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Aim & Scope

Aims

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Contents

Efficient Shoot Induction from Apical Meristem Culture in Olive (Olea europaea L.)

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A B S T R A C T

Olive propagation by tissue culture method is an efficient method for rapid asexual propagation, production of disease-free plants, and access to propagating materials throughout the year for olive plants. In this study, meristematic tissue explants of the Koroneiki cultivar from olive trees were cultured in different basal media included MS (Murashige and Skoog), OM (Olive Medium) and DKW (Driver and Kuniyki), without plant growth regulators. Then, the explants were cultured in basal media supplemented with auxins [NAA (Naphthalene acetic acid, IAA (Indole acetic acid) and IBA (Indole butyric acid)] and cytokinins [BA (Benzyl adenine), KIN (Kinetin), 2ip (Isopentenyl adenine), TDZ (Thidiazuron), and ZEA (Zeatin)]. The superiority of DKW basal media and ZEA growth regulator for inducing branching and longitudinal growth of branches for the Koroneiki cultivar was proven. With increasing concentrations of growth regulators, especially BA, was the dominant response than other responses such as meristem growth and shoot induction.

Keywords: Apical meristem; driver and kuniyki medium; olive; zeatin.

1. INTRODUCTION

The olive (Olea europaea L.) tree is one of the oldest and the most symbolic cultivated plants in the Mediterranean region. The annual yield of olive is estimated at 10 million tons, most of which is used for oil production and less than 10% consumed as table olive. As regards the world trend in the last 30 years, production and consumption of olive oil have increased together. It is unlikely that this trend will change in the near future, considering the recent introduction or increase of olive cultivation and olive-oil consumption in countries such as Japan, Australia, China and South Africa. The traditional area of olive cultivation is the Mediterranean basin, which accounts for 95% of the olive orchards of the world, and where, mainly in Spain, Italy and Greece, almost 99% of the world's olive oil and more than 80% of its table olives are produced IOOC, (2001); Jain and Ishii, (2003).

One of the serious threats to olive trees is olive diseases, especially viral diseases. The viruses cause severe diseases resulting in great yield losses and reduced olive quality. Elimination plant viral agents through meristem culture, which is considered one of the methods of plant cell, tissue, and organ culture, is one of the best methods for producing virus-free plants and plant materials Alam et al. (2004). The plants propagated by this method often have faster growth, higher resistance to plant diseases, higher quality, and greater reproductive capacity Yahyaoui et al., (2021).

Given that olive propagation is often asexual (cuttings), pathogens are easily transmitted through propagation materials such as rootstocks, scions, and cuttings, that is caused to contamination of orchards. In fact, Olive trees are mainly propagated using semi-hardwood cuttings. This procedure has contributed over the years not only to the dissemination of the best olive materials, but also to the spread of pathogens, predominantly viruses Xylogianni et al., (2021). Using healthy and genetically authentic scions, is one of the most important and main steps to control viral contamination. As mentioned above, one of the effective methods for defense against viral contamination of plants is the meristem culture method.

There are also other methods, such as thermotherapy and chemotherapy, to combat plant viruses. These methods and the meristem culture method are often used individually or together against viral diseases of plants. The goal of the present study is to optimize the culture method of the meristem zone of olive young branches in order to achieve methods for producing olive virus-free plants using the meristem culture method.

2. MATERIALS AND METHODS

2.1 Plant material and Sterilization

The plants tested in this study, which included 4year-old scions of olive, were purchased from Fadak Garden, located in Qom province, 15 km from the old Qom-Kashan Road. The scions were placed in the greenhouse of Iranian Research Institute of Plant protection. For preparation of explants, the apical parts of the (young) branches were cut and the first one centimeter of their apex was separated and sterilized as follows. Due to the thinness and high sensitivity of tissues, diluted commercial sodium hypochlorite was used to sterilize them as follow:

1- 60 minutes, 1% sodium hypochlorite
2- 30 minutes, 2% sodium hypochlorite
3- 15 minutes, 3% sodium hypochlorite

Then, the explants were washed 4 to 5 times with sterile distilled water to completely eliminate the effect of the sterilizing agent (sodium hypochlorite). The explants were cultured on free hormone medium included MS Murashige and Skoog (1962), DKW Driver and Kuniyki (1984) and OM Rugini (1984). Then a 0.8 to 1 mm portion from completely sterilized meristematic zone of young branches were separated with a sterile scalpel and cultured on media supplemented with Plant Growth Regulators as: [1 to 4 mg/l BA, KIN, 2ip, TDZ and ZEA from cytokinin(s) and 0.1 to 0.4 mg/ IBA from auxin(s)]. All media contained 30 g/L sucrose and 7.5 g/L agar. Media pH was adjusted to 5.8 before adding agar and before autoclaving (120°C and 1.5 atmosphere) using HCl and NaOH 1N. Cultures incubated at 25 ± °C with a 16 h photoperiod provided by cool white fluorescent lamps (38mol/M2S

2.2 Statistical Analysis

Experiments arranged in a randomized complete block design, with 28 explants (4 petri dish each with 7 explants) per treatment with four replications. Observation was based on, the mean number of explants (callus or regenerated shoot) reactant. Statistical analysis was done using Duncan's multiple range tests.

3. RESULTS

3.1 Establishment of Explants

The results of sterilizing olive explants, which included delicate and sensitive tissues of the plants branches (including meristem and meristematic tissues), showed that the use of commercial sodium hypochlorite (2%) for 30 minutes was more suitable than other treatments, because the percentage of sterilization and survival of explants was higher. The effect of different concentrations of commercial sodium hypochlorite and its different exposure times on olive explants is summarized in Table 1.

As mentioned in the Materials and Methods section, these sterilized tissues were first cultured in hormone-free basal culture media (MS, OM and DKW) In order to control contamination as much as possible, after ensuring that the tissues were sterile, the 0.8 to 1 mm distal sections were separated under sterile conditions in a laboratory hood and transferred to the above media supplemented with plant growth regulators or plant hormones (Fig 1).

3.2 The Effect of Basal Culture Media and Different Plant Growth Regulator Treatments on (Continued) Meristem Growth Callus and Shoot Induction

After successfully establishing the explants in the culture media, the effect of basal media as well as the kind and concentration of Plant Growth Regulator was very clear. This effect was most evident on callus formation Fig. 2, meristem growth, and shoot induction.

Table 1. Sterilization of olive explants using commercial Sodium Hypochlorite

Sterilizing treatment	Hypochlorite Sodium (%)	Exposure time (minutes)	Sterilization and survival (%)
1	1	60	85
2	2	30	98
3	3	15	95

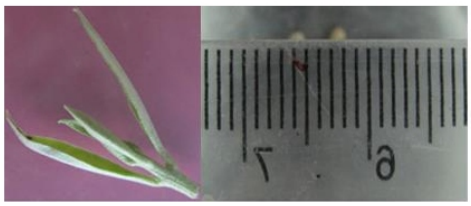

Fig. 1. Steps for preparing and culture olive meristem tissues in hormone-free culture media

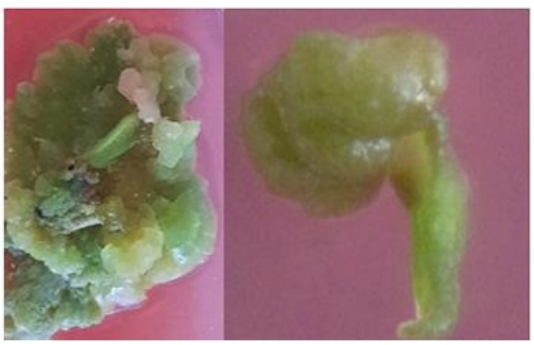

Fig. 2. Callusing in DKW medium with 2 mg/l BA and 0.2 mg/l IBA

The results showed that, the effect of cytokinin(s) and their concentrations started from a minimum amount, then increasing the concentration, increased the response somewhat and concentrations greater than the maximum (used in this study) decreased the response. Addition of 3 mg/L ZEA with or without 0.3 mg/l IBA had the effect on meristem growth and plantlet formation (aerial organ) in terms of the number of explants showing response (9a (Table. 2).

Other cytokinins had less effect in terms of both the initiation of the reaction and shoot regeneration from callus differentiation. Also, in all the treatments studied, ZEA caused the longitudinal growth of shoots to a greater extent than other cytokinins, with the exception of media containing 2ip that the longitudinal growth of the resulting shoots was greater than that of other treatments (data were not shown). Also, KIN and 2ip had obvious and positive effects on main factors examined in this study (callus induction and shoot regeneration from differentiation of callus tissues) respectively.

Different concentrations of TDZ used had no obvious effect on meristem growth, callusing and shoot formation. It is important to note that the positive effect of growth regulators ZEA and KIN on DKW medium and 2ip on OM medium were studied. In the MS basal medium, shoot regeneration were also observed with plant meristem culture. In MS basal medium, the most effective growth regulator for the desired reactions was BA. Another difference between the MS basal medium enriched with BA was the greater growth of calluses after the explants were established in the culture media. Because in the present study the best responses were observed in DKW basal medium and most of the explants with callusing and shoot regeneration belonged to it, only the effect of this basal medium enriched with cytokinins and auxins is summarized in Table 2 and Fig. 3 (A – E).

Table 2. The mean number of explants (with new shoots or calli) on DKW basal media experimented

Percent response	Concentrations (mg/l)	Plant Growth Regulators	Treatment number
1.9^{def}	1 + 0.1	BA + IBA	1
1.8^{def}	2 + 0.2	BA + IBA	2
1.9^{def}	3 + 0.3	BA + IBA	3
2.5^{cdef}	4 + 0.4	BA + IBA	4
4.75^{bcd}	1 + 0.1	ZEA + IBA	5
7.5^{ab}	2 + 0.2	ZEA + IBA	6
9^{a}	3 + 0.3	ZEA + IBA	7
3.9^{cde}	4 + 0.4	ZEA + IBA	8
1.7^{def}	1 + 0.1	2ip + IBA	9
1.7^{cde}	2 + 0.2	2ip + IBA	10
3.5^{cde}	3 + 0.3	2ip + IBA	11
4.4^{bcd}	4 + 0.4	2ip + IBA	12
0^{f}	1 + 0.1	TDZ + IBA	13
0^{f}	2 + 0.2	TDZ + IBA	14
1.12^{ef}	3 + 0.3	TDZ + IBA	15
1.02^{ef}	4 + 0.4	TDZ + IBA	16
4.6^{bcd}	1 + 0.1	KIN + IBA	17
7.37^{ab}	2 + 0.2	KIN + IBA	18
5.6^{bc}	3 + 0.3	KIN + IBA	19
1.6^{def}	4 + 0.4	KIN + IBA	20

Means values within a column with different letters are significantly different at P=0.015 according to Duncan's Multiple Range Test

Fig. 3 (A- E). A- Establishment of olive meristem in DKW medium containing 3 mg/L ZEA. B and C - in DKW medium containing 4 mg/L ZEA D - in DKW medium containing 4 mg/L KIN and E- In DKW medium containing 4 mg/l ZEA

4. DISCUSSION

The reason for using the meristem culture method in this project and other research conducted with this goal (production of healthy plant materials) is utilization of the shoot apical meristem properly. This characteristic is the totipotency of shoot apical meristem cells. It is important that, all of the shoot apical meristem cells do not have similar totipotency. The highest level of totipotency is observed in leaf generative centers. So, depending on the size of the isolated tissue and incision place, different reactions occur, from callus formation to callus differentiation into stem and root, complete plantlet, and meristem repair. With cut of 0.1 to 0.5 mm from the meristem apex, a little callus is formed at first, and then stem and root are formed from the callus differentiation.

The totipotent cells of this organ due to very little differentiation and incomplete differentiation of vascular tissues and also the lack of some cell surface receptors which leads to plant recognition microbial agents, are used to produce plants free of any pathogen Wang and Charles (1991), Galun (2007) and Ball (1960).

The results showed that by separating 0.8 to 1 mm sections of meristem apex and to culture them on media, first callus and then aerial organs were produced.

Siripatr et al. (2011), observed a shoot-forming response by culturing the meristem of passion flower (Passiflora edulis) on MS medium containing 1 to 3 mg/L BA. The above researchers used MS medium containing 0 to 1.2 mg/L IBA for rooting of regenerated shoots. The results of these researchers differ from the present study in terms of the lack of callus formation before the development of aerial organs.

Another study on strawberry (Fragaria chiloensis (L.) Duch.), showed that on MS basal medium enriched with BA shoot induction is predominant reaction. In this study, the presence of BA in the medium increased shoot formation also, its usage, had low levels (near zero) of explant losses due to oxidation Quiroz et al., (2017).

Yao et al. (2022) stated that for induction of new shoots (by passing through the callus phase) of Salvia miltiorrhiza (a type of sage) on MS medium, application of 0.5 mg/L BA, 0.1 mg/L NAA, and 0.1 mg/L GA3 is essential. The researchers used 0.5 MS medium and 1 mg/L NAA to root the new shoots. They also noted that calli generated from the shoot apical meristem are superior to other plant tissues and aerial organs in terms of their induction and production of new organs. This research work is in agreement with the present research in terms of callus induction immediately after meristem culture.

The positive effect of ZEA and BA on growth and formation of aerial organs from meristems of Koroneiki cultivar olive, compared to other cytokinins used in this study, was another important result of the experiments. The growth regulator BA plays a very important role in all cell, tissue and plant organ culture research, with emphasis on effect of it on shoot induction (newly emerging branches).

There is also a lot of evidence about the positive role of ZEA, which confirms the results of this project, for example, Benelli and De Carlo in 2018 stated that 10 µ ZEA not only case to induction of olive apical buds but also causes longitudinal growth of shoots. (Benelli and De Carlo, 2018).

Another report has shown that 4/18 mµ ZEA has a negative effect on olive growth factors (Haddad et al., 2018). Obviously, this conclusion does not confirm the results of this project.

Ali et al. (2009) showed that 4 mg/L ZEA caused a significant increase in shoot formation in olive plants compared to concentrations of 1, 2, and 3 mg/L.

In 2021, researchers pointed to the positive growth-regulating effect of ZEA in different MS and OM basal media for Arbaquin and Muscat olive cultivars Mirzaei et al., (2021). The mentioned subject shows that even on other basal media (compared to the DKW basal media used in this study) such as MS and OM which have been used for tissue culture of other olive cultivar, ZEA plays an important role.

5. CONCLUSION

According to the gardeners and olive scions' growers, tissue culture is a superior and preferred method for olive propagation due to the difficulties for the traditional methods especially difficulties related to different methods for rooting of different cuts olive cultivars. Meristem culture is both a way to overcome traditional propagation methods of this plant and an introduction virusfree olive plants production.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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Endophytic Bacteria as Biocontrol Agents Against Bacterial Leaf Blight and Growth Promoters in Rice

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ABSTRACT

This study aimed to isolate and characterize endophytic bacteria from rice leaves for their potential to suppress Bacterial Leaf Blight (BLB) and promote plant growth. A total of 45 endophytic bacterial isolates were obtained from the healthy leaf samples of MTU-1010, BPT-5204 and NLR-34449 cultivars and out of which, 19 isolates were from Nellore district, 26 isolates were from Chittoor district. The endophytic bacterial count from BPT 5204 cultivar collected from Madibaka village of Yerpedu mandal, Chittoor district showed highest count of 7.8 × 106/g leaf. Whereas samples collected from the Nelaballi village of Pellakuru mandal, Nellore district of MTU 1010 variety recorded lowest colony forming units 2.4 × 106/g of leaf. 45 Endophytic bacterial isolates were evaluated for the antagonistic efficacy by Agar well Diffusion method. Among 45 isolates, EMP-5 recorded highest zone of inhibition with 16.8 mm and 12 isolates recorded with inhibition zone diameter of more than 10.00 mm. Morphological characterization of the 12 isolates revealed that dominance of Gram negative bacteria with rod shaped cells over the Gram positive bacteria. With regard to Plant Growth Promoting (PGP) traits of 12 isolates, EMP-5 showed positive to all PGP trait namely, IAA production, Phosphate solubilization, Siderophore production, HCN, Ammonia Production, Amylase activity and Protease production. These findings highlight the potential EMP-5 endophytic bacteria as eco-friendly biocontrol and biofertilizer agents for rice cultivation.

Keywords: Bacterial leaf blight; endophytic bacteria; Plant Growth Promoting (PGP) traits and paddy.

1. INTRODUCTION

The low productivity of rice in India can be attributed to several biotic and abiotic factors and among the biotic factors, diseases form a major constraint in achieving the potential grain yield. Many bacterial diseases are known to infect rice viz., Bacterial leaf blight (Xanthomonas oryzae pv. oryzae), Bacterial leaf streak (Xanthomonas oryzae pv. oryzicola), Bacterial panicle blight (Burkholderia glumae), Bacterial brown stripe (Acidovorax avenae subsp. avenae) etc. Out of this bacterial leaf blight of paddy (BLB) is of economic importance. The disease mostly occurs in epidemic proportions in many parts of the world, incurring upto 50 per cent crop loss (Gnanamanickam et al., 1999). Crop loss assessment studies revealed that BLB occurrence depends on the crop stage, degree of cultivar susceptibility and to majorly environmental conduciveness in which it occurs.

The yield loss due to BLB is attributed to increase in chaffiness, decrease in grain weight and the number of panicles per plant (Ahmed and Singh, 1975). Endophytic bacteria have been virtually found in every plant studied, where they colonize the internal tissues of their host plant and can form a range of different relationships including symbiosis, mutualism, commensalism etc. Bacterial endophytes colonize an ecological niche comparable to that of phytopathogens, which makes them appropriate as biocontrol agents (Berg et al., 2005). Hence the quest for innovative antibacterial biocontrol agents remains a major challenge and such biocontrol agents are essential to control the BLB inflicted damages in rice. In recent years, co-inoculation of endophytic bacteria in rice is playing a key role for controlling the disease damage and availability of nutrients under sustainable agriculture production system (Swathi et al., 2023, Tu et al., 2024, Faisal Yousuf et al. 2025). There is an imperative need to identify the potential endophytic isolates with plant growth promoting (PGP) traits and disease suppressing ability.

2. MATERIALS AND METHODS

The laboratory experiments pertaining to the present research work were conducted in the Department of Plant Pathology at S.V. Agricultural College, Tirupati.

2.1 Isolation of Bacterial Leaf Blight Pathogen from Disease Sample

Xanthomonas oryzae pv. oryzae, the causal agent of bacterial leaf blight was isolated from the diseased plants collected from Nellore district of Andhra Pradesh. Infected leaves of rice was excised with sterile scalpel and made into small bits. The leaf bits were surface sterilized with one per cent sodium hypochlorite for three minutes and then washed with sterile distilled water. Later the infected leaf bits after proper drying on sterile blotting paper was transferred onto Nutrient Agar (NA) medium and incubated at room temperature (25-270C) for 72 h (Jabeen et al., 2012).

2.2 Isolation of Endophytic Bacteria

Healthy leaves washed with water to remove the dust and there after 2 g of leaves chopped into pieces of 4-6 mm used for isolation. The disinfection and isolation was done as the procedure of Araujo et al. (2001). Briefly, the leaves were disinfected superficially by following the five step protocol as 70 per cent alcohol for 1 minute, 2.5 per cent sodium hypochlorite for 4 minutes, ethanol for 30 sec and finally 3 rinses in sterile distilled water. To confirm the disinfection protocol, aliquots of the sterile water used in the final rinse was plated on 10 per cent Tryptone Soya Agar (TSA) medium at 28 0C for 3 days and the plates were examined for the presence or absence of bacterial colonies. Such of the leaf samples were discarded showing colonies in the incubated Petri plates. Remaining of the surface sterilized leaf bits were macerated with 5 ml of phosphate buffer in a sterilized mortar and pestle. The macerated tissues were diluted serially in the sterile distilled water. From this, 6 dilutions (10-1 to 10-6) were made by transferring 1 ml of the suspension to successive sterile water columns. From last two series of the dilution, 0.1 ml was taken and plated onto TSA medium. The plates were incubated at 280C for 1–10 days until growth was observed, upon which the numbers of Colony Forming Units (cfu) were counted according to the following formula.

$$\text{Number of cfu/g} = \frac{\text{Number of colonies}}{\text{Amount plated} \times \text{dilution}}$$

2.3 Evaluating the Antagonistic Activity of Endophytic Bacteria Against Xanthomonas oryzae pv. oryzae

The Antagonistic efficacy of endophytic bacterial isolates against Xanthomonas oryzae pv. oryzae was evaluated by using agar well diffusion method. The level of Xanthomanas oryzae pv.oryzae growth inhibition was determined by measuring the difference between the inhibition zone formed and diameter of the pathogen growth. (Yasmin, 2016).

2.4 Morphological Characterization of Endophytic Bacteria

Morphological characterization was done on the basis of shape, elevation, texture, margin, colour, size and pigmentation etc. of colonies and gram staining of endophytic bacteria grown on the medium (Bathlomew, 1962).

2.5 Characterization of Potential Endophytic Bacterial Isolates for Plant Growth Promoting Traits

2.5.1 IAA production

Indole Acetic Acid test was performed by inoculating the endophytic bacterial isolates into one per cent tryptone broth for 48 h at 30 ± 20C, followed by addition of Kovac's reagent (0.5 ml). Appearance of cherry red colour ring confirms IAA production. (Kovac's, 1959)

2.5.2 Hydrogen Cyanide (HCN) Production

HCN production of endophytic bacterial isolates was tested qualitatively. The endophytic bacteria were streaked on King's B medium amended with glycine (Bakker and Schipper, 1987).

2.5.3 Ammonia production

Endophytic bacterial isolates were tested for the production of ammonia in peptone water. Freshly grown endophytic bacterial isolates were inoculated into 10 ml of peptone water in each test tube and incubated at 28 ± 20C for 48-72 h. Nessler's reagent (0.5 ml) was added to each tube and observed for the development of faint yellow to dark brown colour as a positive indication for ammonia production. (Cappuccino and Sherman et al., 1992).

2.5.4 Phosphate solubilisation

Endophytic bacterial isolates were screened for their potential to solubilize insoluble calcium phosphate on Pikovskaya agar medium as described by Pikovskaya (1948).

2.5.5 Amylolytic and proteolytic activity

2.5.5.1 Starch hydrolysis

Endophytic bacterial isolates were screened for amylolytic activity by starch hydrolysis test on starch

agar plates. Appearance of the yellow zone around the bacterial growth indicated starch hydrolysis while blue zone surrounding the bacterial growth, indicated that starch is present and has not been hydrolyzed (Sahu et al., 2005).

2.5.5.2 Protease production

Proteolytic activity was tested by inoculation of 10 µl into 6 mm wells made on skim milk agar medium (containing 5 g pancreatic digest of casein, 2.5 g yeast extract, 1 g glucose, 15 g agar and 100 ml of 7 per cent skim milk solution per liter and incubated at 28°C upto 4 days and observed for the formation of the halo zone around the colonies and a clear zone around the cells indicated positive proteolytic activity (Chaiharan and Lumyong 2009).

2.5.6 Siderophore production

Endophytic bacterial isolates were assayed for qualitative siderophore production on the Chromoazurol Succinate (CAS) agar medium described by Schwyn and Neilands (1987).

3. RESULTS AND DISCUSSION

3.1 Isolation of the Pathogen

Bacterial leaf blight causal agent Xanthomonas oryzae pv. oryzae was isolated from the diseased plants collected from the Agricultural Research Station, Nellore. Infected leaf bits surface sterilised and inoculated on Nutrient Agar (NA) Medium resulted in mucoid, round and smooth bacterial colonies on incubation at 28 ± 2 0C for 48-72 hours. The pathogen was purified by streak plate method and preserved at -20 0C in glycerol stocks.

3.2 Isolation of Endophytic Bacteria

A total of 45 endophytic bacterial isolates were isolated from the healthy plants of cultivars viz; MTU 1010, BPT 5204 and NLR 34449 which are popularly cultivated in Nellore and Chittoor districts of Andhra Pradesh (Table 1).

a) Isolation of endophytic bacteria from Nellore district

A total of 19 endophytic bacterial isolates were isolated from the samples collected from nine villages spread across six mandals (Table 1). Among the 19 endophytic bacterial isolates, three isolates from ARS, Nellore (EBA-1, EBA-2, EBA-5) of Nellore mandal, four isolates (EBL-1, EBL-2, EBL-3, EBL-6) of Leguntapadu village of Kovurmandal, two isolates from Venkannapuram (EBV-4, EBV-8) and two isolates from Rajupalem villages (EBR-1, EBR-2) of Kodavalurmandal, two isolates (EBV-1, EBV-2) from Vidavalur village of Vidavalurmandal, two isolates (EBK-1, EBK-3) from Konetirajupalem village and two isolates (EBP-1, EBP-3) from Palemkota villages of Venkatagirimandal and two isolates (EBN-1, EBN-2) from Nelaballi village of Pellakurmandal of the Nellore district (Table 2).

b) Isolation of endophytic bacteria from Chittoor

Similarly a total of 26 endophytic bacteria isolates were isolated from the leaf samples collected from four villages spread across three mandals of the Chittoor district (Table 2). Among the 26 endophytic bacterial isolates, nine isolates from Mallayapalli village (EMP-1, EMP-2, EMP-3, EMP-4, EMP-5, EMP-6, EMP-7, EMP-8, EMP10) of Ramachandrapuram mandal, four isolates (MB-1, MB-2, MB-3, MB-4) from Madibaka village of Yerpedumandal, nine isolates from Urandur village (EUD-1, EUD-2, EUD-3, EUD-4, EUD-6, EUD-7, EUD-8, EUD-9, EUD-10) and four isolates (EP-1, EP-2, EP-3, EP-4) from Pudi villages of Srikalahasthi mandal.

The endophytic bacterial count from the collected samples is presented in terms of cfu/g of leaf and presented in Table 2. Among them, leaf sample of BPT 5204 cultivar collected from Madibaka village of Yerpedu mandal showed highest count of 7.8 × 106/g followed by 7.7 × 106/g leaf of BPT 5204 variety collected from Mallayapalli village of Ramachandrapuram mandal of Chittoor dist. Leaf samples collected from the Nelaballi village of Pellakuru mandal, Nellore district of MTU 1010 variety recorded lowest colony forming units 2.4 × 106/g of leaf.

Table 1. Details of Leaf samples collected from different parts of Chittoor and Nellore district

S. No	District	Mandal	Village	Variety	Latitude	Longitude
1		Yerpedu	Madibaka	BPT-5204	13.6429	79.5970
2			Pudi	NLR-34449	13.767715	79.718184
	Chittoor	Srikalahasthi				
3			Urandur	BPT-5204	13.738249	79.678254
4		Ramachandrapuram	Mallayapalli	BPT-5204	13.554371	79.303225
5		Kovur	Leguntapadu	MTU-1010	14.516979	80.028167
6		Nellore rural	Agricultural Research Station	NLR-34449	14.430182	79.998646
	Nellore	Vidavalur	Venkannapuram	BPT-5204	14.604499	80.066097
8		Kodavalur	Vidavalur	BPT-5204	14.577647	80.002935
			Rajupalem	MTU-1010	14.550652	79.992596
9		Pellakur	Nelaballi	MTU-1010	13.798089	79.764836
10		Venkatagiri	Konetirajupalem	NLR-34449	13.937336	79.626318
			Palemkota road	BPT-5204	13.962785	79.56164

Table 2. List of endophytic bacterial isolates collected from different mandals of Nellore and Chittoor Districts

S. No.	District	Mandal	Village	Variety	Designated Isolates	CFU's × 10^6/g of leaf
1		Yerpedu	Madibaka	BPT-5204	MB-1, MB-2, MB-3, MB-4	7.8^{f}
2		Srikalahasthi	Pudi	NLR-34449	EP-1, EP-2, EP-3, EP-4	5.0^{c}
3	Chittoor		Urandur	BPT-5204	EUD-1, EUD-2, EUD-3, EUD-4, EUD-6, EUD-7, EUD-8, EUD-9, EUD-10	4.0^{b}
4		Ramachandrapuram	Mallayapalli	BPT-5204	EMP-1, EMP-2, EMP-3, EMP-4, EMP-5, EMP-6, EMP-7, EMP-8, EMP-10	7.6^{f}
5		Kovur	Leguntapadu	MTU-1010	EBL-1, EBL-2, EBL-3, EBL-6	7.2^{ef}
6		Nellore rural	Agricultural research Station	NLR-34449	EBA-1, EBA-2, EBA-5	7.65^{f}
7		Vidavalur	Venkannapuram	BPT-5204	EBV-1, EBV-2	6.3^{d}
8	Nellore	Kodavalur	Vidavalur	BPT-5204	EBV-4, EBV-8	6.5^{de}
9			Rajupalem	MTU-1010	EBR-1, EBR-2	6.5^{de}
10		Pellakur	Nelaballi	MTU-1010	EBN-1, EBN-2	2.4^{a}
11		Venkatagiri	Konetirajupalem	NLR-34449	EBK-1, EBK-3	4.4^{bc}
			Palemkota road	BPT-5204	EBP-1, EBP-3	3.7^{b}
					C.D at 5 %	0.80
					SEm(±)	0.27
					C.V %	8.29

*Mean of three replications

**Means in a column followed by same super script letters are not significantly different according to DMRT.

Nagendran et al. (2013) who reported the isolation of endophytic bacteria on Tryptic Soya Agar Medium. Similarly Alam et al. (2017) isolated endophytes on Nutrient Agar Medium. Yousefi et al. (2018) isolated 63 bacterial endophytes by using serial dilution method from internal tissues of rice plants samples collected from different parts of north of Iran on Tryptic Soya Agar Medium. Swathi et al. (2023) Isolated 52 bacterial endophytes from rice on Nutrient agar, Triptic soy agar, Pseudomonas isolation agar and Yeast extract mannitol agar.

3.3 In vitro Evaluation of Endophytic Bacteria Against Bacterial Leaf Blight Pathogen

A total of 45 endophytic bacterial endophyte isolates were evaluated for their antagonistic efficacy against bacterial leaf blight (BLB) pathogen Xanthomonas oryzae pv. oryzae (Fig. 1). The diameter of inhibition zone formation ranged from 0.0 mm to 16.8 mm. Among 45 isolates, EMP-5 and EBK-3 isolates showed highest antagonistic efficacy with inhibition zone of 16.8 mm and 16.6 mm respectively. This was followed by EBA-5 with 15.9 mm isolate. The present study results are in accordance with Azman et al. (2017) who screened 93 bacterial strains against BLB pathogen. Among them, 16 isolates showed positive antagonistic activity indicated by the inhibition zone from 3.33 to 15.00 mm. Similarly Nagendran et al. (2013) screened forty endophytic bacterial isolates against Xoo to test their efficacy of inhibition and the results showed that isolates FZB24, EPB 9, EPB 10, EPCO 29 and EPCO 78 showed the maximum inhibition halo of 20 mm diameter. Yousefi et al. (2018) tested 39 bacterial isolates against Xoo strain. The maximum inhibitory activity was recorded for OS59 with an inhibition zone of 32.67 mm. Do., (2022) results indicated that the rice root endophytic bacteria (ND06 and ND10) produced a significant inhibition on the growth of M. oryzae with growth inhibition of 62.87% and 64.25%, respectively.

The Endophytic bacterial isolates viz.,EMP-5, MB-4, EBV-8, EMP-1, EUD-3, EBA-5, EMP-2, EBK-3, MB-1, EBA-2, EMP-10, EBR-2 which showed inhibition zone of above 10.00 mm diameter were considered as potential and studied for morphological features like colony form, margin of the colony, elevation, colour of the colony, its surface, opacity of the colony and reaction to Gram staining.

3.4 Morphological Characterization of Potential Endophytic Bacteria

The 12 potential endophytic isolates were grown on NA medium at 28 ± 2 ºC for 48 h to record morphological characters. Among 12 isolates, EBA-5, EMP-1, EBA-2, EBK-3, EBV-8 bacterial isolates produced medium sized, circular, raised, undulate and rough, yellowish green colonies with Gram negative reaction. While EUD-3, EMP5 and MB-4 isolates produced punctiform, white, flat, irregular, shiny rods of Gram negative reaction. MB-1 isolate showed white colonies with smooth, irregular, crateriform, undulate and small rods with Gram positive. EMP-10, EBR-2 showed small, circular, tan coloured, mucoid, shiny, rough edge raised, short rods with Gram positive reaction. Whereas, EMP-2 isolate showed small colonies of cream colour, entire, convex and cocci shaped cells with Gram negative reaction (Table 3).

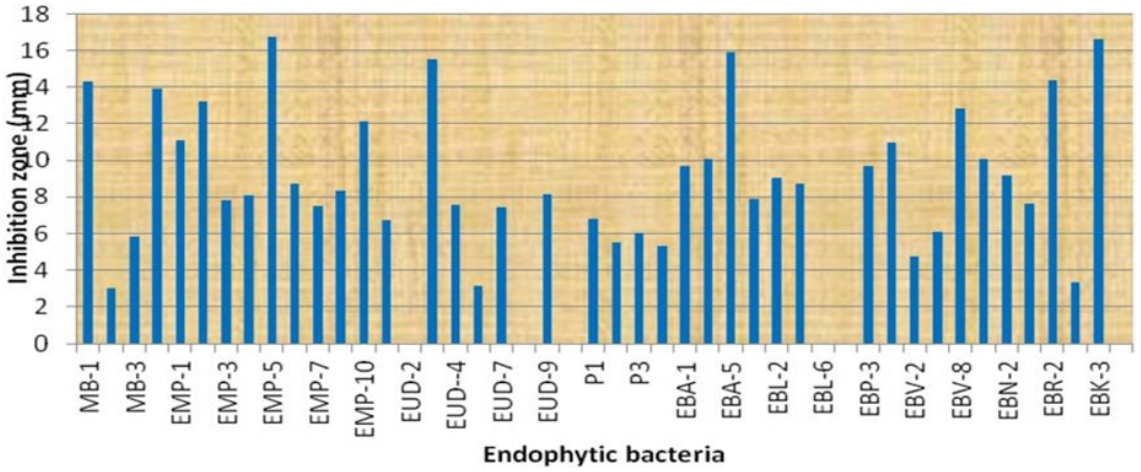

Fig. 1: Efficacy of endophytic bacteria against BLB pathogen (Xanthomonasoryzaepv.oryzae) under In vitro condition

Table 3. Morphological characteristics of Endophytic bacterial isolates

S.No.	Bacterial isolates	Colony size	Shape	Colour	Appearance	Gram reaction	Bacterial Shape
1	EMP-5	Punctiform	Irregular	White	Shiny, smooth, flat, entire	Gram Negative	Short Rods
2	MB-4	Punctiform	Irregular	White	Shiny, smooth, flat, entire	Gram Negative	Long Straight Rods
3	EBV-8	Medium sized	Circular	Yellowish green	Rough, raised, undulate	Gram Negative	Rods
4	EMP-1	Medium sized	Circular	Yellowish green	Rough, raised, undulate	Gram Negative	Rods
5	EUD-3	punctiform	Irregular	White	Shiny, smooth, flat, entire	Gram Negative	Short Rods
6	EBA-5	Medium sized	Circular	Yellowish green	Rough, raised, undulate	Gram Negative	Rods
7	EMP-2	Small	Circular	Cream	convex, entire, rough, raised	Gram Negative	Cocci
8	EBK-3	Medium sized	Circular	Yellowish green	Rough, raised, undulate	Gram Negative	Rods
9	MB-1	Medium sized	Irregular	Off white	Smooth, irregular, crateriform, undulate	Gram Positive	Small Rods
10	EBA-2	Medium sized	Circular	Yellowish green	Rough, raised, undulate	Gram Negative	Rods
11	EMP-10	Small	Circular	Tan	Convex, mucoid, entire, shiny, rough edge, raised	Gram Positive	Short Rods
12	EBR-2	Small	Circular	Tan	Convex, mucoid, entire, shiny, rough edge, raised	Gram Positive	Rods

Among the twelve isolates of endophytic bacteria, three isolates were Gram positive and nine isolates were Gram negative. In the Gram positive all isolates are rods in shape. Whereas among Gram negative eight isolates were with rod shaped cells and one isolate showed cocci shaped cells. Morphological characters of the isolates revealed the dominance of gram negative bacteria over the gram positive bacteria. An earlier study reported a predominance of Gram negative bacteria in the tissues of rice grains (Ashfaq et al., 2013, Yousefi et al. 2018).

3.5 Characterization of Potential Endophytic Bacteria for Plant Growth Promoting Traits

The Endophytic bacterial isolates viz., EMP-5, MB-4, EBV-8, EMP-1, EUD-3, EBA-5, EMP-2, EBK-3, MB-1, EBA-2, EMP-10, EBR-2 which showed inhibition zone of above 10.00 mm diameter were considered as potential and studied for plant growth promoting traits viz., IAA production, phosphate solubilization, siderophore production, Ammonia, HCN, starch hydrolysis and protease production.

3.5.1 Indole Acetic Acid (IAA) production

Out of 12 endophytic bacterial isolates, four bacterial isolates i.e., EMP-5, EMP-2, EMP-10 and EUD-3 producion of high amount of IAA with dark red ring colour indicated as +++. Whereas EBR-2, EBA-2

isolates produced medium amount of IAA with red ring indicated as ++. Isolates viz., MB-4, EBA-5, EBV-8 showed negative reaction by forming green colour ring indicated as in Table 4. In plant growth and development, indole acetic acid (IAA), a phytohormone belonging to auxin class plays an important role. Synthesis of IAA by bacteria on interaction with plant is well documented. Similar results were obtained by Alam et al. (2017) isolated six endopytic bacteria from rice and the maximum IAA was produced by BRRh-2, followed by BRRh-1.

3.5.2 Hydrogen cyanide (HCN) production

Among 12 isolates, five isolates viz., EMP-5, MB1, MB-4, EBR-2 and EBA-5 showed the strong reaction of HCN production while Isolates viz., EMP-2, EBA-2, EMP-10 and EMP-1 showed moderate reaction of HCN production. Three isolates viz., EBK-3, EUD-3, EBV-8 showed negative reaction by exhibiting no change in colour from yellow to orange (Table 4).

3.5.3 Ammonia production

Among 12 isolates, MB-1, MB-4, EBK-3, EBR-2, EMP-10 and EBV-8 produced strong dark brown colour to yellow showing significant ammonia production while four isolates viz., EMP-2, EBA5, EMP-5 and EMP-1 produced faint yellow indicating moderate ammonia production. Two isolates EUD-3 and EBA-2 produced dark green indicating no ammonia production (Table 4).

3.5.4 Phosphate solubilisation

Out of the 12 isolates, ten isolates showed positive reaction by producing clear halo zone around the colony ranging from 0.0 mm to 3.20 mm diameter (Table 4). The highest phosphate solubilization of 3.20 mm was shown by EMP-5 isolate while the lowest phosphate solubilization was shown by EBA-2 and EMP-1 isolate of 0.00 mm.

Phosphorus (P) is the essential macronutrient that plays a vital role in many physiological activities such as cell division, photosynthesis and development of root system. These results are in agreement with Sev et al. (2016), who reported that among 18 rice endophytic bacteria tested for phosphate solubilization halo zone from 3.0-4.5 mm diameter on Pikovskaya's medium (Ahmad and Singh 1975).

3.5.5 Protease production and Amylase activity

12 endophytic bacterial isolates were evaluated for the protease production. Protein hydrolysis in terms of halo zone among the bacterial endophytes ranged from 0.4 cm to 3.5 cm. (Table 4). Among the 12 endophytic bacterial isolates, EMP-2 isolate showed a halo zone of diameter 3.5 cm after 5 days of incubation while MB-4 showed lowest diameter of halo zone of 0.4 cm. Enzymatic and antimicrobial activities are indirect mechanisms exhibited by bacterial endophytes for plant growth. These enzymes are responsible for hydrolytic actions which enable endophytes to penetrate plant tissue and establish symbiotic relationship between endophytes and host. Similar studies on bacterial endophytes of Curcuma have shown the production of amylases by the Bacillus (Kumar et al., 2016, Regina et al., 2018).

Table 4. The Different PGPR traits shown by endophytic bacterial isolates (Quantitative)

S.No	Isolates	IAA production	HCN	Ammonia	Amylase	Phosphate Solubilization Diameter of Halo Zone (mm	Siderophore production (mm)	Protease Activity (cm)
1	EMP-5	+++	+++	++	++	3.2	3.0	2.3
2	MB-1	+	+++	+++	-	2.6	2.1	1.4
3	MB-4	-	+++	+++	-	2.8	2.8	0.4
4	EBK-3	+	-	+++	-	1.0	2.5	1.6
5	EBR-2	++	+++	+++	+++	2.7	3.1	3.0
6	EMP-2	+++	++	++	+++	3.0	2.7	3.5
7	EBA-2	++	++	-	+++	0.0	2.5	1.1
8	EBA-5	-	+++	++	++	0.7	2.5	3.0
9	EMP-10	+++	++	+++	+++	2.4	1.6	3.0
10	EMP-1	+	++	++	+++	0.0	1.6	1.7
11	EUD-3	+++	-	-	+++	1.4	2.1	1.0
12	EBV-8	-	-	+++	+++	1.3	2.5	2.0

IAA production: Strong - dark red (+++), Moderate - light red (++), Weak –dark brown ring (+), Negative- dark green ring (-).

HCN production: Strong-orange to red colour (+++), Moderate- light orange (++), Negative- yellow (-).

Ammonia production: Strong- dark brown to yellow (+++), moderate – faint yellow (++), negative- dark green (-).

Amylase production: Strong –clear yellow zone (+++), moderate –light yellow (++), Negative blue zone (-) surrounding the bacterial growth.

Similarly, 12 endophytic bacterial isolates, seven isolates viz., EBA-2, EMP-2, EMP-1, EBV-8, EUD-3, EMP-10 and EBR-2 formed a clear yellow zone indicating strong amylase activity whereas EMP-5 and EBA-5 showed light yellow halo zone around indicating amylase activity. Remaining three isolates viz., EBK-3, MB-1 and MB-4 produced blue color around bacterial growth with iodine forming color complex with starch indicating no amylase (Table 4).

3.5.6 Siderophore production

In our present investigation, all the 12 endophytic bacterial isolates plated on Chromo azurol–S agar medium amended with ferric chloride showed halo zone with orange colour after incubation of five days which is considered as a positive response. The halo zone produced by the isolates ranged from 1.6 mm to 3.1 mm. EBR-2 isolate showed maximum siderophore production with halo zone of 3.1 mm diameter followed by isolate EMP-5 with 3.0 mm diameter (Table 4). Siderophores are low molecular weight iron binding protein compounds involved in the process of chelating ferric iron (Fe+3) in the soil. When iron availability in the soil is limited, microbial siderophores provide iron and enhance the plant growth.

Plant Growth Promoting (PGP) traits of 12 isolates, EMP-5 showed positive to all PGP trait namely, IAA production, Phosphate solubilization, Siderophore production (3.0 mm), HCN, Ammonia Production, Amylase activity (light yellow) and Protease production (2.3 mm).

Swathi et al. (2023) characterized 52 bacterial endophytes based on biochemical, plant growth promoting activities, nutrient solubilization and molecular characterization and compatibility four potential isolates EBP26, EBP39, EBP47 and EBP52 identified as B.subtilis, Pseudomonas fluorescence, Pseudomonas sp. and Pantoea dispersa developed as bacterial consortium. The results were in agreement with Tu et al (2024) conducted in vitro assays showed Bacillus velezensis LS123N inhibited spore germination and normal growth of germ tubes of B. oryzae, produced multiple

hydrolases, siderophores, IAA and had the ability to solubilize phosphorus compounds.

Faisal Yousuf et al. (2025) isolated four endophytic bacteria (IIRR-F1, IIRR-F2, IIRR-F3 and IIRR-F4) from the rice roots and evaluated for a variety of PGP characteristics and observed IIRR-F1 and IIRR-F2 were capable of producing IAA, Phosphate solubilization ability was detected in isolates IIRR-F2 and IIRR-F4, Siderophore production was observed in isolate IIRR-F2.

4. CONCLUSION

Plant disease suppression and growth promotion are complex interactive processes that may be addressed by plant heightening using endophytic bacteria possessing plant growth promoting traits. The results strongly emphasize that endophytic bacteria (EMP-5) characterized in the study could be efficiently used for management of bacterial leaf blight and for plant growth promotion. The isolate has the potential to be developed into a bio-inoculant for sustainable rice production.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Assessment of Heterosis and Combining Ability for Quality Traits in Tomato (Solanum lycopersicum L.) Using Line × Tester Analysis

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ABSTRACT

The present study Explored about heterosis and combining ability analysis for quality traits in tomato (Solanum lycopersicum L.) Using Line × Tester analysis. Heterosis and combining capacity are two vital contemplations within the utilization of heterosis, which can be utilized to produce amazing hybrid asset candidates and is exceptionally vital in customary hybrid breeding. The combining capacity and heterosis of eight major agronomic characteristics were analyzed in 8 tomato guardians and 15 crosses between them. As well as EC- 163605, a recognized and official great assortment that's as of now offering well on the showcase was utilized as a control to conduct a control heterosis examination, with the objective of selecting perfect parents with tall combining capacity and modern hybrids with product esteem, plant tallness, natural product distance across, add up to abdicate per plant. The comes about appears that both additive and non-additive hereditary impacts are included within the expression of the characteristics and the additive hereditary impact is prevailing in characteristic legacy. In spite of the fact that common combining capacity (GCA) and specific combining capacity (SCA) were not correlated, and the quality of heterosis depends on SCA, the entirety of the parental GCA values (GCA sum) did predict heterosis for a few characteristics with higher predictive accuracy than did SCA. The variance attributable to GCA and SCA, which provide a relative measure of additive and non-additive gene effects, respectively, is estimated using the combining ability analysis. Because most yield components are known to be polygenic, plant breeders would need to perform phenotypic assessments on as many parents as feasible to determine their genetic makeup.

Keywords: Combining ability; heterosis; line × tester; tomato; vegetable crops.

1. INTRODUCTION

Heterosis and combining ability are two important considerations in the utilization of heterosis, which can be used to generate excellent hybrid resource candidates and is very important in conventional hybrid breeding (Reddy et al., 2023). Analyzing heterosis and combining ability are two important considerations in the utilization of heterosis; it is the first step in breeding inbred lines to develop commercial hybrids. Progeny selection is one of the most important stages in plant breeding, but producing excellent progeny depends on the chosen parents. Combining ability is useful for successfully predicting the genetic capabilities of parental lines and crosses (Liu et al., 2021; Izzo et al.,

2022). Tomato is generally treated as “defensive nourishment” in India, tomato is developed in almost 0.87 million hectares uninterested parts and is well adjusted to shifted climatic conditions of the nation. Its generation is about 16.81 million tons, and its efficiency is 19.5mt./ha. (Anonymous, 2011). In created nations it is commonly devoured new; over 80% of the tomato utilization comes from handled items such as juice, glue, puree, it has tall nutritional esteem; one medium new tomato (135g) gives 47% Recommended Dietary Allowance (RDA) of vitamin C, 22% RDA vitamin A and 25 calories. The mash and juice are edible, a promoter of gastric emission and blood purifier. It has antiseptic properties against intestinal infections. The line x Tester mating design is essentially an important for development of top cross analysis in that multiple testers are used as opposed to just one in top cross. Together and individually, they all contribute a shared genetic background that the inbreds' genotype is measured against A line is tested due to the utilization of several testers in vegetable crops (Singh et al., 2024). Tomato may be a great appetizer and its soup is said to be a great cure for quiets enduring constipation. It is one of the finest vegetables which keeps our stomach and digestive tract in arrange. Different breeding techniques have been supported considering the breeding conduct of trim species. Out of these hybrids, breeding is noticeable and utilized within the enhancement of vegetable crops. Heterosis in tomato was, to begin with, watched by Hedrick and Booth (1968) for higher abdicate and more number of natural products per plant. Choudhary et al. (1965) emphasized the broad utilization of heterosis to step up tomato generation. The heterosis sign in tomato is in the frame of the more noteworthy energy, faster development and improvement, earliness in development, and expanded efficiency (Yordanov, 1983). So, an expedient change can be brought about by misusing heterosis for different surrendercontributing characteristics as well as earliness. Combining capacity features a prime significance in plant breeding since it gives information for the determination of guardians conjointly gives information with respect to nature of the quality activity. The information on hereditary structure and mode of legacy of distinctive characters makes a difference breeder to utilize appropriate breeding methodology for their advancement (Kiani et al. 2007). The concept of combining ability was introduced by Sprague and Tatum (1947).

2. MATERIALS AND METHODS

The examination entitled “line x tester mating arrange for abandon and yield characteristics in tomato (Solanum lycopersicum L.)” was carried out at the Test Develop, Mata Gujri College, Fatehgarh Sahib, Punjab, in the midst of winter Season, 2020-2021 and 2021-2022. The subtle components of exploratory area, texture utilized and strategies utilized in the midst of course of appear the examination. Eight different tomato cultivars /lines viz., EC-163605, EC631364, EC-164563, EC-145057, EC-620395, EC-249504, EC-631379, EC-620427 were crossed in a line x tester, so get 15 cross combination. The seedlings of guardians were raised in February, 2021 and encourage transplant in inquire about cultivate to endeavour crosses and create F1's.

2.1 Estimation of Heterosis

The data were subjected to statistical analysis according to Steel and Torrie, (1980) Heterosis was examined over the superior parent (heterobeltiosis), over the mid parent and over the standard variety, i.e., Standard checks (economic heterosis), following the method described by Kempthorne (1957): -

$$\text{Mid parent} = \frac{\bar{F}_1 - \overline{MP}}{\overline{MP}} \times 100$$

$$\text{Heteroeltiosis} = \frac{\bar{F}_1 - \overline{BP}}{\overline{BP}} \times 100$$

$$\text{Economic check} = \frac{\bar{F}_1 - \overline{EC}}{\overline{EC}} \times 100$$

Where,

$\bar{F}_1$ = Mean value of the F_1generation
$\overline{MP}$ = Mean performance of mid parent;
$\overline{BP}$ = Mean value of the better parent in the respective cross combination
$\overline{EC}$ = Mean value of the economic cultivar (check).

2.2 Estimation of Combining Capacity Impacts

The combining ability analysis was carried out by the procedure given by Griffing (1956). Method-2 and Model-1 was adopted for the present study. Method-2 includes P inbreds (parents) and P (P-1)/2 F1s, in all P (P+1)/2 different genotypes which form a set of treatments. Model-1 is also known as fixed effect model in which inferences drawn are applicable only to the lines (treatments) involved in the experiment and not beyond these errors. The statistical model for combining ability analysis under Model-1 is: -

$$Y_{ijk} = \mu + g_i + g_j + S_{ij} + \frac{1}{b}\sum_i \sum_k e.ijk$$

For i, j = 1, 2,….., P (number of parents);

K = 1, 2,….., b (number of replications),

Where,

μ = Populationmean
g_i= General combining ability effect of i^{th} parent
g_j= General combining ability of j^{th} parent
S_{ij}= Specific combining activity effect of ij^{th} combination
Such that $S_{ij} = S_{ji}$
E_{ijk}= The environmental effect pertaining to ijk^{th} observation.

The restrictions imposed on the model are:

$$\sum_i gi = o \text{ and } \sum_j Sij + Sii = o \text{ for each}$$

2.3 Estimation of the General Combining Ability and Specific Combining Ability Effects

The following formulae were adopted to determine the G.C.A. and S.C.A.:

General combining ability (GCA effects of i^{th} parent was calculated as:

$$\hat{g}_i = \frac{1}{p+2}(Y_{io} + Y_{oi} - \frac{2}{p}Y_{oo}$$

Specific combining ability (SCA effects of ijthcross was calculated as

$$\hat{S}_{ij} = \frac{1}{p+2}(Y_{io} + Y_{oi} + Y_{jo} + Y_{oj}) + \frac{2}{(p+1)(p+2)}Y_{oo}$$

3. RESULTS AND DISCUSSION

Gauges of cruel squares for all the characters considered were exceedingly noteworthy demonstrating wide hereditary contrasts among the genotypes. Heterosis was estimated in Table 5. Average fruit weight showed positive heterosis over mid-parent range varies from EC-620427 x EC-163605 to EC-145057 x EC- 164563 EC620427 x EC-163605 to EC-249504 x EC164563 showed significant negative heterosis over better parent for average fruit weight heterosis for the trait fruit weight was reported by many authors as Scott et al. (1986). Fruit shape index revealed Table 6 positive heterosis range varies from EC-145057x EC- 163605 to EC- 249504 x EC- 631379 showing significant positive heterosis over better parent for fruit shape index. Fruit diameter revealed Table 5 positive heterosis over mid-parent range varies from EC-620427 x EC-163605 to EC-145057 x EC- 164563. Positive heterosis over better parent EC-620427 x EC-163605 (232.31) to EC249504 x EC-164563.Ascorbic acids revealed Table 5 run shifts from the EC- 620427 x EC- 163605 to EC- 249504 x EC-164563. Lycopene substance appears the positive heterosis run changes from the EC- 249504 x EC-163605 to EC-620395 x EC-631379. Positive heterosis over mid parent range varies from EC-631379 x EC163605 to EC-620395 x EC-631379. Heterosis appeared the higher pericarp thickness revealed in Table 4 over better parent positive range varies from EC-631364 x EC-163605 to EC249504 x EC-164563. Heterosis appeared in the total soluble solids revealed Table 7 positive heterosis over mid parent range varies from EC- 631364 x EC-164563to EC-620395 x EC631379. Positive heterosis over better parent range varies from EC-631364 x EC-164563 to EC-631364 x EC-163605. Titrable acidity recorded in Table 4 positive heterosis over mid parent range varies from EC- 631364 x EC163605 to EC-620395 x EC-631379. Heterosis appeared in the EC-620395 x EC-164563 to EC620427 x EC-163605 and showed significant positive heterosis over better parents for Titrable acidity. Lycopene content recorded in Table 5 positive heterosis over mid parent range varies from EC- 249504 x EC-163605 (163.42) to EC620395 x EC-631379 (19.07). Lycopene content ranges vary from EC-249504 x EC- 164563 and showed significant negative heterosis over mid parent for lycopene content. EC-620427 x EC163605 to EC-620395 x EC-631379 showed significant positive heterosis over better parents for lycopene content. Total yield per plant revealed Table 7 shows significant positive heterosis over the mid-parent range varies from EC-620427 x EC-163605 to EC-249504 x EC- 164563. EC- 620427 x EC-163605 to EC- 249504 x 631379 shows significant positive heterosis over better parent for total yield per plant. Singh and Singh (1993) and Ahmed et al. (1988) also reported heterosis over better parents in yield per plant or total yield in tomato.

Heterosis for the trait of fruit weight was reported by many authors as Scott et al. (1986). Singh and Singh (1993) and Ahmed et al. (1988) also reported heterosis over better parents in yield per plant or total yield in tomato. Shankarappa et al. (2008), Kumar et al. (2006), Singh et al. (2007), and Singh, et al. (2008) also reported heterosis over fruit shape index.

3.1 Examination of Common Combining Capacity Impact of Distinctive Characteristics in Parents

The estimate of GCA effects revealed that out of 8 parents, Table number 3 average fruit weight EC-631379 and EC- 620427 recorded significant and positive GCA effects. While the (EC- 163605) to (EC- 145057) exhibited significant negative GCA effects for this trait. Fruit diameter range varies from EC- 163605 and EC- 620395 recording significant and positive GCA effects. On other hand, (EC-164563) to (EC- 631364) exhibited significant negative GCA effects for this trait. Fruit shape index EC- 163605 and EC- 620427 recorded significant and positive GCA effects. On other hand, (EC-631379) to (EC- 620395) exhibited significant negative GCA effects for this trait. Pericarp thickness EC- 631364 and EC- 620427 recorded significant and positive GCA effects. On another hand, EC-249504 negative GCA effect for this trait. Total soluble solids EC- 145057 recorded significant and positive GCA effects. On other hand, (EC- 631364) to (EC- 145057) exhibited significant negative GCA effects for this trait. The ascorbic acidity range varies from EC- 163605 and EC- 145057 recording significant and positive GCA effects. On other hand, (EC- 620395) to (EC- 631364) exhibited significant negative GCA effects for this trait. Titrable acidity range varies EC- 163605 and EC- 620427 recorded significant and positive GCA effects. On other hand, (EC- 164563) to -0.085 (EC- 631364) exhibited significant negative GCA effects for this trait. Lycopene content range varies from EC- 631364 and EC- 620427 recorded significant and positive GCA effects. On other hand, (EC-620395) to (EC- 249504) exhibited significant negative GCA

Table 1. Analysis of variance forparents and hybrid and check various characters in tomato

Source of variation	DF	Plant Height (cm)	Days to first flowering	Days to 50% Flowering	No. of Pri. Branches	No. of Sec. Branches	No.of Fruit / Plant	Average fruit wt (g)	Fruit Diameter (mm)
Replicates	2	31.403	15.406 **	7.667 *	0.565	0.304	10.764	3.652	15.014 *
Treatments	22	1311.907 ***	31.028 ***	28.589 ***	23.692 ***	9.269 ***	482.456 ***	775.477 ***	441.814 ***
Error	44	22.177	2.057	1.727	1.565	1.213	5.514	6.319	3.257
Total	68	439.714	11.823	10.593	8.694	3.793	159.973	255.086	145.489

Source of variation	DF	Fruit Shape Index	No. Fruit / Cluster	Pericarp thickness (mm)	TSS (Brix)	Ascorbic acidity (100g)	Titratable Acidity (%)	Lycopene Content	Total yield per plant (g)
Replicates	2	0.001	0.115	0.081	0.171	2.885	0.002	0.130	19508.800
Treatments	22	0.328 ***	2.184 ***	4.571 ***	4.008 ***	113.052 ***	0.146 ***	4.787 ***	2198712.000 ***
Error	44	0.005	0.115	0.169	0.179	9.024	0.008	0.102	26122.780
Total	68	0.110	0.785	1.591	1.418	42.499	0.052	1.618	728824.900

Table 2. Analysis of variance forparents and hybrid and check various characters in tomato

Source of variation	DF	Plant Height (cm)	Days to first flowering	Days to 50% Flowering	No. of Pri. Branches	No. of Sec. Branches	No.of Fruit / Plant	Average fruit wt (g)	Fruit Diameter (mm)
Replicates	2	9.867	5.600 *	4.956	0.422	0.867	9.398	6.822	7.756
Cross	14	752.200 ***	43.952 ***	38.898 ***	21.041 ***	0.248 ***	189.728 ***	337.728 ***	425.103 ***
Line effect	4	1031.311	59.278	58.589	44.478	10.478	287.974	308.945 ***	1281.144 **
Tester effect	2	468.367	16.200	11.822	7.622	6.067	4.485	112.156	24.422
Line x Tester effect	8	683.478 ***	43.223 ***	35.822 ***	12.678 ***	7.678 ***	186.915 ***	409.322 ***	98.478 ***
Error	28	5.224	1.338	0.164	1.637	1.486	3.929	5.084	3.875
Total	44	243.109	15.091	13.165	7.756	2.609	63.295	111.074	138.301

Table 3. General Combing Ability effects of parents for different characters

	Average fruit wt (g)	Fruit Diameter	Fruit Shape Index	Pericarp Thickness	TSS (Brix)	Ascorbic acidity(100g)	Titratable Acidity (%)	Lycopene content	Total yield per plant (g)
EC- 249504	-3.622 ***	-11.289 ***	0.036	-1.522 ***	-0.333 *	2.002	-0.070 *	-0.806 ***	-707.222 ***
EC- 631364	3.267 ***	-13.733 ***	-0.039	0.913 ***	-0.050	-4.610 ***	-0.085 **	0.400 ***	-633.778 ***
EC- 620427	5.267 ***	3.711 ***	0.264 ***	0.918 ***	1.024 ***	2.534 *	0.161 ***	0.527 ***	367.222 ***
EC- 145057	-8.511 ***	8.156 ***	0.038	-0.160	-0.753 ***	3.169 **	-0.061 *	0.162	38.778
EC- 620395	3.600 ***	13.156 ***	-0.299 ***	-0.150	0.112	-3.095 **	0.055	-0.283 *	935.000 ***
EC- 163605	-2.111 **	1.356 **	0.039 *	-0.186	0.148	1.885 *	0.115 ***	0.415 ***	-13.533
EC- 631379	3.089 ***	-0.178	-0.150 ***	0.081	-0.089	-1.169	-0.031	-0.053	170.200 ***
EC- 164563	-0.978	-1.178 *	0.111 ***	0.106	-0.059	-0.716	-0.084 ***	-0.362 ***	-156.667 ***
CD 95% GCA(Line)	1.716	1.232	0.049	0.281	0.289	2.051	0.060	0.218	110.359
CD 95% GCA(Tester)	1.330	0.955	0.038	0.217	0.224	1.589	0.047	0.169	85.484

Table 4. Specific combining abilityeffects of hybrids for different characters

Hybrids	Average fruit	Fruit Diameter	Fruit Shape Index	Pericarp	TSS (Brix)	Titratable Acidity (%)	Titrable	Lycopene	Total yield per
EC-249504 x EC-163605	-7.778 ***	0.089	0.017	-1.467 ***	-1.566 ***	-5.642 **	0.177 **	2.051 ***	-200.911 *
EC-249504 x EC-631379	-0.311	4.956 ***	0.072	-0.474	0.208	9.648 ***	-0.110 *	-0.227	182.022
EC-249504 x EC-164563	8.089 ***	-5.044 ***	-0.089 *	1.941 ***	1.358 ***	-4.005 *	-0.067	-1.825 ***	18.889
EC- 631364 x EC-163605	1.333	4.533 ***	-0.395 ***	0.782 **	-0.202	-0.147	0.085	-1.934 ***	331.311 **
EC- 631364 x EC-631379	-8.533 ***	-2.600 *	-0.097 *	-0.322	-0.616 *	-2.937	0.111 *	-0.282	-460.756 ***
EC- 631364 x EC-164563	7.200 ***	-1.933	0.492 ***	-0.460	0.818 **	3.084	-0.196 ***	2.216 ***	129.444
EC- 620427 x EC- 163605	14.333 ***	-7.911 ***	-0.174 ***	0.066	0.654 *	6.083 **	-0.011	0.505 *	176.644
EC- 620427 x EC- 631379	7.467 ***	-1.711	0.038	0.439	0.268	-3.540	-0.021	0.113	263.911 **
EC- 620427 x EC- 164563	-21.800 ***	9.622 ***	0.136 **	-0.506 *	-0.922 ***	-2.543	0.032	-0.618 **	-440.556 ***
EC-145057 x EC- 163605	-6.889 ***	2.978 **	0.578 ***	0.871 **	1.134 ***	-0.779	-0.132 *	-0.013	-243.911 *
EC-145057 x EC- 631379	4.578 **	-2.489 *	-0.120 **	-0.003	-0.299	-2.709	-0.015	0.052	82.689
EC-145057 x EC- 164563	2.311	-0.489	-0.458 ***	-0.868 **	-0.836 **	3.488	0.147 **	-0.039	161.222
EC- 620395 x EC-631379	-3.200 *	1.844	0.107 *	0.360	0.439	-0.461	0.035	0.343	-67.867
EC- 620395 x EC- 164563	4.200 **	-2.156 *	-0.081	-0.108	-0.418	-0.024	0.084	0.265	131.000
CD 95% SCA	2.973	2.134	0.086	0.486	0.501	3.553	0.104	0.377	191.147

Table 5. Estimates of mid parent (MP), better parent (BP) and standard parent heterosis for different quality in tomato

Hybrids	Fruit diameter			Average fruit weight			Ascorbic acidity		
	Mean	Mid	Better	Mean	Mid	Better	Mean	Mid	Better
EC-249504 x EC-163605	30.67	-3.66	-31.85 **	41	92.19 **	39.77 **	29.3	48.97 **	18.8
EC-249504 x EC-631379	34	-11.30 **	-24.44 **	53.67	50.47 **	27.78 **	41.54	77.18 **	68.41 **
EC-249504 x EC-164563	23	-42.02 **	-48.89 **	58	53.30 **	25.18 **	28.34	18.87 *	14.89
EC- 631364 x EC-163605	32.67	83.18 **	75.00 **	57	222.64 **	159.09 **	28.19	38.60 **	8.41
EC- 631364 x EC-631379	24	-1.37	-24.21 **	52.33	63.54 **	24.60 **	22.34	-7.33	-14.06
EC- 631364 x EC-163563	23.67	-7.79	-31.07 **	64	87.32 **	38.13 **	28.82	17.58	10.83
EC- 620427 x EC- 163605	37.67	69.92 **	46.75 **	72	311.43 **	232.31 **	41.56	107.77 **	64.05 **
EC- 620427 x EC- 631379	42.33	47.67 **	33.68 **	70.33	120.94 **	67.46 **	28.88	21.47 *	14.01
EC- 620427 x EC- 164563	52.67	75.56 **	53.40 **	37	8.82	-20.14 **	30.33	25.47 **	19.74
EC-145057 x EC- 163605	53	110.60 **	67.37 **	37	89.74 **	44.16 **	35.33	53.60 **	12.77
EC-145057 x EC- 631379	46	45.26 **	45.26 **	53.67	58.62 **	27.78 **	30.35	13.34	-3.14
EC-145057 x EC- 164563	47	42.42 **	36.89 **	47.33	31.48 **	2.16	37	36.15 **	18.09 *
EC- 620395 x EC- 163605	55.33	137.14 **	97.62 **	55	118.54 **	48.65 **	30.33	54.21 **	22.97 *
EC- 620395 x EC-631379	55.33	85.47 **	74.74 **	58	46.84 **	38.10 **	26.33	12.32	6.76
EC- 620395 x EC- 164563	50.33	61.50 **	46.60 **	61.33	47.20 **	32.37 **	27.22	14.18	10.36

Table 6. Estimates of mid parent (MP), better parent (BP) and standard parent heterosis for different quality in tomato

Hybrids	Lycopene content			fruit shape index			Pericarp thickness		
	Mean	Mid	Better	Mean	Mid	Better	Mean	Mid	Better
EC-249504 x EC-163605	5.86	163.42 **	117.99 **	1.32	65.34 **	55.91 **	2.49	-46.28 **	-53.31 **
EC-249504 x EC-631379	3.11	1.08	-10.29	1.19	39.06 **	24.04 **	3.75	-23.26 **	-29.69 **
EC-249504 x EC-164563	1.2	-65.91 **	-72.48 **	1.29	47.05 **	28.67 **	6.19	22.45 **	16.06 *
EC- 631364 x EC-163605	3.08	-8.39	-37.93 **	0.83	1.42	-1.57	7.17	102.07 **	82.22 **
EC- 631364 x EC-631379	4.26	1.15	-14.06 *	0.94	7.6	-1.39	6.34	66.68 **	42.72 **
EC- 631364 x EC-163563	6.45	38.26 **	30.13 **	1.79	99.63 **	79.33 **	6.22	56.76 **	30.29 **
EC- 620427 x EC- 163605	5.64	163.09 **	123.06 **	1.36	54.46 **	49.08 **	6.46	50.25 **	38.50 **
EC- 620427 x EC- 631379	4.78	59.53 **	37.98 **	1.38	47.86 **	44.25 **	7.1	56.00 **	52.21 **
EC- 620427 x EC- 164563	3.74	8.45	-14.41 *	1.74	82.20 **	74.00 **	6.18	30.96 **	29.45 **
EC-145057 x EC- 163605	4.76	117.02 **	81.22 **	1.88	122.44 **	122.44 **	6.19	59.33 **	57.24 **
EC-145057 x EC- 631379	4.36	43.00 **	25.67 **	1	10.54	4.18	5.58	34.97 **	25.75 **
EC-145057 x EC- 164563	3.96	13.05	-9.53	0.92	-0.36	-8	4.74	10.18	-0.7
EC- 620395 x EC- 163605	3.72	38.98 **	3.53	0.94	6.19	1.43	5.08	9.53	-4.81
EC- 620395 x EC-631379	4.2	19.07 **	16.98 *	0.89	-6.01	-7.32	5.96	21.90 **	11.69
EC- 620395 x EC- 164563	3.82	-4.18	-12.73 *	0.96	-0.52	-4	5.51	9.07	3.37

Table 7. Estimates of mid parent (MP), better parent (BP) and standard parent heterosis for different quality in tomato

Hybrids	Total soluble solids			Titrable acidity			Total yield per plant		
	Mean	Mid	Better	Mean	Mid	Better	Mean	Mid	Better
EC-249504 x EC-163605	3.5	-20.00 **	-25.00 **	0.87	100.00 **	47.73 **	1257.67	58.86 **	-5.75
EC-249504 x EC-631379	5.04	8.86	7.93	0.43	-27.98 *	-29.73 *	1824.33	30.23 **	24.33 *
EC-249504 x EC-164563	6.22	65.93 **	33.21 **	0.42	19.25	-27.84 *	1334.33	21.47 *	0
EC- 631364 x EC-163605	5.15	49.54 **	26.04 **	0.76	267.74 **	171.43 **	1863.33	318.88 **	190.84 **
EC- 631364 x EC-631379	4.5	21.75 *	-1.96	0.64	70.67 **	3.78	1255	19.07	-14.47
EC- 631364 x EC-163563	5.96	111.85 **	110.85 **	0.28	118.18 *	110	1518.33	102.00 **	76.00 **
EC- 620427 x EC- 163605	7.08	79.92 **	73.31 **	0.91	80.79 **	25.23 *	2709.67	472.26 **	288.20 **
EC- 620427 x EC- 631379	6.45	54.20 **	40.70 **	0.75	12.16	3.67	2980.67	175.31 **	103.13 **
EC- 620427 x EC- 164563	5.29	60.16 **	39.91 **	0.75	77.25 **	3.67	1949.33	149.81 **	125.97 **
EC-145057 x EC- 163605	5.78	52.71 **	41.55 **	0.57	19.72	-15	1960.67	272.87 **	144.27 **
EC-145057 x EC- 631379	4.11	1.82	-10.39	0.54	-16.36	-19.5	2471	117.71 **	68.40 **
EC-145057 x EC- 164563	3.6	14.15	3.35	0.65	63.71 **	-3	2222.67	166.93 **	157.65 **
EC- 620395 x EC- 163605	5.49	14.45 *	-0.36	0.7	136.16 **	124.73 **	3037.67	194.78 **	67.64 **
EC- 620395 x EC-631379	5.71	13.17 *	3.69	0.7	51.80 **	14.05	3216.67	96.18 **	77.52 **
EC- 620395 x EC- 164563	4.89	17.23 *	-11.31	0.7	223.08 **	125.81 **	3088.67	130.96 **	70.46 **

effects for this trait. Total yield per plant varied from EC- 631379 and EC- 620395 recording significant and positive GCA effects. On other hand, (EC- 164563) to (EC- 249504) exhibited significant negative GCA effects for this trait. These findings are in close agreement with Hannan et al. (2007), Kumar et al. (2013), and Shankar et al. (2013) on tomato crops.

The SCA effect is recorded in Table 4. Shows that the average fruit weight range varies from (EC-620427 x EC- 163605) to (EC- 620395 x EC- 164563) show significant positive SCA (EC- 620395 x EC-631379) to (EC- 620427 x EC- 164563) shows significant negative SCA for this trait. Fruit diameter range varies from (EC- 620427 x EC- 164563) to (EC-145057 x EC- 163605) and shows significant positive SCA as well range varies from (EC- 620395 x EC- 164563) to (EC- 620427 x EC-163605) shows significant negative SCA for this trait. The fruit shape index range varies from (EC-145057 x EC- 163605) to (EC- 620395 x EC-631379) shows significant positive SCA as well (EC249504 x EC-164563) to (EC-145057 x EC- 164563) shows significant negative SCA for this trait. Pericarp thickness range varies from (EC249504 x EC-164563) to (EC- 631364 x EC163605) shows significant positive SCA as well varies from (EC- 620427 x EC- 164563) to (EC249504 x EC-163605) shows significant negative SCA for this trait. Total soluble solids cross range varies from (EC- 620427 x EC- 163605) to (EC- 49504 x EC-164563) and shows significant positive SCA as well (EC- 631364 x EC-631379) to (EC-249504 x EC-163605) shows significant negative SCA for this trait. Ascorbic acidity range varies from (EC-249504 x EC-631379) to (EC- 620427 x EC- 163605) shows significant positive sca varies from (EC-249504 x EC-163605) to (EC-249504 x EC-164563) shows significant negative sca for this trait. Titrable acidity range varies from (EC-249504 x EC-163605) to (EC- 631364 x EC-631379) shows significant positive SCA, as well as negative crosses, range varies from (EC-631364 x EC-164563) to (EC-249504 x EC-631379). Lycopene content crosses range varies from (EC-631364 x EC-164563) to (EC- 620427 x EC- 163605) and shows significant positive SCA as well range varies from (EC249504 x EC-164563) to (EC- 620427 x EC- 164563) shows significant negative SCA for this trait. Total yield per plant range varies from (EC- 631364 x EC-163605) to (EC- 620427 x EC-631379) shows significant positive SCA as well range varies from EC-249504 x EC-163605 to (EC-631364 x EC-631379) show significant negative SCA for this trait. Some studies also report greater participation of additive effects on the expression of the average fruit weight, such as Amaral Júnior et al. (1999), Garg et al. (2008).

4. CONCLUSIONS

The high yielding F1 hybrid (EC- 620427 x EC- 163605) had an 83.43 percent heterosis for yield above the mid parent and could be recommended for commercial use. The variance attributable to GCA and SCA, which provide a relative measure of additive and non-additive gene effects, respectively, is estimated using the combining ability analysis. Because most yield components are known to be polygenic, plant breeders would need to perform phenotypic assessments on as many parents as feasible to determine their genetic makeup. The general (GCA) and specialized (SCA) combining ability impacts are some practical factors to suit this goal. It was concluded that the SCA combiner of the F1 hybrid (EC-6313 64 x EC-163605) produced a greater yield in tomatoes.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Assessment of Genetic Diversity through Morphometric and Biochemical Traits in Trapa natans var. spinosa Roxb. from Southwestern Uttar Pradesh, Etawah and Auraiya Districts, India

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ABSTRACT

This study evaluated genetic diversity in Trapa natans var. bispinosa Roxb. from southwestern Uttar Pradesh using morphometric and biochemical traits. During this study, twenty genotypes collected from Etawah and Auraiya districts showed significant variation in fruit. The morphometric parameters namely, length varied from 31.54mm to 51.62mm, breadth 47.48mm to 58.19mm, weight 15.27mm to 29.84mm, volume 17ml to 34.58ml, and specific gravity 0.8 to 1.26, expressed remarkable variation. Furthermore, a marked variation was also noted in biochemical parameters viz. pH ranged from 5.31 to 6.75, TSS 2.75 ºB to 6.76 ºB, ascorbic acid 2.75 mg to 6.76 mg/100g, total sugars 2.805 to 5.215 percent, reducing sugar 1.56 to 3.67 percent and the protein varied from 1.1 to 1.8 percent. The samples had green peel with rudimentary spines (e.g., S18, S20) exhibited superior yield traits, making them ideal for production-oriented breeding, whereas the red peel with rudimentary spines (e.g., S11, S12) had the highest ascorbic acid (up to 6.76 mg/100g) and sugar content, indicating their potential for nutraceutical and processing applications. Moreover, the samples had green peel with the both perfect and rudimentary spine dominated over the red peel once with respect of protein content and fruit breadth. Sample 6 (S6) recorded the highest protein (1.83%) and fruit breadth (58.19 mm), favoring fresh consumption. Genotypic differentiation based on spine and peel color provided a practical framework for targeted cultivar development. The study supports conservation, germplasm enhancement, and future breeding strategies for improving yield, nutritional value, and consumer appeal in water chestnut.

Keywords: Genetic diversity; biochemical; morphometric; rudimentary etc.

1. INTRODUCTION

Trapa natans var. bispinosa Roxb. is commonly known as water chestnut, singhara nut, or bull nut, is an annual, aquatic, floating herbaceous plant belonging to the family Trapaceae. The genus Trapa includes three primary species: T. natans, T. bicornis, and T. rossica. Within T. natans, two botanical varieties are distinguished—T. natans var. natans L., which produces four-horned fruits, and T. natans var. bispinosa Roxb. bearing two stout, curved horns and cultivated widely for its edible fruits (Crow & Helliquist, 2000). Originally native to the warm temperate zones of Eurasia and Africa, T. natans var. bispinosa was introduced to the United States in the 1870s as an ornamental plant. Based on color of the peel, water chestnut is categorized into three groups completely green, (2) completely red, (3) green blended with red (Ahmad & Singh, 1998). Though its natural dispersal is restricted due to the heavy, sinking fruits, it has successfully established in northeastern U.S. regions. Now a day, it is cultivated across tropical and subtropical areas including India, Pakistan, Sri Lanka, Indonesia, and several Southeast Asian countries. In India, major production occurs in states such as Uttar Pradesh, Bihar, Tamil Nadu, West Bengal, Assam, Odisha, and Jammu & Kashmir, particularly among communities like the Kashyap, Majwar, and Nishad (Chakor, 1974). Moreover, this aquatic species flourishes in freshwater ecosystems like lakes, ponds, wetlands, and slow-flowing rivers. Fruit development occurs primarily during the winter season (November to January), with nut ripening and natural fruit drop by late January. The fruit is a top-shaped, single-seeded drupe with a fleshy, edible pericarp surrounding a hard, horned endocarp (pyxidium). Furthermore, Adkar et. al, (2014) reported that Trapa bispinosa is rich in essential minerals and bioactive compounds, making it a valuable plant both nutritionally and medicinally. It contains high levels of calcium (Ca), potassium (K), sodium (Na), zinc (Zn), and various vitamins. Phytochemical studies have identified the presence of saponins, phenols, alkaloids, flavonoids, and compounds with strong hydrogen-donating (antioxidant) capacity Kumar et al. (2024).

Given its agronomic importance and nutritional potential, detailed characterization of T. natans var. bispinosa is crucial. The present study aims to investigate the genetic diversity among populations from Etawah, Uttar Pradesh, through the analysis of morphometric traits and biochemical properties, thereby contributing to germplasm evaluation, conservation, and crop improvement strategies.

2. MATERIALS AND METHODS

The evaluation study of the collected samples was performed at the Horticulture Research of Agriculture Science and Technology, Babasaheb Bhimrao Ambedkar University, Lucknow, where physico- chemical parameters of the fruit were analyzed.

The samples of water chestnut (Trapa natans var. bispinosa Roxb.) were collected from two villages of district Etawah viz., Nagla Moti and Narenia, Ekdil and one village of district Auraiya viz., Paliya, Bidhuna. Twenty samples of both green and red colour fruits from different twenty ponds were collected and utilized for exploring the morphometric and biochemical variability present in the collected germplasm.

2.1 Experimental Detail

The individual sample also considered as Treatment. The laboratory experiments were laid out in Completely Randomized Design (CRD). The data were statistically analyzed by the method given by Panse and Sukhatme (1963).

Table 1. Detail of experimental material

Sample	Peel colour	spine	Site of collection
S_1, S_2, S_3, S_4, S_5, S_6, S_7, S_8, S_9, and S_{10}	Green	Perfect	Village Paliya, Bidhuna District Auraiya
S_{11}, S_{12}, S_{13}, S_{14}, S_{15} and S_{16},	Red	Rudimentary	Village Nagla Moti, Ekdil District Etawah
S_{17}, S_{18}, S_{19}, and S_{20}	Green	Rudimentary	Village Narenia, Ekdil District Etawah

Fig. 1. Samples of water chestnut (Trapa natans var. bispinosa Roxb.)

3. RESULTS AND DISCUSSION

3.1 Morphometeric and Physical Attributes in Trapa Genotypes

The significant variations among morphometeric attributes namely spine (presence/absent), fruit length, fruit width, fruit volume and specific gravity in Trapa genotypes were observed during the study. In the collected genotypes, the red peelcolour fruits were found to have only rudimentary spine whereas the green peel fruit had both perfect spine and rudimentary spine. The results are anticipated by the findings of Verma and Panigrahi (2019). The peel colour and spine condition (perfect/rudimentary) are genetic traits. There was a marked variation recorded in fruit length, fruit weight and fruit volume, among the samples collected from different ponds. The fruit length, fruit weight and fruit volume ranged from 31.54mm to 51.62mm, 15.27g to 29.84g and 17ml respectively, with the mean value of 41.78mm, 23.49g and 24.48 ml. Beside this, the fruit breadthranged 47.48 mm to 58.19 mm with the mean value 52.58mm. The fruit breadth was noted maximum in sample 6(S6) with 58.19 mm which had green colour peel and perfect spine. The specific gravity was recorded maximum in sample 11 (S11) with 1.26 with the mean of 0.96 whereas it ranged from 0.85 to 1.26. These variations in water chestnut due to genetic constitution and may also be affected by cultural practices growing condition and maturity time. Our results are also supported by the findings of Pal et al., (2009) where they fruit weight ranging from 17.52g to 37.95g and fruit length 3.61cm to 4.83 cm, Singh et al., (2010) where they found average fruit weight 22.56gm and Dwivedi et al., (2010) recorded the fruit breadth ranged from3.38-6.38 cm, fruit volume ranged from 11.30 ml to 27.80 ml and specific gravity varied from 1.16 to 1.76.

Table 2. Morphometeric and Physical attributes in Trapa genotypes

Samples/Treatment	Peel colour	Spine	Length (mm)	Breadth (mm)	Weight (g)	Volume (ml)	Specific Gravity
S_1	Green	Perfect	42.36	52.517	22.257	21.667	1.027
S_2	Green	Perfect	38.73	56.05	21.577	21.333	1.01
S_3	Green	Perfect	34.66	52.04	19.007	17.583	1.063
S_4	Green	Perfect	34.17	52.63	21.503	22.333	0.96
S_5	Green	Perfect	35.05	56.74	20.810	20.917	0.993
S_6	Green	Perfect	40.67	58.197	24.980	25.75	0.967
S_7	Green	Perfect	37.15	53.823	21.293	21.5	0.99
S_8	Green	Perfect	31.54	49.797	15.277	18.583	0.857
S_9	Green	Perfect	35	47.483	16.773	19.583	0.853
S_{10}	Green	Perfect	34.56	54.977	17.670	17	1.01
S_{11}	Red	Rudimentary	47.24	48.48	26.100	20.5	1.267
S_{12}	Red	Rudimentary	45.80	52.12	28.773	28.917	0.987
S_{13}	Red	Rudimentary	43.59	48.757	22.413	24.167	0.99
S_{14}	Red	Rudimentary	47.07	50.507	25.960	27.917	0.923
S_{15}	Red	Rudimentary	43.92	54.683	27.867	30.5	0.91
S_{16}	Red	Rudimentary	45	48.21	24.713	28.917	0.853
S_{17}	Green	Rudimentary	49.42	52.42	27.867	29	0.91
S_{18}	Green	Rudimentary	51.62	54.22	29.843	34.583	0.857
S_{19}	Green	Rudimentary	47.30	53.657	27.363	29.333	0.96
S_{20}	Green	Rudimentary	50.82	54.473	27.893	29.667	0.937
Mean			**41.78**	**52.58**	**23.49**	**24.48**	**0.96**
C.D.at 5%			**0.817**	**1.387**	**0.838**	**1.07**	**0.073**

Table 3. Biochemical attributes in Trapa genotypes

Samples	pH	Ascorbic acid(mg/100g)	TSS(B)	Total sugar %	Reducing sugar %	Protein %
S_1	6.333	2.773	5.5	3.365	1.675	1.59
S_2	6	3.26	4.233	3.02	1.185	1.55
S_3	**6.75**	3.217	4.533	3.345	2.213	1.72
Samples	**pH**	**Ascorbic acid(mg/100g)**	**TSS(B)**	**Total sugar %**	**Reducing sugar %**	**Protein %**
S_4	6.417	2.84	4.833	4.035	2.55	1.105
S_5	6	3.07	5.267	3.54	2.983	1.22
S_6	5.833	5.357	4.267	3.695	1.725	**1.83**
S_7	6.167	3.123	4.933	3.73	2.825	1.675
S_8	5.833	4.843	5.533	2.805	1.63	1.5
S_9	6.333	2.983	4.433	3.64	2.49	1.4
S_{10}	6.333	2.753	**5.9**	4.03	3.013	1.58
S_{11}	5.417	**6.76**	2.033	4.695	2.69	1.46
S_{12}	5.333	6.24	2.933	**5.215**	**3.67**	1.375
S_{13}	5.867	3.827	3.2	4.905	3.62	1.1
S_{14}	6.333	4.38	3.533	4.49	2.55	1.64
S_{15}	5.567	6.357	3.167	4.385	2.95	1.32
S_{16}	5.667	4.42	2.867	4.8	3.145	1.78
S_{17}	6.083	3.89	2.033	3.82	1.56	1.555
S_{18}	6.75	4.3	2.833	3.485	2.615	1.72
S_{19}	6.583	3.8	3.1	3.24	2.815	1.56
S_{20}	5.317	6.173	2.767	3.39	1.735	1.78
Mean	**6.0458**	**4.2183**	**3.8949**	**3.8815**	**2.482**	**1.523**
C.D. 5%	**0.874**	**0.64**	**0.508**	**0.29**	**0.32**	**0.18**

3.2 Biochemical Attributes in Trapa Genotypes

It is evident that Table 3 shows significant variation in biochemical attributes except only pH among the samples collected from different ponds of District Etawah and Auraiya (UP). The pH value of Trapa

fruit juice was ranged from 5.37 to 6.75 with mean value of 6.04. The highest pH value was recorded in sample 3 (S3) with 6.75. Our results are anticipated by Faruk et al., (2012) where they found ph content ranged from 5.11 in red peel fruits and 5.88 in green peel fruits. Ascorbic acid content among samples was found significantly different which varied from 2.75 mg/100g inS10 to 6.76 mg/100g in S11 with the mean value of 4.21 mg/100g. The results are supported by Rehman et al., (2024) where they found ascorbic acid content 2.1 mg/100g in green colour water chestnut and 1.97 mg/100g in red colour in water chestnut. A considerable variation was also observed in the total soluble solids among the sample collected from different ponds location of districts Etawah and Auraiya. The total soluble solids ranged from 2.03 ºB in S11 to 5.9ºB in S9 with mean value of 3.89 ºB. Our results are validated with the finding of Ram et al. (2010) and Dwivedi et al., (2010).

A considerable variation was also found among the samples with respect to total sugar and reducing sugar content. The total sugar ranged from 2.805 to 5.215 percent with the mean value of 3.8815 percent whereas the sample 12 (S12) which had red peel colour and rudimentary spine was found to have highest (5.215%) total sugar content. The reducing sugar content was also found highest (3.67%) in sample 12 (S12) however, the range of the reducing sugar among the sample was 1.1 to 3.6 percent. The results are also anticipated by the findings of Babu and Dwivedi (2012). However, the quantity of total sugar and reducing sugar found slightly higher than the finding of Babu and Dwivedi (2012). The protein content in Trapa was also found statistically significant during the study. Since, the highest protein content (1.8%) was recorded in sample 6 (S6) but it ranged from 1.1 to 1.8 percent with mean value of 1.523 percent. Our results are also validated by the findings of Rehman et al., (2024) where they recorded the protein content 1.7 percent in green peel and 1.17 percent in red peel genotypes of Trapa.

4. CONCLUSION

Evaluation of Trapa genotypes revealed distinct trends linked to spine type and peel colour. The genotypes with rudimentary spine, especially green peel fruits were consistently excelled in fruit size, weight, and volume, marking them as ideal for yield enhancement. Red peel types with rudimentary spines also showed large dimensions, confirming that spine rudimentation, regardless of peel colour, is associated with superior morphometric traits. Moreover, the perfect spine, green peel genotypes (S1–S10) generally had moderate size metrics, some (e.g., S6) showed notable breadth and volume. Specific gravity was higher in perfect spine types, with the highest in S11 (rudimentary red), indicating denser fruits. Biochemically, red peel with rudimentary spines (S11–S16) dominated in ascorbic acid (up to 6.76 mg/100g) and sugar content, making them valuable for nutritional and processing purposes. Green peel, perfect spine genotypes showed higher TSS, pH, and protein (up to 1.83% in S6), favoring fresh consumption. The green rudimentary group had a balanced profile, with genotypes like S20 showing both high ascorbic acid and protein, combining physical and nutritional benefits.

Overall, rudimentary spine genotypes particularly green peel fruits are promising for yield-focused breeding, while rudimentary red peel fruits suit nutraceutical goals, and green perfect spine types are apt for market preferences in taste and protein.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models

(ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Effect of Pongamia pinnata Leaf Extract as a Priming Agent on the Growth and Development of the Spinach (Spinacia oleracea)

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ABSTRACT

Seed priming is an effective technique to enhance seed germination, seedling vigor, and crop productivity. This study investigates the effects of Pongamia pinnata leaf extract as a natural priming agent on the growth and development of spinach (Spinacia oleracea). Spinach seeds were primed with the extract and compared with non-primed control seeds through laboratory and field trials. Laboratory tests assessed germination percentage, speed, and uniformity on germination sheets, while field trials measured agronomic traits including germination rate, plant height, no of leaf, leaf surface area, leaf length and width and yield traits at 30, 45, and 60 days after sowing (DAS). Results showed that seed priming with Pongamia pinnata extract significantly improved germination rate and seedling uniformity. Primed seeds demonstrated faster and more consistent germination, while the resulting plants exhibited enhanced vegetative growth, including greater plant height, larger leaves, increased leaf area, and improved root development. Additionally, foliar application of the extract at 15-day intervals post-germination further boosted plant growth, indicating a synergistic effect of priming and foliar spraying. This study concludes that Pongamia pinnata leaf extract is an eco-friendly, sustainable alternative to synthetic treatments, promoting early plant establishment and sustained growth. Its dual application through priming and foliar spraying holds potential for improving spinach cultivation, with broader agricultural applications warranting further research.

Keywords: Seed priming; germination; Pongamia pinnata leaf extract; foliar application; agronomic and yield traits.

1. INTRODUCTION

Spinach (Spinacia oleracea) is a globally cultivated leafy vegetable recognized not only for its rapid growth and adaptability but also for its exceptional nutritional profile. An annual crop belonging to the Amaranthaceae family and the subfamily Chenopodioideae, spinach traces its origins to ancient Persia, now modern-day Iran. Over centuries, it has spread throughout the world and become an essential component of various cuisines and farming systems. Its tender, edible leaves, ranging from smooth to crinkled in texture depending on the cultivar, are favored for both culinary and health applications. As a coolseason crop, spinach thrives in temperatures ranging from 15°C to 20°C. (Roughani et al., 2019). In temperate zones, it is typically cultivated during the spring and autumn seasons, while in tropical and subtropical climates, it is commonly grown throughout the winter. The plant shows optimal

performance in well-drained loamy soils rich in organic matter, particularly where soil pH is slightly acidic to neutral (6.0 to 7.5). Although it prefers full sunlight, spinach can tolerate partial shade, making it flexible for diverse cropping environments. India, being predominantly agrarian, relies heavily on crops like spinach to ensure nutritional security. However, increasing population pressure, limited cultivable land, and deteriorating soil health pose significant challenges to agricultural productivity. Unsustainable farming practices have led to soil degradation, including nutrient depletion, loss of organic matter, salinization, acidity, chemical pollution, and waterlogging (Bhattacharyya et al.,2015). These issues not only reduce crop yields but also threaten long-term soil fertility and biodiversity. Therefore, adopting sustainable agricultural practices such as natural seed treatments, crop rotation, and organic inputs is crucial for maintaining soil quality and enhancing crop resilience (Arumugam et al., 2023).

Seed priming is an effective pre-sowing technique that involves controlled hydration of seeds to initiate vital metabolic processes without allowing radicle emergence. This method enhances seed performance by improving germination speed, uniformity, and seedling vigor (Farooq et al., 2019) Various priming techniques include hydropriming (using water), osmopriming (using osmotic solutions like polyethylene glycol), halopriming (using salt solutions), and biopriming (using beneficial microorganisms). These methods activate key enzymes, repair cellular structures, and promote nutrient mobilization, resulting in improved stress tolerance and better crop establishment (Anjos Neto et al., 2020). In crops like spinach, which are sensitive to environmental stresses, seed priming can facilitate faster emergence, uniform growth, and higher yields, making it a valuable practice in sustainable agriculture. Among the natural agents explored for seed priming, Pongamia pinnata (commonly known as karanja) has garnered significant attention due to its bioactive properties. Native to the Indian subcontinent and parts of Southeast Asia, Pongamia pinnata is a leguminous tree well-known for its antifungal, antibacterial, and insecticidal compounds, notably karanjin and pongamol (Purkait et al., 2021). Traditionally used in agroforestry and organic farming, the leaf extract of Pongamia pinnata has shown potential as both a seed priming agent and a foliar spray. These bioactive compounds enhance seed germination, reduce disease incidence, and promote early-stage plant vigor, making them particularly beneficial for organic and low-input farming systems. Incorporating Pongamia pinnata into spinach cultivation, especially through seed priming and foliar application, holds immense potential. Spinach, being susceptible to early-stage diseases, pests, and inconsistent germination under suboptimal conditions, can benefit significantly from natural treatments derived from Pongamia. The bioactive compounds not only enhance seedling resilience but also minimize the need for synthetic agrochemicals, aligning well with organic farming principles and sustainable agriculture. (Alam et al., 2013).

Key field parameters such as soil type, water availability, sunlight, temperature, and nutrient content directly impact spinach growth and productivity. Optimal conditions include loamy, well-drained soil with sufficient organic matter and nutrients—especially nitrogen, phosphorus, and potassium (Nkcukankcuka et al., 2020). Maintaining soil moisture and exposure to sufficient light is crucial for photosynthesis and metabolic activity. Foliar application of biostimulants like Pongamia pinnata enhances physiological processes by directly supplying nutrients and bioactive compounds to plant tissues (Rouphael et al., 2020).

Integrating Pongamia pinnata extract as a natural priming agent and foliar spray represents a sustainable approach to enhancing spinach cultivation. This method promotes faster germination,

strengthens seedling vigor, and supports robust plant growth while reducing reliance on chemical fertilizers and pesticides. As agriculture faces increasing pressure to boost productivity while minimizing environmental impacts, natural solutions like Pongamia pinnata offer practical, ecofriendly alternatives. Adopting such innovations can make spinach cultivation more resilient, sustainable, and environmentally responsible, contributing significantly to food security and ecological sustainability. (Narayanan et al., 2025).

2. MATERIALS AND METHODS

2.1 Experimental Location and Soil Conditions

The research experiment was conducted during the Rabi season at the field of the Department of Amity Institute of Organic Agriculture, Amity University, Noida, Uttar Pradesh (28.5439° N, 77.3331° E). The experiment was carried out on silt loam soil that contains 15–25% clay. This soil is deep, drains slowly, and was formed from layers of fine lake sediments. It is wellsuited for organic farming and for testing natural treatments like Pongamia pinnata extract on spinach growth (Degani et al., 2022); (Joshi et al., 2021).

2.2 Collection and Preparation of Pongamia pinnata Leaf Extract

Fully matured, disease-free leaves of Pongamia pinnata (100 g) were collected from the field of Amity Institute of Organic Agriculture, Noida. The leaves were washed thoroughly with clean water to remove dirt and contaminants and then airdried in a shaded area to preserve bioactive compounds (Singh et al., 2021). After drying, the leaves were ground into a fine powder using an electric grinder. The powdered leaves were then mixed with distilled water in a 1:1 ratio (100 g powder to 100 ml water) to form a consistent paste (sole sap). This extract was utilized for both seed priming and foliar application.

2.3 Seed Treatment

Commercial spinach seeds (Spinacia oleracea) were procured from the Manipur Private Agro Service Shop. The seeds were washed to eliminate surface contaminants and subsequently coated with the freshly prepared sole sap by thoroughly mixing them to ensure complete surface coverage. Soaking the coated seeds for 2 hours allowed for the absorption of bioactive compounds, which can enhance germination and early seedling vigor (Salam et al, 2022). After soaking, the treated seeds were spread out in a cool, shaded area and allowed to air dry overnight, ensuring uniform coating adhesion.

2.4 Germination Test

The germination test was conducted under controlled laboratory conditions to assess the effect of Pongamia pinnata extract on seed germination. Four germination sheets were evenly moistened with distilled water. Treated and untreated seeds (100 each) were placed on separate sheets, covered with an additional moistened layer, and gently rolled The rolled sheets were placed upright in separate beakers to prevent cross-contamination and kept in a dark, stable environment. After 7 days, germinated seeds were counted, and the germination percentage was calculated according to standard protocols.

2.5 Field Preparation and Sowing

The field was prepared manually using a hand hoe to ensure optimal soil conditions. Deep ploughing was performed to loosen compact soil layers and promote aeration. Residual plant material was removed to achieve a fine tilth. The field was divided into 12 equal-sized plots (2 m × 2 m), arranged in a Randomized Block Design (RBD) to accommodate four treatments (T1, T2, T3, T4) with three replications each. Each plot was labelled accordingly (e.g., T1R1, T1R2, T1R3) and contained five straight rows with 15 cm inter-row spacing. Treated and untreated seeds were sown on December 26th, 2024, using the line sowing method to maintain uniform plant population and spacing (10 cm between plants and 15 cm between rows.

2.6 Foliar Application of Pongamia pinnata Extract

To prepare the foliar extract, 100 g of mature Pongamia pinnata leaves were collected, washed, dried, and ground into a fine powder. Two concentrations (1:5 and 1:10, leaf powder to water) were prepared by mixing the powder with water and filtering through Whatman filter paper No. 40 to obtain a clear extract. Foliar spraying commenced on February 8th, 2025, after spinach germination, and continued at 15-day intervals throughout the growing season. The purpose was to control pests and provide growth-promoting nutrients (Krishnasamy et al., 2024).

2.7 Statistical Analysis

Data were analysed using one-way Analysis of Variance (ANOVA) to determine the effect of treatments on spinach growth parameters. When significant differences ($p < 0.05$) were found, Duncan's Multiple Range Test (DMRT) was used for mean separation. The statistical software OPSTAT was used for data analysis, with treatment means considered significantly different when they did not share a common letter.

3. RESULTS AND DISCUSSION

This study examined how Pongamia pinnata leaf extract, applied as both a seed treatment and foliar spray, affected the growth of spinach (Spinacia oleracea). Various growth traits were measured, including seed germination in the lab, plant height, number of leaves, leaf length and width, root length, fresh leaf weight, and dry leaf weight at 30, 45, and 60 days after sowing. The average values for each treatment, shown with graphs, clearly demonstrated differences among treatments using ANOVA analysis. To find which treatments were significantly different, Duncan's Multiple Range Test (DMRT) was used. DMRT is a trusted method for comparing multiple groups while reducing errors and accurately identifying significant differences (Gomez et al., 1984). Presenting the data with averages and graphs made the results easy to understand and reliable (Vasav et al., 2011).

3.1 Germination and Seedling Vigor

The germination rate was significantly higher in treated seeds compared to the control (T1). The combined application (T4) exhibited the highest germination percentage (88.89%) at 60 DAS, followed by seed priming only (T2) with 61%. The control (T1) had the lowest germination rate (46%). This result indicates that seed priming with Pongamia pinnata extract improves germination by

enhancing enzyme activation, water uptake, and membrane stability during the early stages. The synergistic effect observed in T4 (combined priming and foliar application) could be attributed to the combined benefits of early metabolic activation through priming and enhanced nutrient uptake through foliar feeding (Akpor et al., 2022). Previous studies have reported similar improvements in germination when combining seed treatment with foliar application of bioactive extracts (Fig. 1).

3.2 Plant Height

Across all growth stages (30, 45, and 60 DAS), T4 consistently recorded the highest plant height, followed by T3 (foliar application only), while T1 (control) showed the lowest values. The significant increase in plant height under T4 is likely due to the dual action of enhanced seedling vigor from priming and direct nutrient absorption from foliar spraying. Plant height improvements can be linked to the bioactive compounds (karanjin and pongamol) present in Pongamia pinnata extract, which may enhance chlorophyll synthesis and promote photosynthetic efficiency (Bajpai et al., 2009) (Table. 1). The combined application also likely stimulated the production of growth-promoting hormones, such as auxins and gibberellins, resulting in more vigorous stem elongation (Prakash et al., 2021) (Fig. 2).

3.3 Leaf Characteristics (Number, Length, Width, and Surface Area)

The number of leaves, leaf length, and leaf surface area were significantly higher in Treatment 4 (T4) compared to the other treatments. T4, which combined seed priming and foliar spray, resulted in healthier and larger leaves due to increased leaf length and width. Among the individual treatments, Treatment 2 (Seed priming only) showed the highest leaf surface area, suggesting that seed priming helps promote leaf expansion. This may be due to the early activation of growth processes that encourage cell division and enlargement (Farooq et al., 2019; Khan et al., 2015) (Table 1). Although leaf size in Treatment 3 (Foliar spray only) was similar to T4, the difference was not statistically significant. This indicates that foliar spray alone can improve leaf growth, but combining it with seed priming offers additional benefits (Fig. 2).

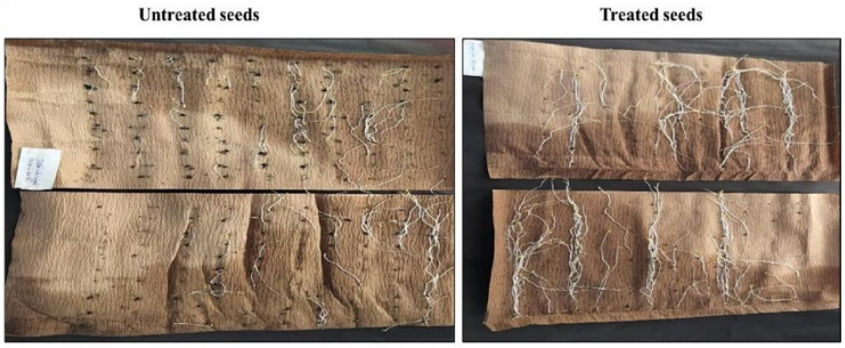

Fig. 1. The seed quality parameters

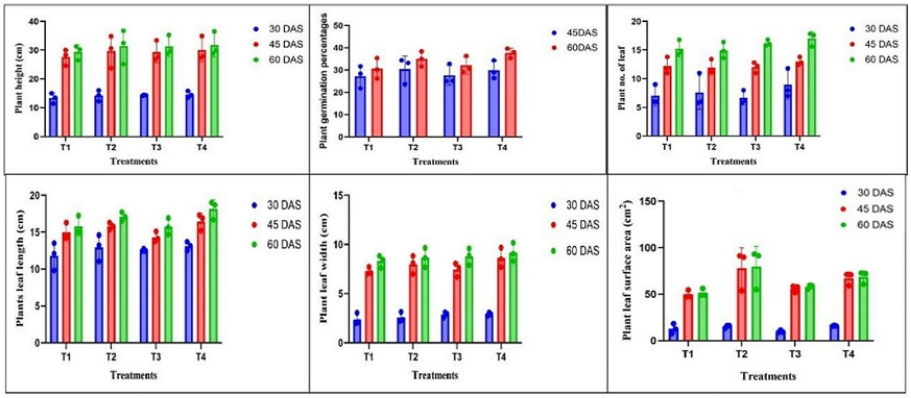

Fig. 2. Yield quality attributes under different DAS duration

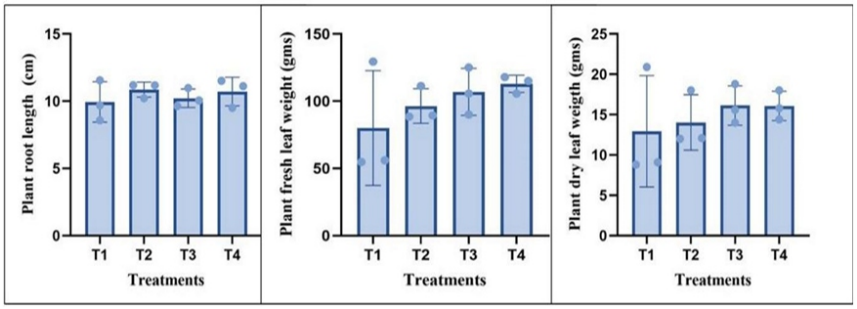

Fig. 3. Biomass production in different treatments

Table 1. Growth parameters

	At 30 DAS					
Treatment	Plant height	Plant number of leaf	Plant leaf length	Plant leaf width		Plant leaf surface area
T1	13.3a	7a	11.807a	2.387a		12.773ab
T2	14.2a	7.6a	12.96a	2.567a		15.217ab
T3	14.3a	6.667a	12.547a	2.867a		9.717b
T4	14.58a	8.933a	13.12a	2.92a		15.83a
	45 DAS					
Treatments	Germination%	Plant height	Plant number of lengths	Plant leaf length	Plant leaf width	Plant leaf surface area
T1	27.267a	27.66a	12.2a	14.993a	7.293a	49.72b
T2	30.4a	29.7a	11.933a	15.807b	7.987a	78.22a
T3	27.667a	29.267a	12a	14.32b	7.467a	55.997ab
T4	2.933a	30.067a	13a	16.467a	8.627a	67.05ab
	A t 60 DAS					
Treatments	Germination%	Plant height	Plant number of lengths	Plant leaf length	Plant leaf width	Plant leaf surface area
T1	46.004b	29.333a	15.2a	15.773b	8.313a	51.277b
T2	88.891b	31.427a	14.933a	17.1b	8.66a	79.753a
T3	57.81b	31.287a	16.2a	15.757b	8.8a	58.03ab
T4	197.705a	31.733a	17a	18.14a	9.187a	68.52ab

Table 2. Yield parameters (After harvesting)

Treatments	Root length	Fresh leaf weight	Dry leaf weight
T1	9.943a	80.1a	80.1a
T2	10.863a	96.433a	96.433a
T3	10.223a	106.93a	106.933a
T4	10.717a	112.9a	112.9a

3.4 Biomass Production (Fresh and Dry Leaf Weight)

The highest fresh and dry leaf weights were recorded in T4, followed by T3, T2, and T1. Although all treatments showed an increase compared to the control, T4 exhibited a significantly higher biomass. The improved leaf biomass under T4 can be attributed to the dual benefits of early germination vigor and sustained nutrient supply from foliar application. The significant increase in fresh and dry leaf weight under combined treatments may be due to enhanced physiological activity and better nutrient translocation facilitated by the bioactive compounds in Pongamia pinnata. Similar effects have been reported with plant-based biostimulants that stimulate biomass accumulation by improving metabolic efficiency and photosynthetic capacity (Du Jardin et al., 2015) (Table 2 and Fig. 3).

3.5 Root Development

Root length did not show a significant difference between treatments, indicating that Pongamia pinnata extract mainly promotes shoot growth rather than root development. This supports the idea that foliar application primarily enhances the above-ground parts of the plant. The limited effect on root length could also be due to nutrient competition between shoots and roots. These findings agree with previous studies showing that biostimulants often improve above-ground biomass more than root growth under certain conditions (Calvo et al., 2014; Gokulapriya et al., 2022).

3.6 Statistical Significance and Correlation

The analysis of variance (ANOVA) revealed significant differences ($p < 0.05$) between treatments for most growth parameters, particularly plant height, leaf number, and leaf area. DMRT results indicated that T4 had significantly superior performance compared to other treatments, especially in leaf biomass and height. The correlation analysis also showed a strong positive relationship between seed priming and enhanced leaf area, indicating that initial seed treatment plays a crucial role in determining vegetative growth potential.

4. CONCLUSION

This study shows that applying Pongamia pinnata leaf extract both as a seed treatment and as a foliar spray significantly improves the growth of spinach plants. The combined treatment (T4) was the most effective, leading to faster germination, taller plants, more leaves, larger leaf area, and greater overall biomass compared to single treatments or no treatment. Treating the seeds helps seedlings start strong, while spraying the leaves supports continued growth by providing extra nutrients. The natural compounds in Pongamia pinnata, like karanjin and pongamol, likely help increase chlorophyll and improve photosynthesis, which boosts leaf and shoot growth. Although root growth did not change much, the increase in shoot size shows that this extract can be a good natural alternative to chemical growth enhancers. Because these results are promising, more studies are needed to confirm the benefits in different environments and with other crops. Using Pongamia pinnata leaf extract in spinach cultivation not only improves plant growth but also supports sustainable farming practices.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Notes: