reported that 93.02 % area with respect to tree outside forest comes under agroforestry in the district. The major tree species planted under agroforestry were *Populus deltoides* (G-48) (92.22 %), *Eucalyptus* sp. (7.04 %) and others (0.74 %). Four agroforestry systems were identified in the district, among which agri-silviculture was the most prevalent (92.39%). Wheat (rabi) and paddy (kharif) were the dominant intercrops grown with poplar plantations. Most farmers (75.19%) practiced boundary plantation of *Populus deltoides* with wheat-paddy rotation, while 24.81% followed block plantation, which was more common in Deoband tehsil. The preference for poplar was mainly attributed to its short rotation period, assured market, and easy availability of planting material.

Based on the pilot survey findings, poplar-based agroforestry systems (boundary and block plantations) integrated with wheat and paddy were identified as the predominant practice in Saharanpur district. Accordingly, the present study focused exclusively on these systems for further economic analysis.

Economics of agroforestry systems practiced by the farmers in all tehsils of the district

Wheat and paddy under poplar based boundary plantation practice

In this agroforestry practice, *Populus*

deltoides (G-48) was planted along the farm boundary with wheat and paddy crops were taken alternative season up to the sixth year. The input cost of A (poplar trees), B (wheat crop), C (paddy crop) and D (rental value of land) of six years (2011-16) wheat and paddy under poplar-based boundary plantation agroforestry practice in all the tehsil are elaborated in table 1.

Input Cost Analysis

Cost A (Poplar trees): The average per-hectare input cost of poplar was highest in Nakur tehsil (₹23,900) and lowest in Behat (₹20,060), mainly due to lower labour costs in Behat. In all tehsils, costs were highest in the first year because of expenses on planting material (ETP) and establishment, while only pruning costs were incurred from the second to fifth year.

Cost B (Wheat crop): Wheat cultivation costs included seed, tillage, irrigation, fertilizers, pesticides, harvesting, and labour. Tillage operations accounted for the highest expenditure, whereas pesticide costs were minimal. Total wheat input cost was highest in Saharanpur tehsil (₹2,41,199) due to higher labour wages, and lowest in Behat (₹2,07,054), with other tehsils showing comparable costs.

Cost C (Paddy crop): Similar to wheat, tillage operations formed the major cost component for paddy. The highest input cost was recorded in Saharanpur (₹2,53,097.50), followed by Rampur Maniharan, Deoband, Nakur, and Behat. Paddy input costs increased progressively from the first to the sixth year across all tehsils.

Cost D (Rental value of land): Land rental value increased gradually over the six-year period in all tehsils, with the lowest cumulative value in Behat (₹2,47,500) and ₹2,92,500 in the remaining tehsils.

Overall, the total cumulative input cost was highest in Saharanpur tehsil (₹8,08,856.50) and lowest in Behat (₹6,90,366.50), reflecting differences in labour and land rental costs across the district.

Table 1. All input cost of boundary plantation poplar with wheat and paddy in all tehsils of Saharanpur district

(A) Operational and management cost of poplar trees (2011-16)					
Particulars	Behat	Deoband	Nakur	Rampur Maniharan	Saharanpur
ETP cost (520 ETP @Rs. 20/ETP)	10400	10400	10400	10400	10400
Transportation	1600	1000	1800	1800	1000
Digging and planting cost (@Rs.2.5/ETP)	1300	1820	1820	2080	1820
Pruning	6760	9620	9880	8840	8840
Total	20060	22840	23900	23120	22060
(B) Operational and management cost of wheat (2011-16)					
Seed (120 kg/ha)	16800	16800	16800	16800	16800
Tillage operations	46000	57000	62200	60200	60000
Irrigation (5 times)	21375	21375	24000	18750	21375
Fertilizers					
Urea @ 300 kg/ha	11400	11400	11400	11400	11400
DAP @150 kg/ha	17400	17400	17400	17400	17400
FYM @240 q/ha	3840	6720	5280	7920	9360
Pesticides (herbicide+fungicide)	1900	6100	3525	3750	3525
Transportation (2 trolleys)	13600	13600	13600	13600	13600
Harvesting @300 kg/ha	21320	24600	24600	24600	24600
Threshing @8 % of total grain yield	31279	31279	31279	31279	31279
Labour cost (Sowing, pesticide spray, irrigation, application of fertilizer and threshing etc)	22140	25740	25740	28080	31860
Total (Rs.)	207054	232014	235824	233779	241199
(C) Operational and management cost of paddy (2011-16)					
Seed (15 kg/ha)	11700	11700	11700	11700	11700
Nursery expenses	6700	9450	8500	10300	13500
Puddling (Puddling one round+Harrowing one round)	46000	57000	57000	63200	59000
Transplanting	20700	20700	20700	24450	25000
Irrigation (5 times)	23000	21375	24000	18750	21375
Fertilizers					
Urea @375 kg/ha	14250	14250	14250	14250	14250
DAP @150 kg/ha	17400	17400	17400	17400	17400
Pesticides(herbicide+fungicide+insecticide)	5000	7275	4000	8500	10020
Transportation	1402.5	1402.5	1402.5	1402.5	1402.5
Harvesting+Threshing	45000	45000	45000	45000	45000
Labour cost	24600	28600	28600	31200	34450
Total	215752.5	234152.5	232552.5	246152.5	253097.5
(D) Rental value of land	247500	292500	292500	292500	292500
Total input (A+B+C+D)	690366.5	781506.5	784776.5	795551.5	808856.5

(II) Output /return

Table 2 showed the output of A (benefit from trees), B (benefit from wheat) and C (benefit from paddy) of wheat and paddy under poplar plantation practice in all the tehsils of Saharanpur district.

Output A (benefit from trees)

From the result, it was revealed that in this practice, total benefit from poplar plantation was highest estimated as Rs. 1,568,960 in Rampur Maniharan, while the lowest was recorded as Rs. 12, 56,960 in Behat tehsil. The sale value of pruned wood was starts from third year and ends at fifth year in all the tehsils. In sixth year,

average 520 trees/ha of poplar were auctioned at the rate of Rs. 600 to 750/q in different tehsils.

Output B (benefit from wheat)

The total maximum benefit from per hectare wheat grain and straw yield was estimated as Rs. 6, 71,623.80 in Rampur Maniharan, while lowest recorded in Saharanpur (Rs. 5, 42,740.81) tehsil. The maximum benefit was occurred in fourth year and minimum was in first year.

Output C (benefit from paddy)

The highest output cost of C of paddy grain and straw yield was recorded in Saharanpur (Rs. 6,64,888.82) tehsil, while lowest was estimated in Behat (Rs. 5,86,414) tehsil in the year of 2011-16.

Table 2. Output of boundary plantation poplar with wheat and paddy in all tehsil of Saharanpur district

(A) Benefit from trees (2011-16)					
Particulars	Behat	Deoband	Nakur	Rampur Maniharan	Saharanpur
Sale of pruned wood (used as fuel) @ Rs. 100/q	8960	8960	8960.00	8960.00	8960.00
Auction of standing trees (520 trees @ Rs. 600/q) (average green timber weight 400 kg/tree at 6 years of age)	1248000	1456000	1393600.00	1560000.00	1497600.00
Total	1256960	1464960	1402560.00	1568960.00	1506560.00
(B) Benefit from wheat (2011-16)					
Grains	372468	407447.70	387651.70	411219.00	281030.63
Straw	237296.4	222483.30	244797.30	260404.80	261710.18
Total	609764.4	629931.00	632449.00	671623.80	542740.81
(C) Benefit from paddy (2011-16)					
Grains	443580.1	497797	482175	503072.5	508285.29
Straw	142833.9	152996.3	146851.7	156449	156603.53
Total	586414	650793.3	629026.8	659521.6	664888.82
Total output (A+B+C)	2453138	2745684.27	2664036	2900105.38	2714189.63

Wheat and paddy under poplar based block plantation practice

Table 3 described the total input cost of wheat and paddy under poplar block plantation in all the tehsils of the Saharanpur district.

(I) Input cost

Cost A (operational and management cost of poplar trees)

The total operational and management cost (cost of ETP, transportation, digging and planting and pruning) of poplar trees was higher (Rs. 28,362.50 to 23,787.50) in block plantation as compare to wheat and paddy under poplar based

boundary plantation. The number of block entire transplanting (625 ETP) was more than the boundary plantation (520 ETP).

In Behat tehsil, the input cost for poplar block plantation is lowest (Rs. 23,787.50), while in other tehsils; the input cost was almost similar within the range of Rs. 26,312.50 to 28,362.50.

Cost B (operational and management cost of wheat crop)

Among the different particulars of raising wheat crop, the maximum input cost was recorded for tillage operations (one round tiller and one round harrowing), while minimum was for pesticides application in all the tehsils. The

highest cost of tillage operations was Rs. 64,000 in Saharanpur tehsil, while the lowest Rs. 46,000 in Behat tehsil. The overall input cost was also lowest in Behat tehsil (Rs. 2,18,948.52) and in other tehsils, the trend in increasing order was Deoband < Nakur < Rampur Maniharan < Saharanpur.

Cost C (operational and management cost of paddy crop)

The total operational and management cost of paddy was recorded higher in Saharanpur tehsil (, followed by Rampur Maniharan, Deoband, Nakur,

and Behat tehsils. In block plantation, costs of inputs are higher as comparison to boundary plantation.

Cost D (rental value of land)

Rental value of land was recorded within the range of Rs. 2, 49,000 to 2, 92,500 in all the tehsils. The total rental value of land was found lowest in Behat tehsil.

Total input cost was calculated maximum in Saharanpur tehsil, followed by Rampur Maniharan, Deoband, Nakur and Behat tehsils.

Table 3. All input cost of block plantation poplar with wheat and paddy in all tehsil of Saharanpur district

(A) Operational and management cost of poplar trees (2011-16)					
Particulars	Behat	Deoband	Nakur	Rampur Maniharan	Saharanpur
ETP cost (625 ETP @Rs. 20/ETP)	12500.00	12500.00	12500.00	12500.00	12500.00
pdefaultTransportation	1600.00	1000.00	1800.00	1800.00	1000.00
Digging and planting cost (@Rs.2.5/ETP	1562.50	2187.50	2187.50	2500.00	2187.50
Pruning	8125.00	11562.50	11875.00	10625.00	10625.00
Total	23787.50	27250.00	28362.50	27425.00	26312.50
(B) Operational and management cost of wheat (2011-16)					
Seed (120 kg/ha)	21000.00	21000.00	21000.00	21000.00	21000.00
Tillage operations	46000.00	57000.00	62200.00	60200.00	64000.00
Irrigation (5 times)	23000.00	21375.00	24000.00	18750.00	18750.00
Fertilizers					
Urea @ 300 kg/ha	15200.00	15200.00	15200.00	15200.00	15200.00
DAP @150 kg/ha	17400.00	17400.00	17400.00	17400.00	17400.00
FYM @240 q/ha	8400.00	11280.00	9120.00	13200.00	14400.00
Pesticides (herbicide+fungicide)	1900.00	8700.00	5525.00	8325.00	8810.00
Transportation (2 trolleys)	13600.00	13600.00	13600.00	13600.00	13600.00
Harvesting @300 kg/ha	21320.00	24600.00	22960.00	24600.00	24600.00
Threshing @8 % of total grain yield	24068.52	24068.52	24068.52	24068.52	24068.52
Labour cost (Sowing, pesticide spray, irrigation, application of fertilizer and threshing etc)	27060.00	30140.00	30140.00	34320.00	40710.00
Total (Rs.)	218948.52	244363.52	245213.52	250663.52	262538.52
(C) Operational and management cost of paddy (2011-16)					
Seed (15 kg/ha)	11700.00	11700.00	11700.00	11700.00	11700.00
Nursery expenses	6700.00	9450.00	8500.00	10300.00	13500.00
Puddling (Puddling one round+Harrowing one round)	46000.00	57000.00	57000.00	63200.00	64000.00

Transplanting	20700.00	20700.00	20700.00	24450.00	25000.00
Irrigation (5 times)	23000.00	21375.00	24000.00	18750.00	29750.00
Fertilizers					
Urea @375 kg/ha	16150.00	16150.00	16150.00	16150.00	16150.00
DAP @150 kg/ha	17400.00	17400.00	17400.00	17400.00	17400.00
Pesticides(herbicide+fungicide + insecticide)	8015.00	10350.00	4000.00	10975.00	12625.00
Transportation	13600.00	13600.00	13600.00	13600.00	13600.00
Harvesting+Threshing	45000.00	45000.00	45000.00	45000.00	45000.00
Labour cost	28290.00	32890.00	32890.00	35880.00	40710.00
Total	236555.00	255615.00	250940.00	267405.00	289435.00
(D) Rental value of land	292500.00	292500.00	249000.00	292500.00	292500.00
Total input (A+B+C+D)	771791.02	819728.52	773516.02	837993.52	870786.02

(II) Output/ return/ benefit

All benefits received from this practice have been elaborated in table 4.

Output A (benefit from trees)

Total output/benefit from poplar trees was recorded at the time of harvesting (sixth year), while value of sale of pruned wood is recorded from third year to fifth year. The total earning from poplar wood was higher in block plantation than the boundary plantation when compared tehsil-wise individually because the number of trees and pruned wood year-wise were higher. The output cost was calculated higher in Rampur Maniharan (Rs. 18, 35,340), while lowest in Nakur

tehsil (Rs. 14, 98,340).

Output B (benefit from wheat)

The maximum benefit from wheat grain and straw was estimated as Rs. 5, 06,148.44 in Deoband tehsil, followed by Rs. 4, 91,561.63 in Rampur Maniharan, Rs. 4, 66,459.06 in Behat, Rs. 4, 58,961.63 in Saharanpur and Rs. 4, 57,628.82 in Nakur tehsils.

Output C (benefit from paddy)

Result has shown that highest benefit from paddy grain and straw was recorded in Deoband tehsil (Rs. 5, 05,229.04), while lowest was estimated as Rs. 4, 55,950.14 in Nakur tehsil.

Table 4. Output of block plantation poplar with wheat and paddy in all tehsil of Saharanpur district

(A) Benefit from trees (2011-16)					
Particulars	Behat	Deoband	Nakur	Rampur Maniharan	Saharanpur
Sale of pruned wood (used as fuel) @ Rs. 100/q	10340.00	10340.00	10340.00	10340.00	10340.00
Auction of standing trees (520 trees @ Rs. 600/q) (average green timber weight 400 kg/tree at 6 years of age)	1500000.00	1690000.00	1488000.00	1825000.00	1750000.00
Total	1510340.00	1700340.00	1498340.00	1835340.00	1760340.00
(B) Benefit from wheat (2011-16)					
Grains	293733.45	312789.50	288252.66	292153.63	289553.63
Straw	172725.61	193358.94	169376.16	199408.00	169408.00
Total	466459.06	506148.44	457628.82	491561.63	458961.63

(C) Benefit from paddy (2011-16)					
Grains	371448.02	392465.02	365002.43	369283.05	369283.05
Straw	98739.75	112764.02	90947.71	94830.70	94830.70
Total	470187.77	505229.04	455950.14	464113.75	464113.75
Total output (A+B+C)	2446986.83	2711717.48	2411918.96	2791015.38	2683415.38

Benefit-cost analysis of wheat and paddy under poplar-based boundary and block plantation

An agroforestry system cannot be practiced or adopted only on the basis of the suitability of the agricultural crops for cultivation in combination with tree species until and unless it is remunerative. Keeping in view the benefit-cost ratio (B:C) has been analysed to ascertain economically profitable combinations feasible for the all blocks of Saharanpur district.

Table 5 indicated that the present worth of future returns was obtained by discounted both input cost as well as output cost at the minimum acceptable benefits on prevailing rate of interest i.e. 10 % per year. Based on input and output analysis the highest B:C was calculated as 3.29:1 in Rampur Maniharan tehsil, followed by 3.23:1 in Behat tehsil, 3.07:1 in Deoband, 3.07:1 in Nakur and 2.98:1 in Saharanpur tehsils. The highest NPV was estimated at 13.66 lakhs/ha in Rampur tehsil, while it was lowest in Behat tehsil (11.53

lakhs/ha) in wheat and paddy under poplar based boundary. However, in Deoband, Nakur and Saharanpur, it was 12.73, 12.21 and 12.05 lakhs/ha, respectively. Under boundary plantation, it was also revealed that the per hectare total actual input cost and output/return of agroforestry farmers of the district was calculated as Rs. 772211.60 and Rs. 2695430.66, respectively. The total net return was calculated as Rs. 1923219.06, while the net present value (NPV) with discounted at 10 % rate of interest was 12.45 lakhs/ha. The discounted cost and benefit invested by the farmers in Saharanpur district was Rs. 578627.28 and Rs. 1823128.33, respectively with the benefit-cost ratio of 3.15:1. It is revealed from the above results that farmers received Rs. 3.15 on the investment of Rs. 1 through cultivating wheat and paddy under poplar based boundary plantation practice in the Saharanpur district.

Table 5. Benefit-cost analysis of boundary plantation poplar with wheat and paddy in all tehsils of Saharanpur district

Poplar+Wheat+Paddy+Rental Value					
Year (0-6 Years)	Behat	Deoband	Nakur	Rampur Maniharan	Saharanpur
Actual cost(Rs./ha) (X)	6,90,366.50	781506.50	784776.50	795551.50	808856.50
Discounted at 10 % (X1)	5,17,190.92	586563.50	589464.86	596876.15	607196.05
Actual benefit (Rs./ha) (Y)	24,53,138.33	2745684.27	2664035.73	2900105.38	2714189.63
Discounted at 10 % (Y1)	16,70,212.75	1860364.42	1810064.25	1963069.92	1811930.31
Net-benefit (Y-X)	17,62,771.83	1964177.77	1879259.23	2104553.88	1905333.13
Discounted at 10 % (Y1-X1)	11,53,021.83	1273800.92	1220599.38	1366193.77	1204734.27
Net Present value (NPV) with discounted at 10 % rate of interest (Y1 - X1) (Y1-X1) (lakhs/ha)	11.53	12.73	12.21	13.66	12.05
Benefit-cost ratio (BCR) with discounted at 10 % rate of interest (Y1/X1)	3.23:1	3.17:1	3.07:1	3.29:1	2.98:1
NPV Boundary Plantation in S.Pur District & BCR	12.45 & 3.15:1				

Under block plantation, the highest benefit-cost ratio was calculated as 2.99:1 in Rampur Maniharan tehsil with the net present value of 12.53 lakhs/ha (Table 6). In Deoband tehsil, the B:C was slightly lower (2.98:1) than Rampur Maniharan, followed by Behat (2.86:1), Nakur (2.82:1) and Saharanpur (2.77:1) tehsils. The trend of NPV was also obtained in following decreasing order i.e. Deoband >

Saharanpur > Behat > Nakur. Chauhan et al., 2012 revealed that the success of agroforestry system/model depends upon its adoption by the farmers who are concerned about its ultimate economic viability. Farmers are planting poplar on their land (bunds or block depending on their resources) for additional income from trees. Inter-cultivation also supports the poplar trees for their better growth due to the various inputs to agricultural crops like fertilizers, irrigation and other management practices. The higher returns in poplar based intercropping are mainly due to higher productivity of poplar than without intercropping (6 and 1).

The per hectare actual cost of poplar based block plantation for farmers of Saharanpur district worked out to Rs. 8,14,763.01, while the actual benefit of Rs. 26,09,010.81. The discounted cost and benefit invested by the farmers in Saharanpur district was Rs. 6,10,929.60 and Rs. 17,63,169.71, respectively with the net present value of 11.52 lakhs/ha. The benefit-cost ratio was calculated as 2.89:1 for wheat and paddy crop under poplar based block plantation in the district.

It is concluded from the above findings that farmers received Rs. 2.89 on the investment of Rs. 1 through cultivating wheat and paddy under poplar based block plantation practice in the Saharanpur district.

(8) and (7) reported that poplar has played a significant role in enhancing the income of the farmers and average economic returns per hectare of poplar based agroforestry is two to five times more than traditional crop rotation.

(9) stated that Poplar based agroforestry is one of the major commercial agroforestry systems practiced by the farmers in Western Uttar Pradesh of India. The financial and economic analyses indicated that Net Present Value (NPV) in case of bund system with an eight years' rotation were Rs. 137,000/-, Rs. 127,000/- and Rs. 118,000/- at discount factors of 8, 10 and 12 percent, respectively, with a Benefit Cost Ratio (BCR) of 2.8 for all the three discount factors.

Trees for industrial use are catching the attention of farmers to grow economically and poplar based agroforestry systems have proven worth in north-western states of India (11; 5; 3).

Table 6. Benefit-cost analysis of block plantation poplar with wheat and paddy in all tehsils of Saharanpur district

Poplar+Wheat+Paddy+Rental Value					
Year (0-6 Years)	Behat	Deoband	Nakur	Rampur Maniharan	Saharanpur
Actual cost(Rs./ha) (X)	771791.02	819728.52	773516.02	837993.52	870786.02
Discounted at 10 % (X1)	580252.14	614809.88	578948.81	628475.75	652161.49
Actual benefit (Rs./ha) (Y)	2446986.83	2711717.48	2411919	2791015.38	2683415.38
Discounted at 10 % (Y1)	1660375.66	1833950.46	1635448.4	1881309.84	1804764.19
Net-benefit (Y-X)	1675195.81	1891988.96	1638402.9	1953021.86	1812629.36
Discounted at 10 % (Y1-X1)	1080123.52	1219140.58	1056499.6	1252834.10	1152602.70
Net Present value (NPV) with discounted at 10 % rate of interest (Y1 - X1) (lakhs/ha)	10.8	12:19	10.56	12.53	11.53
Benefit-cost ratio (BCR) with discounted at 10 % rate of interest (Y1/X1)	2.86:1	2.98:1	2.82:1	2.99:1	2.77:1
NPV Block Plantation in S.Pur District & BCR			11.52 & 2.89:1		

Conclusion

The economic analysis clearly demonstrated the financial advantages of poplar-based agroforestry systems. The present study, titled "Economic Assessment of Agroforestry Systems in Saharanpur District of Western Uttar Pradesh," evaluated the economic feasibility of the most prevalent agroforestry practices in the region, namely poplar-based block and boundary plantations integrated with wheat and paddy crops. The adoption of agroforestry systems depends not only on the biological compatibility of

tree species with agricultural crops but also on their economic viability and profitability.

in the district. Under boundary plantation of poplar with wheat and paddy, the total net return was ₹19,23,219.06, with a net present value (NPV) of ₹12.45 lakh ha⁻¹ at a 10% discount rate. The discounted cost and benefit were ₹5,78,627.28 and ₹18,23,128.33, respectively, resulting in a benefit-cost ratio of 3.15:1. In contrast, the poplar-based block plantation with wheat and paddy recorded a comparatively lower benefit-cost ratio of 2.89:1.

These findings indicate that farmers received ₹3.15 and ₹2.89 for every rupee invested in boundary and block plantations, respectively. Moreover, the widespread adoption of poplar-based agroforestry significantly contributes to meeting the rising industrial wood demand, thereby reducing pressure on natural forests and dependence on imported timber. Based on the present study, it can be concluded that poplar-based boundary plantation integrated with wheat and paddy crops is the most economically viable and sustainable agroforestry practice in Saharanpur district. Owing to similar agro-climatic and topographical conditions, this agroforestry model can be effectively replicated in other regions with comparable landscapes to enhance farm income while simultaneously addressing industrial wood demand.

References

1. Bangarwa, K.S. and Wuehlisch, G.V. 2009. Using exotic poplar in northern India for higher returns in agroforestry. *Asia-Pacific Agroforestry News*, 35: 3-5.
2. Census of India. 2011. District Census handbook, Saharanpur, Village and town Directory. Directorate of Census Operations, Uttar Pradesh, Lucknow.
3. Chandra, J.P. 2011. Development of poplar based agroforestry system. *Indian Journal of Ecology*, 38: 11-14.
4. Chauhan, S.K., Brar, M.S. and Sharma, R. 2012. Performance of poplar (*Populus deltoides* Bartr.) and its effect on wheat yield under agroforestry system in irrigated agro-ecosystem, India. *Caspian Journal of Environmental Sciences*, 10: 53-60.
5. Chauhan, S.K. and Mangat, P.S. 2006. Poplar based agroforestry is ideal for Punjab, India. *Asia-Pacific Agroforestry News*, 28: 7-8.
6. Dhillon, A., Sangwan, V., Malik, D.P. and Luhach, M.S. 2001. An Economic Analysis of Poplar Cultivation. *The Indian Forester*, 127(1):86-90.
7. Dwivedi, R.P., Kareemulla, K., Singh, R., Rizvi, R.H. and Chauhan, J. 2007. Socio-economic analysis of agroforestry system in western Uttar Pradesh. *Indian Research Journal of Extension Education*, 7: 18-22.
8. Joshi, B.C. 1996. Poplar cultivation under agroforestry. In: Session of the International Poplar Commission, 20th, Budapest, 1-4 October 1996. Environmental and social issues in poplar and willow cultivation and utilization: Proceedings. pp. 409-423.
9. Kareemulla, K., Rizvi, R. H., Kumar, K., Dwivedi, R. P. and Singh, R. 2005. Poplar agroforestry systems of Western Uttar Pradesh in Northern India: A socio-economic analysis. *Forests, Trees and Livelihoods*, 15: 375-381.
10. Mercer, D.E., G.E. Frey, and F.W. Cubbage. 2014. Economics of Agroforestry. In: Kant S. and J.R.R. Alavalapati (eds.). *Handbook of Forest Economics*. Earthscan from Routledge. New York, 209 pp.
11. Newman, S.M. 1997. Poplar agroforestry in India. *Forest Ecology and Management*, 90 (1): 13- 17.
12. Saxena, V. 2012. Climate change mitigation and adaptation potential of agroforestry in northern plains of India. Ph.D. thesis, Forest Extension Division, Forest Research Institute University, Dehradun, Uttarakhand, India.
13. Sood, D. 2024. Wood and Wood Products Update 2024. U.S. Department of Agriculture, Foreign Agricultural Service. 2024. GAIN Report IN2024-0026, Office of Agricultural Affairs, Mumbai, June 6, 2024.
14. Statistical Abstract, Uttar Pradesh. 2013. Economics and statistics division, State Planning Institute, U.P., Lucknow.
15. TERI. 2000. Farm forestry extension study report: Saharanpur district. Tata Energy Research Institute, Project No. 99SF62, New Delhi.

Received : May 2025; Revised : June 2025; Accepted : July 2025



GENOMICS-ASSISTED BREEDING FOR DROUGHT TOLERANCE IN WHEAT (*TRITICUM AESTIVUM* L.): RECENT ADVANCES AND FUTURE PROSPECTS

Amit Kuma^{1*}, Shivani¹, Reshu Chaudhary², Bijendra Singh² and R. P. Singh³

¹Department of Biotechnology, KVSCOS, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh, India

²College of Horticulture, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India

³Former Vice Chancellor, Swami Keshwanand Rajasthan Agriculture University, Bikaner

*Corresponding Author Email-amit.agbiotech1581@gmail.com

Abstract

Drought is one of the major abiotic limitations to wheat production worldwide, and the impacts of drought are worsened due to climate change. Drought tolerance consists of many genes with complex physiological and molecular processes, along with strong interactions between genotypes and environments; therefore, traditional phenotypic selection has produced minimal improvement to date. In this review we summarize recently published work (2020-2025) on genomics-assisted methods used to develop drought tolerance in bread wheat (8; 2). QTL mapping and marker-assisted backcrossing has allowed for successful validation of drought-related loci and their transfer into elite lines (3; 7). In addition, the coupling of genome-wide association studies with high-throughput phenotyping and genomic selection have improved predictability of grain yield in water-limited environments (5; 11). Overall, transcription factors from the DREB, NAC, MYB, and WRKY families are still considered the primary regulatory targets, but CRISPR/Cas-based gene editing is now able to provide precise, multiplex gene modifications in polyploid wheat (1; 10). The combination of pan-omics (the study of all omes), gene editing, speed breeding, and predictive modelling provides a realistic approach to developing climate-resilient, high-yielding cultivars. However, the rates at which phenotyping can occur and the speed at which candidate loci can be functionally validated are still the rate-limiting steps on this path (4; 6).

Keywords: *Triticum aestivum*; drought tolerance; genomics-assisted breeding; quantitative trait loci; genomic selection; CRISPR/Cas; transcription factors; pan-omics.

Introduction

Wheat (*Triticum aestivum* L.) is a cornerstone of global food security, supplying a major share of the calories and protein consumed by the human population. Sustaining and increasing wheat output is, however, becoming progressively more difficult as agriculture competes for a shrinking and increasingly unreliable freshwater supply. Sub-optimal water availability for plant growth and transpiration-drought-is the foremost abiotic limitation to wheat production, and most climate-change projections forecast greater aridity and more frequent water deficits across important

wheat-growing regions (3). Closing the yield gap without amplifying the environmental costs of intensive irrigation and fertilisation has therefore emerged as a central objective of modern cereal improvement (6).

Conventional breeding has historically relied on direct phenotypic selection for drought-adaptive traits such as deep rooting, water-use efficiency and stay-green. While effective, this strategy is slow, resource-intensive and strongly constrained by the low heritability and pronounced genotype-by-environment interactions that characterise the drought

response (9). The maturation of genomics over the past five years-anchored by a high-quality wheat reference genome and complementary pangenome assemblies-has opened more precise and rapid routes to dissecting and deploying the genetic basis of drought tolerance (8; 2). This review summarises these recent advances, spanning genetic resources, QTL mapping and marker-assisted selection, genome-wide association studies and genomic selection, regulatory candidate genes, genome editing, and the integration of multi-omics with high-throughput phenotyping.

Drought Stress and the Complexity of Tolerance in Wheat

Drought disrupts wheat growth and development through coordinated changes in metabolism, gene expression and physiology. Persistent water deficit triggers stomatal closure, reduced relative water content, loss of turgor, impaired photosynthesis and, ultimately, yield penalties that are most damaging when stress coincides with sensitive developmental windows such as jointing, anthesis and grain filling (6). Genotypic screening consistently reveals wide variation in morphological, physiological and agronomic responses to water limitation, with tolerant lines distinguished by superior water-use efficiency and photochemical performance-evidence of substantial exploitable genetic diversity (9).

From a breeding standpoint, the practical goal is not mere survival under extreme stress but the maintenance of a harvestable yield under realistic, water-limited field conditions. Drought tolerance is a quantitative trait with a complex phenotype and polygenic control; the relevant loci typically have small individual effects, low heritability and considerable interaction with the environment, which is precisely why marker-based and genomic approaches-capable of tracking many loci simultaneously-are so valuable for this trait (3).

Genomic Resources: From a Single Reference to Wheat Pangenomes

Genomic dissection of drought tolerance in wheat was long hampered by the sheer size and complexity of its allohexaploid genome (~16 Gb).

The release of a chromosome-level reference assembly of the cultivar Chinese Spring provided the first comprehensive framework for locating and characterising drought-relevant alleles. This was followed rapidly by the assembly of multiple additional cultivars, revealing extensive structural and presence-absence variation in modern breeding germplasm (8). Graph-based pangenome resources now capture this variation across many sequenced genomes, allowing breeders to query haplotype diversity and identify favourable alleles that a single reference would miss (2). Collectively, these resources have shifted wheat research from a multi-omics to a pan-omics paradigm, in which genomic, transcriptomic, proteomic, metabolomic and phenomic layers are integrated to decode complex traits such as drought adaptation (10).

QTL Mapping and Marker-Assisted Selection

Quantitative trait locus mapping remains a foundational tool for connecting genomic regions to drought-adaptive phenotypes. Comprehensive reviews of the literature have catalogued numerous QTLs associated with diverse physio-morphological traits expressed at different growth stages, and have highlighted that broad germplasm collections-rather than a narrow elite core-are the richest sources of useful drought-tolerance alleles (3). Beyond discovery, the practical value of these loci is realised when they are introgressed into adapted, high-yielding backgrounds.

A clear demonstration is the marker-assisted backcross transfer of QTLs governing biomass, canopy temperature, thousand-kernel weight, normalised difference vegetation index and yield into the popular Indian variety HD3086, using foreground selection across successive backcross generations to recover positive, homozygous lines with improved performance under moisture deficit (7). Such examples confirm that, despite the historical difficulty of applying marker-assisted selection to a low-heritability trait, well-validated and environment-stable marker-QTL associations can be deployed effectively to enhance climate resilience in elite wheat.

Genome-Wide Association Studies and Genomic Selection

Genome-wide association studies (GWAS) exploit historical recombination across diverse

panels to resolve marker-trait associations at higher resolution than bi-parental QTL mapping. Dense single-nucleotide polymorphism (SNP) genotyping of spring wheat panels has identified and validated novel loci for seedling-stage drought indices, with several chromosomes recurring as stable genomic regions across independent studies and populations-an encouraging sign of transferability for marker-assisted improvement (5).

Because drought tolerance and yield are governed by many small-effect loci, genomic selection (GS)-which predicts breeding values from genome-wide marker effects rather than a few large QTLs-is increasingly favoured for advancing populations. Integrating GS with high-throughput, image-based phenotyping and machine-learning models markedly improves the prediction of drought resistance and grain yield, while helping to relieve the phenotyping bottleneck that constrains large-scale field evaluation (11). The combination of GWAS for locus discovery and GS for whole-genome prediction now forms the analytical backbone of climate-resilient wheat breeding pipelines (4).

Transcription Factors and Candidate Genes

Transcription factors (TFs) are particularly powerful targets for manipulating drought tolerance because, unlike most structural genes, they regulate entire downstream cascades and thereby coordinate multi-step stress-response pathways. The dehydration-responsive element-binding (DREB/CBF) family, together with NAC, MYB, WRKY and bZIP regulators, governs the perception and transduction of water-deficit signals and the activation of protective effectors such as late-embryogenesis-abundant proteins, chaperones and osmolyte-biosynthesis enzymes (6). Recent functional genomics in wheat has characterised additional regulators-including members of the NAC and bHLH families implicated in root growth and water-use efficiency-expanding the catalogue of validated candidate genes available for breeding and editing (10). Because many of these loci remain to be functionally confirmed in field conditions, their systematic validation is a priority for translating regulatory knowledge into cultivar improvement (9).

CRISPR/Cas Genome Editing

Genome editing offers a more targeted and rapid alternative to transgenic over-expression for engineering drought tolerance, and its application in wheat has accelerated markedly. The polyploid wheat genome, with its multiple homoeologous copies of most genes, is well suited to multiplex CRISPR/Cas9 strategies that can simultaneously edit several homoeoalleles. For instance, multiplex CRISPR/Cas9 editing of the negative-regulator gene *TaSal1* produced heritable knockouts associated with altered stomatal behaviour and improved water-related phenotypes, illustrating the feasibility of precise trait modification in hexaploid wheat (1). Editing approaches are increasingly directed at regulatory genes and their cis-elements to fine-tune, rather than simply abolish, drought-responsive expression, and are now embedded within broader pan-omics-guided design frameworks for next-generation wheat (10).

Integration of Multi-Omics and High-Throughput Phenotyping

No single data layer fully captures the architecture of drought tolerance. The integration of genomics with transcriptomics, proteomics, metabolomics and phenomics provides a systems-level view of how wheat perceives, signals and adapts to water deficit, and underpins the transition to pan-omics-based crop design (10). Realising the value of these datasets depends critically on phenotyping capacity, which has become the principal cost and rate-limiting step in genetic analysis of drought. Field- and image-based high-throughput phenotyping, combined with environmental covariates and machine-learning models, is now being deployed to characterise physiological components of the drought response at scale and to feed predictive breeding models (11). Trait-characterisation frameworks that link agronomic, physiological and molecular information to specific genomic interventions-marker-assisted selection, speed breeding, genomic selection and gene editing-are accelerating the delivery of resilient cultivars (4).

Conclusions and Future Perspectives

Over the period 2020-2025, genomics-assisted breeding has substantially strengthened the toolkit available for improving drought tolerance

in wheat. A high-quality reference genome and pangenome resources have democratized allele discovery in a notoriously complex genome (8; 2); QTL mapping and marker-assisted backcrossing have moved validated loci into elite varieties (3; 7); GWAS and genomic selection have improved both locus resolution and whole-genome prediction (5; 11); and CRISPR/Cas editing has enabled precise manipulation of regulatory candidate genes in polyploid wheat (1).

The frontier now lies in integration. Combining pan-omics datasets, predictive modelling, speed breeding and gene editing within unified design pipelines promises to compress breeding cycles and to target the genotype-dependent, multi-season nature of drought adaptation more effectively (10; 6). Two challenges remain decisive: increasing the throughput and precision of phenotyping, and functionally validating the large and growing list of candidate loci under realistic field conditions (4; 9). Addressing these will be essential to convert genomic knowledge into climate-resilient, high-yielding wheat cultivars for water-limited environments.

References

1. Abdallah, N. A., Elsharawy, H., Abulela, H. A., Thilmony, R., Abdelhadi, A. A., & Elarabi, N. I. (2022). Multiplex CRISPR/Cas9-mediated genome editing to address drought tolerance in wheat. *GM Crops & Food*, 16, 1-17. <https://doi.org/10.1080/21645698.2022.2120313>
2. Bayer, P. E., Petereit, J., Danilevich, M. F., Anderson, R., Batley, J., & Edwards, D. (2022). Wheat Panache: A pangenome graph database representing presence-absence variation across sixteen bread wheat genomes. *The Plant Genome*, 15(3), e20221. <https://doi.org/10.1002/tpg2.20221>
3. Khadka, K., Raizada, M. N., & Navabi, A. (2020). Recent progress in germplasm evaluation and gene mapping to enable breeding of drought-tolerant wheat. *Frontiers in Plant Science*, 11, 1149. <https://doi.org/10.3389/fpls.2020.01149>
4. Roychowdhury, R., Das, S. P., Gupta, A., Parihar, P., Chandrasekhar, K., Sarker, U., Kumar, A., Ramrao, D. P., & Sudhakar, C. (2024). Accelerating wheat improvement through trait characterization: Advances and perspectives. *Physiologia Plantarum*, 176(5), e14544. <https://doi.org/10.1111/ppl.14544>
5. Sallam, A., Awadalla, R. A., Elshamy, M. M., Börner, A., & Heikal, Y. M. (2024). Genome-wide scanning to identify and validate single nucleotide polymorphism markers associated with drought tolerance in spring wheat seedlings. *The Plant Genome*, 17(1), e20444. <https://doi.org/10.1002/tpg2.20444>
6. Singh, C., Yadav, S., Khare, V., Gupta, V., Patial, M., Kumar, S., Mishra, C. N., Tyagi, B. S., Gupta, A., Sharma, A. K., Ahlawat, O. P., Singh, G., & Tiwari, R. (2025). Wheat drought tolerance: Unveiling a synergistic future with conventional and molecular breeding strategies. *Plants*, 14(7), 1053. <https://doi.org/10.3390/plants14071053>
7. Sunilkumar, V. P., Krishna, H., Devate, N. B., Manjunath, K. K., Chauhan, D., Singh, S., Sinha, N., Singh, J. B., Prakasha, T. L., Pal, D., Sivasamy, M., Jain, N., Singh, G. P., & Singh, P. K. (2023). Marker-assisted selection for transfer of QTLs to a promising line for drought tolerance in wheat (*Triticum aestivum* L.). *Frontiers in Plant Science*, 14, 1147200. <https://doi.org/10.3389/fpls.2023.1147200>
8. Walkowiak, S., Gao, L., Monat, C., Haberer, G., Kassa, M. T., Brinton, J., Ramirez-Gonzalez, R. H., Kolodziej, M. C., Delorean, E., Thambugala, D., et al. (2020). Multiple wheat genomes reveal global variation in modern breeding. *Nature*, 588, 277-283. <https://doi.org/10.1038/s41586-020-2961-x>
9. Wang, Q., Wu, Y., Ozavize, S. F., Qiu, C.-W., Holford, P., & Wu, F. (2024). Genotypic differences in morphological, physiological and agronomic traits in wheat (*Triticum aestivum* L.) in response to drought. *Plants*, 13(2), 307. <https://doi.org/10.3390/plants13020307>
10. Yao, Y., Guo, W., Gou, J., Hu, Z., Liu, J., Ma, J., Zong, Y., Xin, M., Chen, W., Li, Q., Wang, Z., Zhang, R., Uauy, C., Baloch, F. S., Ni, Z., & Sun, Q. (2025). Wheat2035: Integrating pan-omics and advanced biotechnology for future wheat design. *Molecular Plant*, 18(2), 272-297. <https://doi.org/10.1016/j.molp.2025.01.005>
11. Zhang, J., Zhang, D., Fan, Y., Liu, C., Wang, X., & Liu, P. (2024). Integrating high-throughput phenotyping and genome-wide association studies for enhanced drought resistance and yield prediction in wheat. *New Phytologist*, 243(6), 2273-2287. <https://doi.org/10.1111/nph.19942>



GENETIC DIVERGENCE ANALYSIS IN INDIAN MUSTARD [*BRASSICA JUNCEA* (L.) CZERN & COSS] GENOTYPES

Sharad Kumar Singh Redu* and Tejbir Singh

Department of Genetics and Plant Breeding, Kisan P.G. College Simbhaoli, Hapur (U.P.), India

*Corresponding Author Email-sharad13ip@gmail.com

Abstract

A study was carried out with 40 genotypes of Indian mustard [*Brassica juncea* (L.) Czern and Coss.] to examine the degree of genetic divergence within these genotypes. These genotypes were tested in randomized block design with three replications during rabi season of 2022-23 and 2023-24 under early and late planting. The degree of genetic divergence was studied in forty genotypes of Indian mustard [*Brassica juncea* (L.) Czern and Coss.] by using the pooled data of 4 environments (early and late sown). The analysis of variance (ANOVA) showed significant variation for all the thirteen characters (Table 1). Further, the estimates of genotypic and phenotypic variance were maximum for number of siliqua followed by biological yield, plant height, days to 50% flowering and days to maturity indicated sufficient genetic variability available in the material. The analysis was carried out in 40 genotypes of Indian mustard [*Brassica juncea* (L.) Czern & Coss.] using Mahalanobis D2 statistics followed by Rao (1952) for thirteen characters. Genotypes were found to be into seven clusters. The cluster VI had the largest number of genotypes (nine) followed by cluster IV (eight), V (eight), III (six), I (five) and II (four). Results shown that number of primary branches per plant, length of siliqua, plant height, seeds per siliqua and number of secondary branches per plant were the major contributors towards genetic diversity. The maximum intra cluster divergence was in cluster III followed by I, II, VI, V and IV, while maximum inter cluster divergence observed between cluster I and VI followed by cluster II and III, cluster III and VI and cluster I and V which indicates that efficient breeding programme can be formulated to improve yield by hybridization between genotypes of these clusters.

Key words: Genetics divergence, Mahalanobis D2 statistics, Indian mustard, cluster analysis

Introduction

Indian mustard is a member of the genus *Brassica* and the family Brassicaceae (former Cruciferae). It is popularly known as rai, raya or laha is one of the most important oil seed crop of the country. Since ancient times, many spices of mustard plants have been widely formed and used as food, medicine and spices. The plants are tall, erect, heavily branched and the flower has four sepals, four petals with deep yellow to pale yellow in color. It is a natively autogamous plant, but due to environmental factors and the sporadic visits of pollinating insects, there is frequent out crossing,

which can range from 12 to 20% (9). Mustard is sown in rabi season mainly in northwest India, contributes nearly 33 percent to edible oil pool of the country (DRMR, annual report 2023-24). India is a major producer of mustard oil and now become rank 1 in oilseeds production of rapeseed mustard with total production of 13.16 million tonnes (ICAR-DRMR, annual report 2023-24). Rapeseed-mustard production contributes to 33.24 % of total oilseeds production. These crops are cultivated on an area 9.18 million/ha with a total net production of 13.26 million tonnes and with an average yield of 1444 kg/ha (1). In order to quantify the genetic diversity among specific genotypes of

any plant and ascertain genetic affinity or the genetic separation between genotypes using cluster analysis, genetic divergence analysis is a crucial technique (5, 8, 11, 15, 7 and 3). Numerous studies have already assessed the genetic diversity of *Brassica juncea* using phenotypic traits (12, 10 and 13). Analysis and assessment of genetic divergence is very essential to know the spectrum of divergence in any crop. Keep in this view, forty genotypes of Indian mustard were taken in present study to assess the genetic diversity in the pooled data over the four environments using the D2 statistical approach.

Materials and Methods

The research experiment was conducted at research farm, Department of Genetics and Plant breeding, Kisan P.G. College, Hapur (U.P.) during rabi season 2022-23 and 2023-24 under early and late sown conditions thus making a total of four environments. In present investigation, forty genotypes of Indian mustard were taken and planted under randomized block design with three replications and each plot was consisted of single row of 3 meter length. The inter and intra row spacing were 30 cm and 15 cm, respectively. All the recommended packages of agricultural practices were adopted to raise a good crop. Five plants of each genotype were randomly selected for recording of data. The data were recorded for the following 13 characters namely days to 50% flowering, days to maturity, plant height (cm), number of primary branches per plant, number of secondary branches per plant, biological yield (g), number of siliqua per plant, length of siliqua (cm), seeds per siliqua, thousand seed weight (g), harvest index (%), oil content (%) and seeds yield per plant (g). The oil content was recorded by using Fourier Transform Near-Infrared Spectroscopy (FT-NIRS) at Directorate of Rapeseed Mustard, Bharatpur, Rajasthan. Mahalanobis D2 statistics analysis was used for assessing genetic diversity among 40 genotypes of Indian mustard [*Brassica juncea* (L.) Czern & Coss.]. The clustering of D2

values was formed by using Tocher's method as described by Rao (1952) while the intra and inter cluster distances were calculated using formula given by Singh and Chaudhary (1985).

Results and Discussion

The pooled analysis of variance (ANOVA) showed significant variation for all the thirteen characters studied (Table 1). The mean range were 46-70.65 for biological yield, 18.51- 29.88 for harvest index, 9.62- 18.40 for seed yield and 32-42.86 for oil content. The estimates of genotypic and phenotypic variance were maximum for number of siliqua followed by biological yield, plant height, days to 50% flowering and days to maturity indicated sufficient genetic variability available in the studied material of Indian mustard (Table 2).

Table 1. Analysis of variance for 13 characters in forty genotypes of Indian mustard.

S. No.	Source of variance	Mean sum of squares		
		Replication	Treatment	Error
	Degree of freedom	2	39	78
1	Days to 50% flowering	0.90	111.11**	1.09
2	Days to maturity	10.28	104.42**	1.61
3	Plant height (cm)	3.32	132.18**	7.07
4	No. of primary branches	0.023	0.161**	0.036
5	No. of secondary branches	1.11	0.86**	0.10
6	Biological yield (g)	9.33	147.76**	2.41
7	No. of siliqua per plant	115.10	2912.01**	70.55
8	Length of siliqua (cm)	0.024	0.060**	0.013
9	Seeds per siliqua	0.39	5.73**	0.25
10	Thousand seed weight (g)	0.01	3.39**	0.01
11	Harvest index (%)	14.54	18.27**	1.29
12	Oil content (%)	0.01	16.27**	0.01
13	Seed yield (g)	0.58	12.28**	0.15

Table 2. Mean range and estimates of genotypic and phenotypic variance for thirteen characters in Indian mustard.

S. no.	Genotypes	Mean	Min.	Max.	var (g)	var (p)
1.	Days to 50 % flowering	59.59	48.08	70.58	36.67	37.76
2.	Days to maturity	156.32	146.58	168.08	34.27	35.88
3.	Plant height (cm)	174.40	159.50	187.05	41.70	48.78
4.	No. of Primary branches/ Plant	5.56	5.16	6.27	0.04	0.08
5.	No. of secondary branches/ Plant	10.85	9.72	12.03	0.25	0.35
6.	Biological Yield (g)	56.10	46.00	70.65	48.45	50.86
7.	No. of Siliqua per plant	339.60	282.32	417.13	947.15	1017.70
8.	Length of Siliqua (cm)	4.73	4.45	5.11	0.02	0.03
9.	Seeds per Siliqua	15.79	13.72	18.97	1.83	2.07
10.	Thousand seed weight (g)	4.52	3.33	7.29	1.13	1.14
11.	Harvest Index (%)	23.82	18.51	29.88	5.66	6.95
12.	Oil Content (%)	39.15	32.00	42.86	5.42	5.43
13.	Seed yield (g)	12.94	9.62	18.40	4.04	4.20

Based on the D2 values, all 40 genotypes of Indian mustard were grouped into 6 clusters using the Tocher's method. The clustering pattern of genotypes is presenting in Table 3. Out of 6 clusters, cluster VI (Jawahar Mustard, CS-54, Aravali, Varuna, KMR-15-2, RLM 619, BR-40, KMR-16-2, RH-40-6) being the largest one with 9 genotypes followed by cluster IV (PM-28, RH-30R-17-18, KMR-18-3, PM-29, Pusa Agrani, KMR-17-3, Gujrat Mustard, KMR-40-60) and V (Pusa Mahak, Kranti, Jagan Nath, DRMR-IJ-31, Vaibhav, KMR-17-4, RH-819, KMR-19-4) with 8 genotypes each, cluster III (KMR-850, Pusa Bahar, KMR-14-1, KMR-17-2, KMR-18-4, KMR-18-1) with 6 genotypes, cluster I (Azad Chetna, Pusa Tarak, TKM 19-1, Pusa Jai Kisan, KMR-17-5) with 5 genotypes and cluster II (KMR-19-5, Pusa Bold, KMR-15-1, KMR-16-3) with 4 genotypes (Table 3). This clustering pattern indicated that there was a considerable diversity among the genotypes. The grouping of genotypes from same geographical origin into different clusters may be due to the different genetic background and wide divergence in features. Similar results were observed by Nagda et al., 2018 and Nandi et al., 2021.

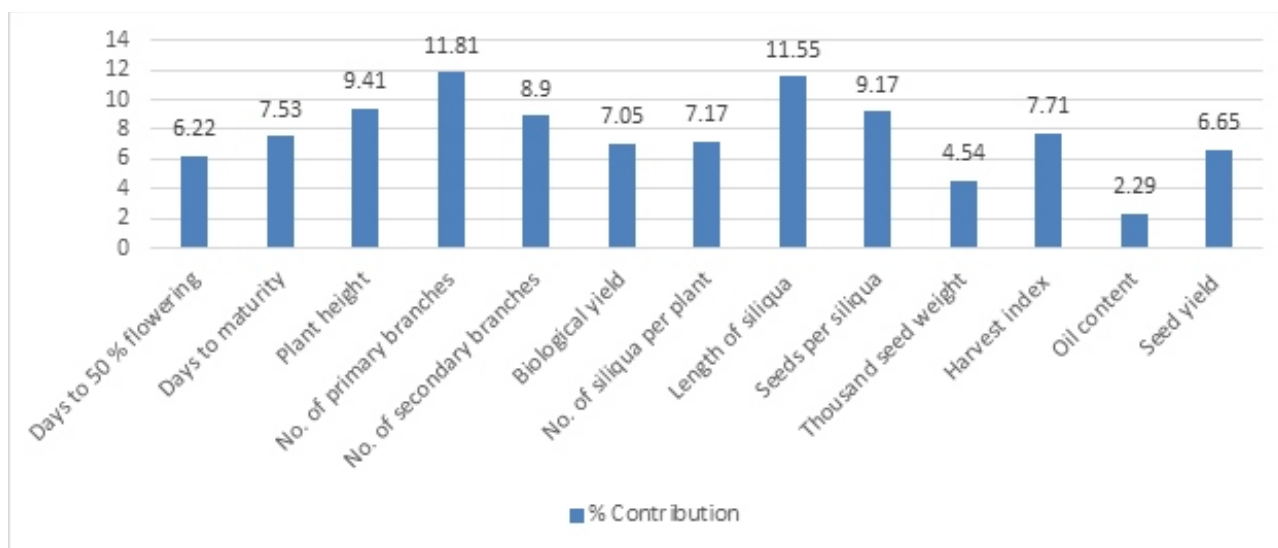
Table 3: Distribution of forty genotypes of Indian mustard in different clusters.

Cluster no.	No. of genotypes	Name of genotypes
I	5	Azad Chetna, Pusa Tarak, TKM 19-1, Pusa Jai Kisan, KMR-17-5
II	4	KMR-19-5, Pusa Bold, KMR-15-1, KMR-16-3
III	6	KMR-850, Pusa Bahar, KMR-14-1, KMR-17-2, KMR-18-4, KMR-18-1
IV	8	PM-28, RH-30R-17-18, KMR-18-3, PM-29, Pusa Agrani, KMR-17-3, Gujrat Mustard, KMR-40-60
V	8	Pusa Mahak, Kranti, Jagannath, DRMR-IJ-31, Vaibhav, KMR-17-4, RH-819, KMR-19-4
VI	9	Jawahar Mustard, CS-54, Aravali, Varuna, KMR-15-2, RLM 619, BR-40, KMR-16-2, RH-40-6

The character viz. number of primary branches/ plant (11.81), length of siliqua (11.55), plant height (9.41), seeds per siliqua (9.17) and number of secondary branches/ plant (8.90) were the major contributors towards genetic divergence contributed more than 50 % towards the total divergence (Table 4 and Figure 1). Parallel findings were reported by Tahira et al., 2017, Rout et al., 2018, Nagda et al., 2018 and Nandi et al., 2021.

Table 4: Contribution of different characters towards genetic divergence in Indian mustard.

S.No.	Characters	% Contribution
1.	Days to 50% flowering	6.22
2.	Days to maturity	7.53
3.	Plant height	9.41
4.	No. of primary branches/ plant	11.81
5.	No. of secondary branches/ plant	8.90
6.	Biological yield	7.05
7.	No. of siliqua per plant	7.17
8.	Length of siliqua	11.55
9.	Seeds per siliqua	9.17
10.	Thousand seed weight	4.54
11.	Harvest index	7.71
12.	Oil content	2.29
13.	Seed yield	6.65

**Fig. 1: % contribution of various characters towards genetic divergence**

The cluster means for different characters are present in Table 5. Cluster I possessed high mean values for length of siliqua (4.88), harvest index (26.95) and oil content (41.17); cluster III for number of secondary branches/ plant (11.51), biological yield (65.54), number of siliqua per plant (379.19), seeds per siliqua (16.84) and seed yield (15.56); cluster VI for days to 50% flowering (67.70), days to maturity (163.15), plant height (178.38) and cluster II for thousand seed weight

(5.26). Cluster I and III possessed the same highest mean value for number of primary branches/ plant (5.75). These results showed that the genotypes with high mean values for the corresponding characters clustered together inside the clusters with high cluster mean for the corresponding characters. The cluster mean indicates good plants for hybridization programme could be selected on mean basis. These results are supported by (6).

Table 5: Cluster mean values for seed yield and its components in Indian mustard.

No.	No. Characters/ Clusters	I	II	III	IV	V	VI
1.	Days to 50% flowering	53.82	58.79	61.36	57.56	55.17	67.70
2.	Days to maturity	150.12	154.08	160.92	154.03	152.48	163.15
3.	Plant height (cm)	168.61	169.80	172.54	177.03	174.59	178.38
4.	No. of primary branches/ plant	5.75	5.30	5.75	5.66	5.46	5.45
5.	No. of secondary branches/ plant	10.90	10.43	11.51	10.71	11.24	10.35
6.	Biological yield (g/plant)	59.43	50.60	65.54	52.24	58.96	51.28
7.	No. of siliqua per plant	361.64	349.66	379.19	320.55	337.36	315.40
8.	Length of siliqua (cm)	4.88	4.58	4.80	4.70	4.69	4.72
9.	Seeds per siliqua (g)	16.58	16.01	16.84	15.60	14.69	15.70
10.	Thousand seed weight (g)	4.04	5.26	4.77	4.30	4.47	4.53
11.	Harvest index (%)	26.95	24.86	24.60	23.70	21.28	23.46
12.	Oil content (%)	41.17	40.49	37.65	41.16	37.57	38.05
13.	Seed yield (g/plant)	15.13	12.35	15.56	12.11	12.18	11.67

The values of inter-cluster distances were greater than intra-cluster distances suggesting the presence of considerable diversity among the clusters (Table 6). The intra cluster distances varied from 2.165 to 2.962 within cluster and inter cluster distances from 2.618 to 5.220. The cluster III exhibited maximum intra cluster distance (2.962) which indicated that genotypes of this cluster are more diverse than the other clusters. Maximum inter-cluster distance was observed between cluster I and VI (5.220) indicating wider genetic diversity between the genotypes of these cluster. Therefore, this could be concluded that the genotypes belong to more diverse cluster could be used for successful crop breeding programme. Similar results were suggested by (2), (8) and (11).

Table 6: Average intra (bold values) and inter-cluster distances between different clusters

Cluster No.	I	II	III	IV	V	VI
I	2.906					
II	4.026	2.610				
III	3.418	4.693	2.962			
IV	3.421	2.618	4.311	2.165		
V	4.165	3.316	3.928	2.670	2.549	
VI	5.220	3.291	4.663	2.908	3.712	2.582

Conclusion

The genotypes of a single cluster show closeness in genetic makeup whereas genotypes of different cluster show distinctness in their genetic makeup. In simple words, the more diverse genotypes are always desirable while planning a crop breeding programme. So, selection of

genotypes from distant clusters will be genetic worthful in hybridization programme. The inter cluster distance indicated that the genotypes of more diverse clusters were I and VI followed by cluster II and III, cluster III and VI and cluster I and V. The results indicated that worthful crop breeding programme can be formulated to improve yield by hybridization between genotypes of these diverse clusters.

References

1. Anonymous 2023. Directorate of Economics and Statistics, Department of Agriculture and Cooperation, Agricultural Statistics at a Glance. pp 52.
2. Bind, D., Singh, S.K. and Dwivedi, V.K. (2015). Assessment of genetic diversity and other genetic parameters in Indian mustard [*Brassica Juncea* (L.) Czern&Coss]. Indian Journal of Agricultural Research. 49(6): 554-557.
3. Gupta, A., Chauhan, S., Tyagi, S.D. and Sigh, S. (2023). Genetic variability, heritability, genetic advance and cluster analysis in indian mustard (*Brassica juncea* L. Czern & Coss.) under timely and late sown conditions. International Journal of Plant & Soil Sciences. 35(19): 292-301.
4. ICAR-DRMR, Data for the year2023-24. 3rd Advance Estimates, DA & FW
5. Kaur, H. and Banga, S.S. (2015). Genetic divergence in novel determinate variants of resynthesized Indian mustard (*Brassica juncea* (L.) Czern & Coss). Indian Journal of Genetics. 75(2): 260-263.

6. Kumar, M., Singh, K.P. and Singh, D. (2007). Genetic divergence analysis for seed yield, its parameters and oil content in Indian mustard [*Brassica juncea* (L.) Czern & Coss]. Research on Crops 8: 183-185.
7. Margam, B.K., Chakraborty, N.C. and Sadhu, A. (2022). Assessment of genetic diversity based on agro morphological traits in Indian mustard [*Brassica juncea* (L.) Czern. & Coss.] germplasm. Electronic Journal of Plant Breeding. 13(3): 1019-1023.
8. Nagda, R., Dubey, N., Avinashe, H. and Tamatam, D. (2018). Assessment of genetic diversity in mustard genotypes. Plant Archives. 18(2): 1466-1470.
9. Nandi, S., Rout, S., Sur, B., Chakraborty, M., Hijam, L., Roy S.K. and Gupta, A. (2021). Assessment of genetic parameters, trait association and diversity analysis in Indian mustard [*Brassica juncea* (L.) Czern and Coss]. Biological Forum -An International Journal. 13(2): 95-109.
10. Reddy, S.N.K. and Shrivastav, S.P. (2022). Studies on genetic variability and divergence in mustard (*Brassica Juncea* L.). International Journal of Plant & Soil Science. 34(20): 565-574.
11. Rout, S., Das, A., Sur, B., Umamaheswar, Sadhu, N.S., Ghimiray, T.S., Mondal, H.A., Hijam, L., Chakraborty, M. and Roy, S.K. (2019). Genetic divergence in Indian mustard [*Brassica juncea* (L.) Czern&Coss] under sub-himalayan region. Journal of Crop and Weed. 15(2): 18-24.
12. Saikrishna, A., Arunkumar, B., Khan, H., Kuchanur, P.H. and Patil, A. (2021). Genetic diversity studies in Indian mustard [*Brassica juncea* (L.) Czern & Coss.] For yield and its component traits. The Pharma Innovation. 10(10): 534-538.
13. Sur, B., Rout, S., Single, S., Mandal, R., Nath, S., Maying, B., Sadhu, S., Chakraborty, M., Hijam, L., Debnath, M.K. and Roy, S.K. (2023). Evaluation of mustard genotypes [*Brassica juncea* (L.) Czern and Coss] for quantitative traits and character association of seed yield and yield components at sub-Himalayan region of West Bengal (India). Plant Science Today. 10(1): 166-178.
14. Tahira, Arshad, M., Khan, M.A., Khan, M.A. (2017). Cluster analysis, association and path coefficient analysis for seed yield improvement in rapeseed. Pakistan Journal of Agricultural Research. 30(4): 315-322.
15. Tarkeshwar, Nath, S., Mishra, G., Chaudhary, A.K., Gupta, R., Gupta, A.K. and Vimal, S.C. (2022). Genetic diversity analysis in indian mustard [*Brassica juncea* (L.) Czern and Coss.] genotypes. Biological Forum -An International Journal. 14(2): 1571-1574.

Received : May 2025; Revised : June 2025; Accepted : July 2025



ULTRA HIGH-DENSITY PLANTATION (UHDP) IN INDIA: CURRENT STATUS, OPPORTUNITIES, AND FUTURE PROSPECTS

Sandeep Kumar¹ and Everest Shiwach²

¹Department of Botany, Meerut College, Meerut, Uttar Pradesh, India

²Department of Botany, D.N. College, Meerut, Uttar Pradesh, India

***Corresponding Author Email-**sandeep.kumar@mcm.ac.in

-everestmalik@gmail.com

Abstract

The horticulture sector plays a crucial role in Indian agriculture by contributing to nutritional security, employment generation, and economic development. However, increasing population pressure, shrinking landholdings, and climate-related challenges have necessitated the adoption of innovative cultivation systems capable of enhancing productivity while ensuring sustainability. Ultra High-Density Plantation (UHDP) has emerged as a promising orchard management strategy that combines increased planting density with scientific canopy management, drip irrigation, fertigation, and crop regulation practices. Initially developed for mango cultivation, UHDP has subsequently been adopted in guava, pomegranate, apple, citrus, and several other fruit crops. The technology facilitates early fruiting, higher productivity per unit area, efficient resource utilization, and improved economic returns. This review summarizes the principles, technological components, achievements, challenges, and future prospects of UHDP in India, with particular emphasis on its application in major fruit crops.

Keywords: Ultra High-Density Plantation, UHDP, High-Density Planting, Mango, Guava, Apple, Citrus, Pomegranate, Canopy Management, Precision Horticulture

Introduction

India is the second-largest producer of fruits globally and possesses immense potential for horticultural development. Fruit crops contribute significantly to agricultural GDP and provide livelihood opportunities for millions of farmers. Despite this achievement, productivity of many fruit crops remains below global standards due to traditional orchard systems characterized by low planting density, delayed bearing, excessive vegetative growth, and inefficient utilization of land and resources (7).

Rapid urbanization and fragmentation of agricultural land have increased the need for intensive production systems capable of maximizing yield per unit area. High-Density Planting (HDP) and Ultra High-Density Plantation (UHDP) systems have emerged as

effective approaches for achieving this objective. UHDP integrates closer spacing, canopy management, drip irrigation, fertigation, and crop regulation practices to enhance orchard productivity and profitability (3).

The successful implementation of UHDP in mango cultivation has encouraged its adoption in other fruit crops such as guava, pomegranate, citrus, and apple. Consequently, UHDP is increasingly recognized as an important component of sustainable horticultural intensification in India (11).

Concept and Principles of Ultra High-Density Plantation

Ultra High-Density Plantation refers to the cultivation of fruit trees at substantially higher planting densities than conventional systems

while maintaining manageable canopy size through scientific pruning and training. The primary objective is to maximize light interception, improve resource-use efficiency, and increase productivity per unit area (12).

Table 1. Comparison of Orchard Systems

Parameter	Conventional	HDP	UHDP
Spacing (m)	10 × 10	5 × 5	3 × 2 or 2.5 × 2.5
Trees ha ⁻¹	~100	~400	674-1600
Bearing begins	5-7 years	3-4 years	2-3 years
Canopy management	Minimal	Essential	Mandatory
Irrigation	Flood	Drip preferred	Drip mandatory
Productivity	Low	Moderate	Very high

The success of UHDP depends upon five major components:

High Plant Density

Increasing plant population improves land-use efficiency and total yield per hectare. Although individual tree yield may decrease, cumulative orchard productivity increases substantially (14).

Canopy Management

Canopy management is the foundation of UHDP. Regular pruning and training maintain optimum tree architecture, improve sunlight penetration, and facilitate pest management (4).

Drip Irrigation

Drip irrigation supplies water directly to the root zone, minimizing losses through evaporation and runoff. This significantly enhances water-use efficiency (Kumar, 2024).

Fertigation

Fertilizers applied through irrigation water improve nutrient uptake efficiency and reduce nutrient wastage. Fertigation ensures precise nutrient delivery according to crop requirements (3).

Crop Regulation

Growth regulators such as paclobutrazol are commonly used to regulate vegetative growth and promote flowering, particularly in mango orchards (11).

UHDP in Mango Cultivation

Mango (*Mangifera indica* L.) represents the most successful example of UHDP adoption in India. Traditional mango orchards are generally planted at 10 × 10 m spacing, accommodating approximately 100 trees ha⁻¹. In contrast, UHDP systems employ spacings such as 3 × 2 m or 2.5 × 2.5 m, resulting in populations exceeding 1,600 trees ha⁻¹ (7).

Important cultivars suitable for UHDP include Amrapali, Mallika, Alphonso, Kesar, Dashehari, Totapuri, Ambika, and Arunika (15).

Canopy management is particularly important in mango. Trees are maintained at heights of approximately 2-2.5 m through annual pruning, allowing uniform light penetration and easier harvesting. Research conducted by ICAR institutions has demonstrated that UHDP mango orchards can begin commercial fruit production within two to three years of planting and achieve yields significantly higher than conventional orchards (11).

Application of paclobutrazol further enhances flowering and yield by suppressing excessive vegetative growth. Studies have reported productivity increases ranging from 200-400% under well-managed UHDP systems (3).

UHDP in Guava

Guava (*Psidium guajava* L.) is highly suitable for UHDP because of its precocious bearing habit and excellent response to pruning. Traditional orchards are planted at 6 × 6 m spacing, whereas UHDP systems may employ spacings as close as 2 × 1 m, accommodating approximately 5,000 plants ha⁻¹ (14).

Cultivars such as Allahabad Safeda, Lalit, Shweta, and Lucknow-49 have demonstrated excellent performance under intensive planting systems. Regular pruning is essential for maintaining canopy architecture and ensuring adequate sunlight penetration (8).

Studies conducted at ICAR-CISH and IARI have shown that UHDP guava orchards produce significantly higher yields and superior fruit quality compared with conventional orchards.

Productivity levels of 40-60 t ha⁻¹ have been reported under intensive management practices (14).

UHDP in Pomegranate

Pomegranate (*Punica granatum* L.) is another fruit crop that responds favorably to intensive cultivation systems. Traditional orchards generally employ spacing of 5 × 5 m, whereas UHDP systems utilize spacing of 3 × 2 m or 4 × 2 m, accommodating 1,250-1,667 plants ha⁻¹ (9).

Major cultivars grown under UHDP include Bhagwa, Ganesh, and Mridula. Scientific canopy management combined with drip irrigation and fertigation improves fruit size, rind coloration, and marketable yield (5).

Research has demonstrated productivity levels exceeding 20-25 t ha⁻¹ under intensive orchard management. Moreover, closer spacing facilitates efficient pest and disease management, thereby improving overall orchard profitability (10).

UHDP in Apple

High-density apple orchards have revolutionized fruit production in temperate regions. Modern apple UHDP systems utilize dwarf rootstocks such as M9 and M26, enabling planting densities of 2,000-4,000 trees ha⁻¹ compared with only 250-300 trees ha⁻¹ in conventional orchards (13).

Training systems such as Tall Spindle and Vertical Axis maximize light interception and improve fruit quality. Commercial fruit production begins within 2-3 years of planting, compared with 7-8 years under traditional systems (13).

Studies conducted in Himachal Pradesh and Jammu & Kashmir have reported substantial increases in productivity, fruit coloration, and market value under UHDP apple systems (16), (1).

UHDP in Citrus

Citrus fruits, including sweet orange, mandarin, lemon, and lime, also benefit from intensive orchard systems. Conventional orchards are typically planted at spacings ranging from 6 × 6 m to 8 × 8 m. Under UHDP, spacing may be reduced to 3 × 2 m, 4 × 2 m, or 4 × 3 m depending upon

cultivar vigor and rootstock characteristics (6)

Major citrus cultivars suitable for UHDP include Nagpur Mandarin, Kinnow Mandarin, Mosambi, Valencia Orange, and Acid Lime. Drip irrigation and fertigation significantly improve nutrient uptake and water-use efficiency (2).

Research indicates that citrus orchards managed under intensive planting systems can achieve productivity increases of 30-60% compared with conventional orchards while maintaining desirable fruit quality attributes (6).

Economic Benefits of UHDP

The primary advantage of UHDP is increased productivity per unit area. Additional benefits include:

- Early fruit bearing.
- Higher yield per hectare.
- Improved fruit quality and uniformity.
- Efficient utilization of water and fertilizers.
- Reduced harvesting costs.
- Enhanced profitability.
- Greater suitability for mechanization.

Table 2. Comparative Performance of UHDP in Major Fruit Crops

Crop	Conventional Density	UHDP Density	Yield Increase (%)
Mango	100	1333-1667	200-400
Guava	278	2500-5000	150-300
Pomegranate	400	1250-1667	100-200
Apple	250-300	2000-4000	150-250
Citrus	156-278	833-1666	30-60

The increased productivity and earlier returns generally compensate for the higher initial investment associated with drip irrigation and orchard establishment (3).

Challenges and Constraints

Despite its numerous advantages, UHDP faces several challenges:

1. High initial establishment costs.
2. Requirement for skilled pruning and canopy management.
3. Dependence on drip irrigation infrastructure.
4. Intensive nutrient management requirements.
5. Increased risk of disease outbreaks under poor canopy ventilation.

6. Need for regular technical supervision.

Successful adoption therefore requires continuous farmer training and extension support (7).

Future Prospects

The future of UHDP in India is highly promising. Advances in precision horticulture, remote sensing, artificial intelligence, automated fertigation systems, and climate-smart agriculture are expected to further improve orchard productivity and sustainability.

Future research should focus on:

- Development of dwarf cultivars and rootstocks.
- Precision nutrient management.
- Mechanized pruning technologies.
- Water-efficient irrigation systems.
- Climate-resilient orchard management practices.

Expansion of UHDP to additional fruit crops can play a major role in increasing national fruit production while ensuring efficient utilization of land and water resources.

Conclusion

Ultra High-Density Plantation has emerged as one of the most important technological innovations in Indian horticulture. By integrating high plant density with scientific canopy management, drip irrigation, fertigation, and crop regulation, UHDP has significantly enhanced productivity and profitability in mango, guava, pomegranate, apple, and citrus cultivation. Although the system requires greater technical expertise and initial investment, its long-term benefits outweigh these constraints. Wider adoption of UHDP, supported by research institutions, extension agencies, and government programs, can contribute substantially to sustainable horticultural development and improved farmer livelihoods in India.

References

1. Banyal, S. K., & Sharma, S. K. (2011). *Effect of fertigation and root stocks on yield and quality of apple under high density plantation*. *Indian Journal of Horticulture*, 68(3), 419-424
2. Castle, W. S. (2010). Citrus rootstocks and high-density planting systems. *HortTechnology*, 20(1), 107-113.
3. Chaudhari, A. U., Krishna, B., Soman, P., & Balasubrahmanyam, V. R. (2019). Development of a package for intensive cultivation of mango using ultra-high density planting (UHDP), drip and fertigation technologies for higher productivity. *International Journal of Agriculture Sciences*, 11(23), 9280-9284.
4. Gopu, B., Balamohan, T. N., Soman, P., & Jeyakumar, P. (2014). Canopy management in mango (*Mangifera indica* L.) cv. Alphonso with reference to flowering, yield and quality characters under ultra high density planting. *Journal of Applied Horticulture*, 16(1), 50-53. <https://doi.org/10.37855/jah.2014.v16i01.07>
5. Haneef, M., Kaushik, R. A., Sarolia, D. K., Mordia, A., & Dhakar, M. (2014). Irrigation scheduling and fertigation in pomegranate cv. Bhagwa under high density planting system. *Indian Journal of Horticulture*, 71(1), 45-48.
6. Hayat, F., Iqbal, S., Coulibaly, D., Razzaq, M. K., Nawaz, M. A., Jiang, W., & Shi, T. (2022). A mini review of citrus rootstocks and their role in high-density orchards. *Plants*, 11(21), 2876. <https://doi.org/10.3390/plants11212876>
7. Kumar, N. (2019). High density planting in mango: Prospects and problems. *Advanced Agricultural Research and Technology Journal*, 3(1), 47-53.
8. Mitra, Sisir & Gurung, M.R. & Pathak, Pradyot. (2008). GUAVA PRODUCTION AND IMPROVEMENT IN INDIA: AN OVERVIEW. *Acta Horticulturae*. 59-66. 10.17660/ActaHortic.2008.787.4.
9. Pal, R. K., Babu, K. D., Singh, N. V., Maity, A., & Gaikwad, N. (2014). Pomegranate research in India: Status and future challenges. *Progressive Horticulture*, 46(2), 184-201.
10. Pawar, D. D., Dingre, S. K., & others. (2014). Pomegranate, drip irrigation, fertigation, high density planting. *Indian Journal of Horticulture*
11. Rajan, S., Srivastava, K. K., & Kumar, D. (2021). Increasing mango productivity through high-density planting. *Indian Horticulture*, 66(4), 18-20.
12. Ram, S., & Sirohi, S. C. (1991). Feasibility of high density orcharding in Dashehari mango. *Acta Horticulturae*, 291, 207-212. <https://doi.org/10.17660/ActaHortic.1991.291.23>
13. Robinson, T. L. (2011). High-density apple orchard systems. *Acta Horticulturae*, 903, 633-646.

14. Singh, G., & Singh, A. K. (2007). High density planting in guava. *Acta Horticulturae*, 735, 235-241. DOI: 10.17660/ActaHortic.2007.735.31.
15. Srivastava, K. K., Kumar, D., & Shukla, P. K. (2024). Transforming conventional mango orchard to high-density planting for high productivity and quality mango production. *Indian Horticulture*, 69(4), 22-24.
16. Srivastava, K. K., Kumar, D., Singh, D. B., Singh, S. R., Mir, J. I., & Sharma, O. C. (2021). Increasing apple productivity through high-density planting. *Indian Horticulture*, 66(4), 18-20

Received : May 2025; Revised : June 2025; Accepted : July 2025



IMPACT OF ELECTRIC VEHICLES ON TRAFFIC AND EMISSIONS

Aakansha Goswami¹, Bijendra Singh² and R. P. Singh³

¹Professor, Vikrant University, Gwalior

²Dean, College of Horticulture, SVBPUAT, Meerut

³ Former Vice Chancellor, Bikaner Agriculture University, Rajasthan

*Corresponding Author Email-@gmail.com

Abstract

In order to estimate carbon-emission specific road networks while taking electric cars (EVs) into account, this study develops a method. Oil-fueled vehicles (OFVs) prioritise minimising trip time, while EVs additionally take battery charge state and charging station locations into account when choosing a route. A mixed equilibrium traffic assignment model is used to determine the traffic volume for each link in the network. The three factors of traffic volume, average speed, and road type are then used to generate a carbon-emission estimating technique. Two networks are used in a case study. Due to EVs' propensity to select routes with charging stations, it has been discovered that the road network's travel time has increased by 27%. Perceived risk, safe electricity quantity, and anticipated charging electricity all influence EV route choices. EVs can lower carbon dioxide emissions, but they cannot lower road network energy usage. Furthermore, it was shown that the placement of charging stations significantly affects traffic flow. The overall trip time, total carbon emissions, and balance of charging station utilisation metrics in the transportation network have all comparatively decreased once charging station locations were optimised. These include a 0.2% reduction in total journey time, a 1.85% reduction in total carbon emissions, and a 0.95% reduction in the balance of charging station use. The study is useful for forecasting traffic flow and carbon emissions, as well as for building road networks and deciding where to put charging heaps.

Introduction

Transportation is the main source of carbon emissions, and several nations have implemented carbon reduction regulations in response to global warming. The United States' "Long-Term Strategy Pathways to Net-Zero Greenhouse Gas Emissions by 2050," China's "The Action Plan for Peak Carbon Dioxide Emissions by 2030," and the United Kingdom's "Clean Growth Strategy" are a few examples. Reducing carbon emissions requires encouraging travellers to select eco-friendly means of transportation. It is anticipated that electric cars (EVs) will lower air pollution and carbon emissions, which will prompt significant development of related transportation infrastructure. Oil-fueled cars (OFVs) and electric vehicles (EVs) will coexist in a mixed traffic flow

during the transition until EVs eventually completely replace OFVs.

Additionally, there are several issues with EVs that must be resolved: First, there aren't many charging stations, therefore it's important to figure out how to make the most of them; Second, EVs can only go a short distance, which makes drivers nervous. Lastly, EV users' driving habits differ from those of OFVs, which may have new effects on the road network. As a result, academics are actively researching subjects like energy use, charging behaviour, and EV path selection.

Cai et al. [1] suggested two charging strategies in those studies on EV charging behaviour: system optimisation and user optimisation. They noted that while the system optimisation method concentrates on coordinating and regulating

charging behaviour on a larger scale, the user optimisation strategy seeks to direct individual charging behaviour. In order to investigate charging strategies for connected EVs, Liu [2] created a charging process function and vehicle energy consumption model. By simulating EV trip characteristics using the Monte Carlo approach, Wang et al. [3] established a model to forecast EV charging demand. Yang et al. [4] examined the effects of driving distance and trip duration on high-power charging for private EVs. An algorithm for charging advice based on geometry was created by Wang et al. [5] and is appropriate for intelligent charging services. In order to mimic charging decisions at the destination, Pan et al. [6] created a charging choice behaviour model for EV drivers based on a logit model. Using survey data, Yang et al. [7] suggested a nested logit model and a polynomial logit model to compare the travel habits of EV and OFV drivers. Wang et al. [8] examined EV drivers' charging habits while taking satisfaction and heterogeneity into account. A multistage optimisation approach was presented by Wang et al. [9] to give EV drivers intercity trip charging options. In order to reduce societal costs, Xiong et al. [10] developed an optimisation model for charging stations. They used a K-level nested quantum response equilibrium model to examine how EV drivers make decisions while charging. He et al. [11] examined the impact of EVs' constrained driving range and charging needs on route selection; however, they did not take into account the position planning of charging piles in the hybrid traffic network and instead utilised a linear charging function to calculate charging time. To examine EV driving routes and their effects on traffic network performance, Agrawal et al. [12] created a multiclass dynamic user equilibrium model.

In order to balance EV route selection and charging waiting time, Chen et al. [13] suggested a bi-level programming methodology to optimise the location and capacity of charging facilities. Drivers' preferences change from journey time and distance before using EVs to route accessibility afterward, according to Jensen et al. [14]. It suggests that the assumptions of path difficulties and the EV traffic planning model would need to be changed. In order to reduce the

overall travel time, Pourazarm and Cassandras [15] investigated the EV path problem in networks with charging sites. A path selection strategy for EVs that integrates charging time and variable travel time consideration based on distance limit and EV charging demand was proposed by Shao et al. [16].

Within the field of energy consumption literature, Cai [17] created a path planning model that included a nonlinear charging function and the EV energy consumption model. Song [18] suggested an energy consumption model that takes into account the relationship between battery energy consumption and the vehicle driving index under different driving circumstances. Based on energy usage statistics, He et al. [19] proposed an emission model for EVs and OFVs. Using real driving condition data and taking into account the relationship between speed and link, Zhang and Yao [20] created an energy consumption factor model. In order to examine the impact of EV penetration on road traffic conditions and energy consumption, Yao et al. [21] looked into models of electricity and petrol usage. Zhong et al. [22] addressed suboptimal charging pricing schemes within fixed and elastic demand user equilibrium frameworks by incorporating environmental capacity limitations into mixed EV and OFV network traffic to reduce emissions in protected areas. Yao et al. [23] highlighted the critical impact of the energy consumption coefficient in road energy consumption by analysing differences in energy consumption models across different road configurations using vehicle-specific electricity as an intermediate variable.

In conclusion, while previous research has examined how charging piles are categorised in relation to travel, it has not examined traffic equilibrium assignment taking into account the hierarchical arrangement of charging piles within mixed traffic flows. Regarding the traffic equilibrium assignment research, previous studies modelled the EV route selection by restricting the EV's range, neglecting to take user perception deviation and the state of charge (SOC) into account. Furthermore, it is uncertain how EVs will affect the road network, and more research is needed to determine how to evaluate network carbon emissions.

This work creates a mixed user equilibrium (M-UE) traffic assignment model to examine path selection, charging behaviour, charging station utilisation, and traffic flow distribution in order to fill these research gaps. Traveller risk perception, perceived time deviation, and beginning SOC are among the factors considered. By adding minimal remaining charge and desired charge parameters, a unique approach to characterise charging behaviour is put forth. A carbon-emission approach based on the mixed equilibrium assignment model is also created.

The rest of this document is structured as follows: A carbon-emissions approach and a mixed traffic equilibrium assignment model are presented in Section 2. A case study is presented in Section 3, and significant findings are outlined in Section 4.

Methodology

The influence of EVs on traffic flow assignment and carbon emissions on the road network is the primary focus of this study. Due to range anxiety and the need to think about charging, EV drivers choose different routes than fuel-powered vehicles. In order to explain how EVs affect flow distribution, a new traffic assignment model must be proposed. The impact of EVs on the road network is then examined by setting up a carbon emission calculation model based on traffic volume derived from the traffic assignment model.

Mixed Traffic Equilibrium Assignment Model

Several assumptions about the kinds of vehicles and their behaviour within the network are made in order to simplify the model in this study. EVs and conventional OFVs make up the network. Based on their personal experience, OFV users select the route that takes the least amount of time. EV customers, on the other hand, take into account both travel and charging times when making decisions. Depending on distance and SOC, EVs must be charged at stations situated at the network's connection nodes. EV users and OFV users are the two user types taken into consideration. While OFVs are presumed to have enough petrol to finish their journey, EVs may

need to charge while travelling to ensure they reach their destination. The perceived trip time and danger perceptions of EV users differ. Furthermore, the traffic network has a finite number of charging stations.

Conclusion

In order to evaluate the effect of EVs on traffic assignment, the study creates a mixed equilibrium model that takes travellers' beginning battery level, perceived time deviation, and risk perception into account. In order to estimate CO₂ emissions in the network, a carbon emission model is also constructed based on traffic assignment. Sensitivity studies for road type, safe remaining electricity, desired charging electricity, and risk perception are performed in two test cases to examine the effects of EV features on the network. The results show that because EVs prefer routes with charging stations, they will lengthen the overall travel time on the road network. The road network's journey time has grown by 27%. Vehicle routing decisions are influenced by the charging state's location and the user's sense of risk. The overall journey time, total carbon emissions, and balance of charging station utilisation all fell by 0.2%, 1.85%, and 0.95%, respectively, when we relocated a charging station. Additionally, the kind of road and average speed have a significant impact on carbon emissions. Mixed network charge times are 113.83% longer than those of main roads, although they are 21.96% and 17.29% shorter than those of subsidiary and branch roads, respectively.

The majority of current research focuses on static road networks, which are frequently virtual rather than actual networks. Future studies could use real-world data to investigate traffic assignment and billing strategies in dynamic traffic flow networks. Additionally, a hybrid stochastic user equilibrium model might be used for additional research, taking into account users' uncertainty regarding trip duration. Simultaneously, the analysis of charging station optimisation may enhance road traffic efficiency and reduce future psychological "charging anxiety" among EV customers.

References

1. Cai J., Chen C., Chen D., and Yong S., R Research on Electric Vehicle Charging Guidance Strategy, *Modern Electronics Technique*. (2021) 44, no. 1, 137–143.
2. Liu C., Study on Optimization of On-Way Charging Strategy for Connected Electric Vehicles, 2020, Lanzhou Jiaotong University.
3. Wang H., Zhang M., and Yang X., Prediction of Electric Vehicle Charging Demand Based on the Influence of Temperature, *Electrical Measurement and Instrumentation*. (2017) 54, no. 23, 123–128.
4. Yang Y., Tan Z., and Jiao G., Analysis of Factors Affecting Fast Charging of Electric Vehicles Based on Connected Data, *Transportation Systems Engineering and Information*. (2020) 20, no. 05, 86–92.
5. Wang Y., Bi J., Zhao X., and Guan W., A Geometry-Based Algorithm to Provide Guidance for Electric Vehicle Charging, *Transportation Research Part D: Transport and Environment*. (2018) 63, 890–906, <https://doi.org/10.1016/j.trd.2018.07.017>, 2-s2.0-85050857428.
6. Pan L., Yao E., and MacKenzie D., Modeling EV Charging Choice Considering Risk Attitudes and Attribute Non-Attendance, *Transportation Research Part C: Emerging Technologies*. (2019) 102, 60–72, <https://doi.org/10.1016/j.trc.2019.03.007>, 2-s2.0-85062614426.
7. Yang Y., Yao E., Yang Z., and Zhang R., Modeling the Charging and Route Choice Behavior of BEV Drivers, *Transportation Research Part C: Emerging Technologies*. (2016) 65, 190–204, <https://doi.org/10.1016/j.trc.2015.09.008>, 2-s2.0-84957009552.
8. Wang Y., Yao E., and Pan L., Electric Vehicle Drivers' Charging Behavior Analysis Considering Heterogeneity and Satisfaction, *Journal of Cleaner Production*. (2021) .
9. Wang Y., Bi J., Guan W., Lu C., and Xie D., Optimal Charging Strategy for Intercity Travels of Battery Electric Vehicles, *Transportation Research Part D: Transport and Environment*. (2021) 96, no. 3.
10. Xiong Y., An B., and Kraus S., Electric Vehicle Charging Strategy Study and the Application on Charging Station Placement, *Autonomous Agents and Multi-Agent Systems*. (2020) 35, no. 1, <https://doi.org/10.1007/s10458-020-09484-5>.
11. He F., Yin Y., and Lawphongpanich S., Network Equilibrium Models With Battery Electric Vehicles, *Transportation Research Part B: Methodological*. (2014) 67, 306–319, <https://doi.org/10.1016/j.trb.2014.05.010>, 2-s2.0-84902463353.
12. Agrawal S., Zheng H., Peeta S., and Kumar A., Routing Aspects of Electric Vehicle Drivers and Their Effects on Network Performance, *Transportation Research Part D: Transport and Environment*. (2016) 46, 246–266, <https://doi.org/10.1016/j.trd.2016.04.002>, 2-s2.0-84964345488.
13. Chen R., Qian X., Miao L., and Ukkusuri S. V., Optimal Charging Facility Location and Capacity for Electric Vehicles Considering Route Choice and Charging Time Equilibrium, *Computers & Operations Research*. (2020) 113,
14. Jensen A. F., Rasmussen T. K., and Prato C. G., A Route Choice Model for Capturing Driver Preferences When Driving Electric and Conventional Vehicles, *Sustainability*. (2020) 12, no. 3,
15. Pourazarm S. and Cassandras C. G., Optimal Routing of Energy-Aware Vehicles in Transportation Networks With Inhomogeneous Charging Nodes, *IEEE Transactions on Intelligent Transportation Systems*. (2018) 19, no. 8, 2515–2527, <https://doi.org/10.1109/tits.2017.2752202>, 2-s2.0-85051223934.
16. Shao S., Guan W., Ran B., He Z., and Bi J., Electric Vehicle Routing Problem With Charging Time and Variable Travel Time, *Mathematical Problems in Engineering*. (2017) 2017, 1–13, <https://doi.org/10.1155/2017/5098183>, 2-s2.0-85010461179.
17. Cai Y., Research on Electric Vehicle Distribution Path Planning Considering Charging Strategy and Nonlinear Energy Consumption, 2020, South China University of Technology.
18. Song Y., Energy Consumption Modeling and Driving Range Estimation of Pure Electric Vehicles Based on Driving Cycles, 2014, Beijing Jiaotong University.
19. He Y., Zhang P., Shao Y., and Tao Y., CO₂ Emission of Fuel and Pure Electric Vehicle in the Process of Circulation, *Journal of Chongqing Jianshu University*. (2019) 38, no. 7, 126–130.
20. Zhang R. and Yao E., Mesoscopic Model Framework for Estimating Electric Vehicles' Energy Consumption, *Sustainable Cities and Society*. (2019) 47, <https://doi.org/10.1016/j.scs.2019.101478>, 2-s2.0-85062063716.
21. Yao E., Lang Z., Song Y., Yang Y., and Zuo T., Microscopic Driving Parameters-Based Energy-Saving Effect Analysis Under Different

- Electric Vehicle Penetration, *Advances in Mechanical Engineering*. (2013) 5, no. 4, <https://doi.org/10.1155/2013/435721>, 2-s2.0-84893674444.
22. Zhong R., Xu R., Sumalee A., Ou S., and Chen Z., Pricing Environmental Externality in Traffic Networks Mixed With Fuel Vehicles and Electric Vehicles, *IEEE Transactions on Intelligent Transportation Systems*. (2021) 22, no. 9, 5535–5554.
23. Yao E., Yang Z., Song Y., and Zuo T., Comparison of Electric Vehicle's Energy Consumption Factors for Different Road Types, *Discrete Dynamics in Nature and Society*. (2013) 2013, 1–7, <https://doi.org/10.1155/2013/328757>, 2-s2.0-84893695412.
24. Nguyen S. and Dupuis C., An Efficient Method for Computing Traffic Equilibria in Networks With Asymmetric Transportation Costs, *Transportation Science*. (1984) 18, no. 2, 185–202, <https://doi.org/10.1287/trsc.18.2.185>, 2-s2.0-0021427308.

Received : April 2025; Revised : May 2025; Accepted : June 2025