Journal of Environmental Biology
p-ISSN: 0254-8704 • e-ISSN: 2394-0379 • CODEN: JEBIDP

JEB®

Original Research Paper
Volume 46 Issue 2 March 2025 pp. 291-298
DOI: http://doi.org/10.22438/jeb/46/2/MRN-5396

J. Environ. Biol., 2025

Journal website : www.jeb.co.in ★ E-mail : editor@jeb.co.in

Development of *in-vitro* regeneration protocol for fast multiplication of guava (*Psidium guajava* L.) rootstock cv. Sardar

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Received: 23 May 2024 **Revised:** 12 August 2024 **Accepted:** 11 November 2024

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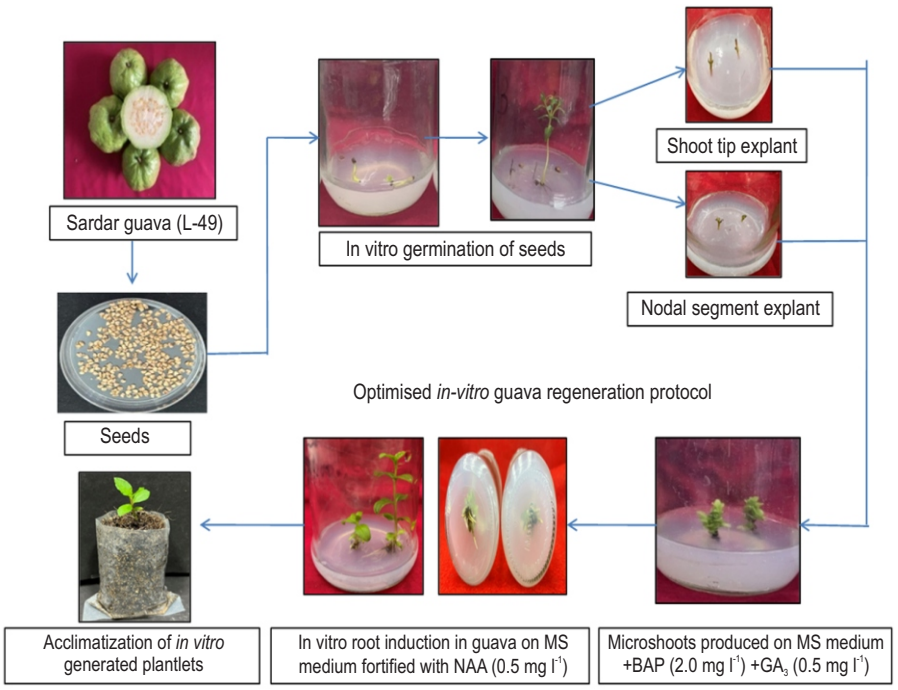
Abstract

Aim: To develop a systematic and robust *in vitro* multiplication method for production of guava rootstock.

Methodology: Nodal segments and shoot tip explants were sourced from *in vitro* and poly house raised seedlings. These explants were inoculated in Murashige and Skoog (MS) basal media supplemented with different concentrations and combinations of growth regulators for establishment, shoot proliferation and root induction.

Results: The supplementation of basal MS media with 6-Benzylaminopurine (BAP) (2.0 mg l^{-1}) + Kinetin (0.5 mg l^{-1}) facilitated the successful establishment of explants sourced from in vitro raised guava seedlings, resulting in higher establishment rates for nodal segments (62.67 %). Optimal shoot proliferation (55.70 %) and shoot length (2.78 cm) was achieved by incorporating BAP (2.0 mg l^{-1}) + gibberellic acid (GA_3) (0.5 mg l^{-1}) into the MS media whereas 1-Naphthaleneacetic acid (NAA) at a concentration of 0.5 mg l^{-1} resulted in the highest levels of root induction (54.63%) and number of roots per shoot (2.94).



Interpretation: Successful *in vitro* regeneration of guava plants was attained by adding 2.0 mg l^{-1} of BAP and 0.5 mg l^{-1} of GA_3 to the MS medium for highest shoot proliferation with NAA @ 0.5 mg l^{-1} for the optimum rooting of plantlets. The rooted plants were effectively acclimatized using a mixture of cocopeat, vermiculite and perlite (2:1:1) as a growing medium.

Key words: Micropropagation, *Psidium guajava*, Plant growth regulators, Tissue culture

How to cite: Singh, H., K. Kaur, G. Kaur, G. Singh and J.S. Brar: Development of *in vitro* regeneration protocol for fast multiplication of guava (*Psidium guajava* L.) rootstock cv. Sardar. *J. Environ. Biol.*, **46,** 291-298 (2025).

Introduction

Guava *Psidium guajava* L. commonly called the "apple of the tropics" belong to family Myrtaceae indigenous to Central America and Southern Mexico. The fruit is highly valued for its exceptional nutritional content. The vitamin C content of guava is around 180 to 300 mg per 100 g of fruit, which is much higher than citrus fruits like oranges and lemons (Angulo-Lopez *et al.,* 2021). Guava cultivation is gaining popularity because of its health benefits and higher returns, however, the nursery production of elite guava plants through patch budding cannot keep pace with the rising demand as propagation of these plants take around two years in nursery to become saleable to orchardists. Guava can also be propagated through air layering which takes around 5-6 months, however, the use of rootstock has a great impact on tree architecture, fruit quality, productivity and resistance to biotic and abiotic stresses (Shivran *et al.,* 2022).

Guava rootstock seedlings take one year to attain the desired thickness for patch budding, a procedure with a success rate of around 85-90 per cent. In addition, a significant amount of labour and land is necessary for raising these young plants. Moreover, seedlings cultivated on seed beds frequently become afflicted with fungal infections, mostly by *Fusarium* spp. (Shukla *et al.,* 2019) and are not true to type and lack uniformity. Therefore, it is crucial to reduce the duration required for raising guava rootstock as well as to produce true to type plants in order to produce a large quantity of elite planting material. Utilising methods such as micropropagation via tissue culture aids rapid multiplication and maintenance of disease free and uniform plants. Micropropagation encompasses various techniques for production of disease-free, genetically identical plants in a very short span of time through establishment on aseptic culture medium (Cardoso *et al.,* 2018). The success of all *in-vitro* micropropagation techniques are predominantly dependent on the choice of explants, the cultivar employed, the composition of the culture medium, and the concentration of plant growth regulators (Ahmadi *et al.,* 2021).

Modifications in the basal medium, calcium ion concentration, auxin or cytokinin concentrations, or culture conditions can be employed to inhibit necrosis in plantlets or *in-vitro* shoots. Murashige and Skoog (Amin and Jaiswal, 1988; Loh and Rao, 1989; Biswas *et al.,* 2007; Aguilar *et al.,* 2017 and Bold's Basal Medium (Tzatzani *et al.,* 2023), have commonly been used for micropropagation of guava. Utilising one or more plant growth regulators (PGRs) such as auxin, cytokinin and gibberellin stimulates the development of shoots and roots. Both juvenile seedlings and mature tissues are utilised as explants for the micropropagation of guava (Ali *et al.,* 2003). However, the growth of mature tissue is hindered (Youssef *et al.,* 2010) because of the release of phenolic compounds, which results in the discoloration of culture media within 12-24 hrs, ultimately inducing the death of explants (Arora *et al.,* 2022). Micropropagation studies have been conducted on guava cultivars using single node cuttings from seedlings raised in greenhouses, shoot tip explants from mature tissues (Amin and Jaiswal 1987; Amin and Jaiswal, 1988; Loh and Rao, 1989; Papadatou *et al.,* 1990; Biswas *et al.,* 2007; Aguilar *et al.,* 2017) and *in-vitro* raised seedlings (Ali *et al.,* 2003). Nevertheless, literature on the *in-vitro* multiplication of guava rootstock cv. Sardar (L-49) is scarce. Hence, the objective of this work was to improve the procedure for *in-vitro* shoot multiplication (utilising MS medium, in combination with BAP or GA_3) and root development (using MS media and NAA or IBA) of *P. Guajava*, specifically the 'Sardar (L-49)' rootstock. A well-defined micropropagation methodology for raising guava rootstock will facilitate its efficient multiplication and contribute to breeding programmes and germplasm conservation.

Materials and Methods

The current investigation was carried out at the Tissue Culture Laboratory of the Department of Fruit Science and School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana during the years 2021 to 2023. Mature guava fruits were collected from seven-year-old healthy trees of the cultivar Sardar (L-49) grafted on its own rootstock and planted at a spacing of 6× 6m. Seeds were extracted and seedlings were raised under *in-vitro* and polyhouse conditions, both, for obtaining explants. Sardar (L-49) rootstock seedlings were selected as the primary source of explants for rapid multiplication using *in-vitro* techniques. The explants were derived from seedlings that were four to five weeks old. The seedlings were thoroughly rinsed under running tap water for 10 min, followed by immersion in an aqueous solution of detergent (Tween 20; Himedia, Mumbai, India) for 3 min, and 3-4 washes with autoclaved distilled water. Subsequently, shoot tips measuring 1-2 cm and nodal segments 2-3 cm in length were carefully extracted (Fig. 1). These explants were initially treated with 0.1% $HgCl_2$ for 3 min, followed by 0.5% bavistin for 10 min (Table 1).

The explants were then rinsed twice with autoclaved distilled water to eliminate any residual toxic substances from mercuric chloride. Thereafter, the explants were surface sterilized in a laminar air flow cabinet and transferred onto autoclaved petri dishes lined with filter paper to absorb excess moisture. Subsequently, the explants were placed in aseptic solutions to initiate the *in-vitro* tissue culture procedure. Nodal segments and shoot tips from *in-vitro* and polyhouse-raised seedlings were collected during three different seasons and placed on basal MS medium supplemented with PGRs to identify the most appropriate season for explant culturing. The percentage of culture establishment was then measured after four weeks of culture. Aseptic guava explants were grown on full strength MS medium (Murashige and Skoog in 1962; Duchefa in Haarlem, Netherlands) supplemented with sucrose (3.0%), myo-inositol (100 mg l^{-1}) (Duchefa, Haarlem, Netherlands) and different growth regulators, viz. BAP, kinetin, GA_3, NAA and IBA in varying concentrations. The pH of the MS media was adjusted to 5.8 using either 1 N HCl or 1 N NaOH. The nutrient media was thereafter sterilized in an autoclave at 121°C and 15 p.s.i. pressure for 20 min (Harvey Sterilemax autoclave, Thermo

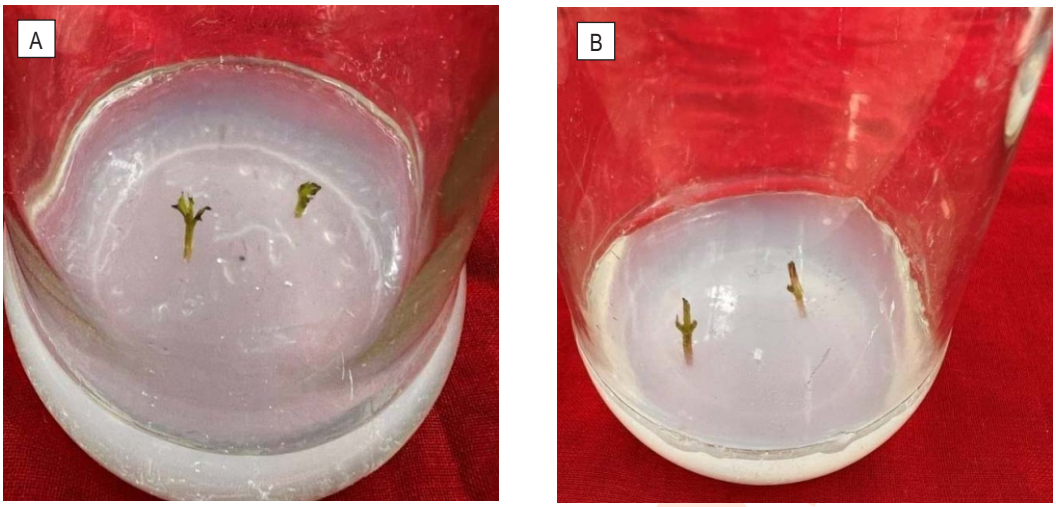


Fig. 1: Explants for *in-vitro* propagation of guava rootstock cv. Sardar guava (A) Shoot tips and (B) Nodal segments containing two lateral buds.

Table 1: Effect of different surface sterilizing chemicals on survival of shoot tips obtained from *in-vitro* raised seedlings of guava rootstock cv. Sardar

Treatments	Survival (%)
Control	0.00
$HgCl_2$(0.1 %) for 1 min	5.90^{g}
$HgCl_2$(0.1 %) for 3 min	13.20^{e}
$HgCl_2$(0.1 %) for 5min	12.50^{ef}
$HgCl_2$(0.1 %) for 7 min	11.77^{f}
$HgCl_2$(0.1 %) for 3 min and Bavistin (0.1 %) for 10 min	14.90^{d}
$HgCl_2$(0.1 %) for 3 min and Bavistin (0.1 %) for 20 min	23.20^{c}
$HgCl_2$(0.1 %) for 3 min and Bavistin (0.5 %) for 10 min	35.37^{a}
$HgCl_2$(0.1 %) for 3 min and Bavistin (0.5 %) for 20 min	34.03^{b}
SEM	3.99
LSD ($p < 0.05$)	1.00

Scientific, USA). Subsequently, the media was transferred to jars (100 ml; Borosil, Mumbai, India) and allowed to cool until solidification. The basal MS media was supplemented with BAP at concentrations of 1.0, 2.0, 3.0, and 4.0 mg l^{-1} (Sigma Cell Culture, minimum 90%, St. Louis, USA), and 0.5 mg l^{-1} Kinetin (SRL Chemicals, Mumbai, India) for the establishment of explants. The cultures were incubated at 25±2°C under a photoperiod of 16 and 8 hrs of darkness, with a photosynthetic photon flux density (PPFD) of 35 µmol m^2 s^{-1} (F140t9d/38, Toshiba), provided by white light. The relative humidity was maintained at 50-60 % for the initiation of explant establishment. Extraction of guava rootstock cv. Sardar (L-49) seeds from ripe fruits was carried out in August, followed by washing and shade drying. After soaking in a 100 ppm GA_3 solution for 24 hrs, the seeds were air-dried in a laminar air flow cabinet (NSW, India) following the removal of outer seed coat. Subsequently, the sterilized seeds were washed with autoclaved distilled water and placed on MS medium in culture jars. The cultured seeds were then incubated at 25°C for 16 hrs under continuous fluorescent light, followed by dark period of 8 hr. The *in-vitro* raised guava seedlings were used as a source of explants, with shoot tips and nodal segments being carefully detached and used under aseptic conditions. The sprouted segments (derived from shoot tips cultivated under *in-vitro* conditions) were aseptically extracted from culture vessels using sterilised forceps after 2-3 weeks to enhance shoot proliferation. The sprouted shoots were separated from the explants using a sterilised surgical blade and forceps. The emerging sections were horizontally or vertically inoculated for regeneration on shoot proliferation medium, and the shoot bud proliferation rate decreased to 14.39%, when the proximal end was placed in contact with the medium as compared to the distal end. The nutrient media included basal MS media augmented with BAP (1.0, 2.0, 3.0, and 4.0 mg l^{-1}) and GA3 (0.5 mg l^{-1}) to achieve effective shoot multiplication.

The mature shoots were removed from the shoot proliferation medium after four weeks of culture. They were then transferred to basal MS medium with different concentrations of NAA (0.5, 1.0, 1.5, and 2.0 mg l^{-1}) and IBA (0.5, 1.0, 1.5 and 2.0 mg l^{-1}) to facilitate rooting of guava rootstock explants. The explants were subcultured at weekly intervals. The rooting parameters were determined three weeks following the incubation period. Guava microshoots with established roots were removed from the growing media and rinsed with clean water to facilitate effective hardening. To prevent any disruption in establishment of the plantlets in the pot bed, the lower leaves were carefully trimmed using a pair of scissors. The established seedlings were relocated to plastic containers (measuring 12×18 inches) filled with a sterilised combination of cocopeat, vermiculite and perlite in 2:1:1 ratio. Transparent polythene bags were employed to cover the pots in order to preserve moisture. The pots were stored

at an ambient temperature of 20–25°C and were watered regularly every 4-5 days. Following a period of 2-3 weeks, the plants were relocated to field settings. The studies were arranged to a completely randomised design (CRD) with three replications per treatment. The analysis of variance (ANOVA) was using SAS (Statistical Analysis System) 9.3 software (SAS, 2011). The statistical analysis involved calculating significant difference between the mean values using the Least Significant Difference (LSD) test at a significance level of $P \leq 0.05$.

Results and Discussion

The highest culture establishment was seen between the months of September to November for both *in-vitro* and polyhouse raised seedlings (Table 2). Furthermore, the maximum rate of establishment was seen for nodal segments (68.47% for *in-vitro* and 41.03% for polyhouse produced seedlings) during September-November, in comparison to shoot tip explants (62.50% for *in vitro* and 37.06% for polyhouse raised seedlings). The timeframe between June and August resulted in the lowest success rate of shoot tip explants (17.13% for *in-vitro* and 11.23% for polyhouse produced seedlings) whereas for nodal segments, the success rate ranged from 23.33% for *in-vitro* to 15.20% for polyhouse raised seedlings. The culture establishment over different seasons is regulated by several factors, such as phenolic exudation, microbial contamination and browning. In this study, the maximum contamination (100 %) was observed in control whereas it varied from 31-44 percent under different treatment combinations. However, the minimum browning (10.97%) was obtained when explants were treated with 0.1 % $HgCl_2$ for 3 min and 0.1 % Bavistin for 10 min for both shoot tip and nodal segments. Phenol exudation in woody species such as guava leads to the browning of the culture, which subsequently reduces the pace of establishment of *in-vitro* culture. But ensuring a sufficient amount of calcium, which is present as a divalent cation Ca^{2+}, might impede the buildup of phenolic compounds (Teixeira da Silva *et al.,* 2020). The peak cultural establishment was seen between the months of September to November for nodal segments obtained from guava seedlings grown *in-vitro.*

The addition of BAP (2.0 mg l^{-1}) and Kinetin (0.5 mg l^{-1}) to MS medium led to the highest success rate in establishing both nodal segments (41.53%) and shoot tip explants (36.33%). In contrast, when MS media was used alone, the establishment rate was significantly lower for both nodal segments (11.33%) and shoot tip explants (7.47%) (Table 3). The use of BAP alone with MS media led to a notably least establishment of explants, in contrast to the combination of kinetin and BAP with MS media. Out of several concentrations of BAP, the addition of BAP at 2.0 mg l^{-1} to the MS medium resulted in the highest success rate of explant establishment whereas decreased success in explants establishment produced on higher concentrations of BAP is attributed to the inhibitory effect of phytohormones when applied in higher concentrations. The *in-vitro* seedlings of guava cultivar Sardar (L-49) were established using different types and concentrations of cytokinins, as shown in Table 2. When BAP (2.0 mg l^{-1}) and Kinetin (0.5 mg l^{-1}) were added to MS medium, the establishment rate of nodal segments increased to 62.67% and shoot tip explants increased to 56.37%. In contrast, when only Basal MS media was used, the establishment rate was significantly lower for both nodal segments (16.47%) and shoot tip explants (12.60%).

Among the two types of explants used, the nodal segments exhibited greater superiority and a higher survival probability compared to shoot tip explants. Alizadeh *et al.* (2012) demonstrated that the combined use of BAP (2.0 mg l^{-1}) and NAA (0.2 mg l^{-1}) resulted in a more efficient establishment of *in-vitro* culture of grapes within a short time frame. Cytokinins are commonly utilised to counteract the apical dominance induced by auxins, since it facilitates the formation of lateral buds (Sadeghi *et al.,* 2015) whereas kinetin has a significant effect on tissue culture by stimulating cell proliferation and production of adventitious shoots (Castilho *et al.,* 2019). Furthermore, a beneficial impact of seedlings raised *in-vitro* on the successful establishment of explants was observed, in contrast to explants obtained from seedlings raised in a polyhouse environment, specifically in the case of guava rootstock cultivar Sardar (L-49). This is due to the fact that explants obtained from polyhouse raised seedlings resulted in contamination and inadequate growth of shoots and roots *in-vitro*. Therefore, in order to conduct further shoot induction and multiplication studies, only explants from *in-vitro* grown guava seedlings were utilised.

The fortification of MS media with BAP and GA_3 had a positive impact on shoot induction and several shoot parameters

Table 2: Effect of seasons on culture establishment (%) for *in-vitro* and polyhouse raised seedlings of guava rootstock cv. Sardar (L-49) (*Psidium guajava*)

Seasons	*In-vitro* raised seedlings establishment (%)		Polyhouse raised seedlings establishment (%)	
	Nodal segment	Shoot tip	Nodal segment	Shoot tip
June -Aug	23.33[c]	17.13[c]	15.20[c]	11.23[c]
Sept- Nov	68.47[a]	62.50[a]	41.03[a]	37.06[a]
Feb - April	35.20[b]	28.33[b]	32.37[b]	22.73[b]
SEM	7.26	7.34	7.59	7.47
LSD (p<0.05)	2.96	3.14	3.16	1.56

Season means followed by same letters within each column are not significantly different at p<0.05

Table 3: Effect of MS medium supplemented with plant growth regulators on explant establishment of guava rootstock cv. Sardar

Treatments	*In-vitro* raised seedlings		Polyhouse raised seedlings	
	Node	Shoot tip	Node	Shoot tip
Basal MS	16.47^{h}	12.60^{h}	11.33^{h}	7.47^{h}
MS + BAP (1.0 mg l^{-1})	20.37^{g}	18.43^{g}	15.30^{g}	12.70^{g}
MS + BAP (2.0 mg l^{-1})	36.17^{d}	32.70^{d}	30.47^{d}	26.50^{d}
MS + BAP (3.0 mg l^{-1})	30.47^{e}	24.33^{e}	25.50^{e}	22.37^{e}
MS + BAP (4.0 mg l^{-1})	24.37^{f}	20.40^{f}	18.50^{f}	15.70^{f}
MS + BAP (2.0 mg l^{-1}) + Kinetin (0.5 mg l^{-1})	62.67^{a}	56.37^{a}	41.53^{a}	36.33^{a}
MS + BAP (3.0 mg l^{-1}) + Kinetin (0.5 mg l^{-1})	48.33^{b}	44.43^{b}	38.60^{b}	34.50^{b}
MS + BAP (4.0 mg l^{-1}) + Kinetin (0.5 mg l^{-1})	40.67^{c}	34.30^{c}	36.30^{c}	31.27^{c}
SEM	5.46	5.16	4.01	3.76
LSD ($p < 0.05$)	1.58	1.59	1.46	1.56

Means followed by same letters within each column are not significantly different at $p<0.05$

Table 4: Effect of different plant growth regulators on shoot induction and multiplication of guava rootstock cv. Sardar under *in-vitro* conditions

Media	Shoot (%)	Average no. of shoots / explants	Average shoot length (cm)	Days for shoot induction	Average no. of leaves/shoot
Basal MS	0^{f}	0^{g}	0^{f}	-	0^{f}
MS + BAP(1.0 mg l^{-1})	22.68^{e}	1.50^{f}	1.53^{e}	28.67^{a}	1.40^{e}
MS + BAP(2.0 mg l^{-1})	47.92^{b}	2.84^{ab}	2.65^{a}	22.33^{d}	3.03^{ab}
MS + BAP(3.0 mg l^{-1})	38.87^{c}	2.46^{cd}	2.35^{bc}	24.33^{c}	2.40^{c}
MS + BAP(4.0 mg l^{-1})	32.60^{d}	2.21^{d}	2.09^{c}	26.33^{b}	1.97^{d}
MS + BAP(2.0 mg l^{-1}) + GA3 (0.5 mg l^{-1})	55.70^{a}	3.04^{a}	2.78^{a}	21.00^{e}	3.17^{a}
MS + BAP(3.0 mg l^{-1}) + GA3 (0.5 mg l^{-1})	41.60^{c}	2.62^{bc}	2.58^{ab}	23.67^{c}	2.73^{bc}
MS + BAP(4.0 mg l^{-1}) + GA3 (0.5 mg l^{-1})	29.10^{d}	1.80^{e}	1.81^{d}	26.67^{b}	1.83^{d}
SEM	6.06	0.35	0.32	0.94	0.37
LSD ($p < 0.05$)	5.78	0.30	0.28	0.84	0.41

Means followed by same letters within each column are not significantly different at $p<0.05$

of guava seedlings, as shown in Table 4 and Fig. 2 (b). Shoot induction and regeneration was not observed when basal MS media was used alone. However, when MS media was supplemented with growth regulators at various doses, a significant impact on shoot induction was noted. The addition of BAP (2.0 mg l^{-1}) + GA_3 (0.5 mg l^{-1}) to the MS medium led to the highest shoot regeneration rate (55.70%) in the shortest time (21.00 days), along with the longest shoot length (2.78 cm), highest number of shoots (3.04) and highest number of leaves (3.17) per shoot. The shoot regeneration observed on adding BAP at a concentration of 2.0 mg l^{-1} in MS medium was lower (47.92%) compared to combination of BAP (2.0 mg l^{-1}) and GA_3 (0.5 mg l^{-1}). Thus, the synergistic impact of these two growth regulators may be associated with increased shoot proliferation in comparison to either BAP or GA_3 alone.

The most important characteristic of cytokinin is to stimulate shoot multiplication and inhibit their elongation. Thus, BAP is extensively utilized in the micropropagation of stone fruits and has favourable outcomes when paired with various growth regulators (Keresa *et al.,* 2019) in order to overcome the inhibition of shoot elongation caused due to cytokinins. Auxins have a role in promoting apical dominance, facilitating cell development, and inducing bud proliferation. The addition of GA_3 to the basal media leads to formation of new shoots and number of shoots per explant (Machado *et al.,* 2020). However, there is limited literature available on the collective impact of cytokinins and GA_3 on *in-vitro* organogenesis. The of results this study validate the synergistic impact of GA_3 and cytokinin on the initiation and proliferation of shoots, which aligns with the findings of Naaz *et al.* (2019) in *Syzygium cumini* and *Syzygium alternifolium,* Blando *et al.* (2013) in *Eugenia myrtifolia* and Freire *et al.* (2018) in *Psidium cattelianum.* BAP has a greater metabolic rate in guava tissue compared to other cytokinins, resulting in an increased rate of shoot multiplication in guava tissue (Mishra *et al.,* 2014). On the contrary, Mahmoud *et al.* (2017) demonstrated that high dosages of benzyl adenine had a negative impact on shoot proliferation in guava.

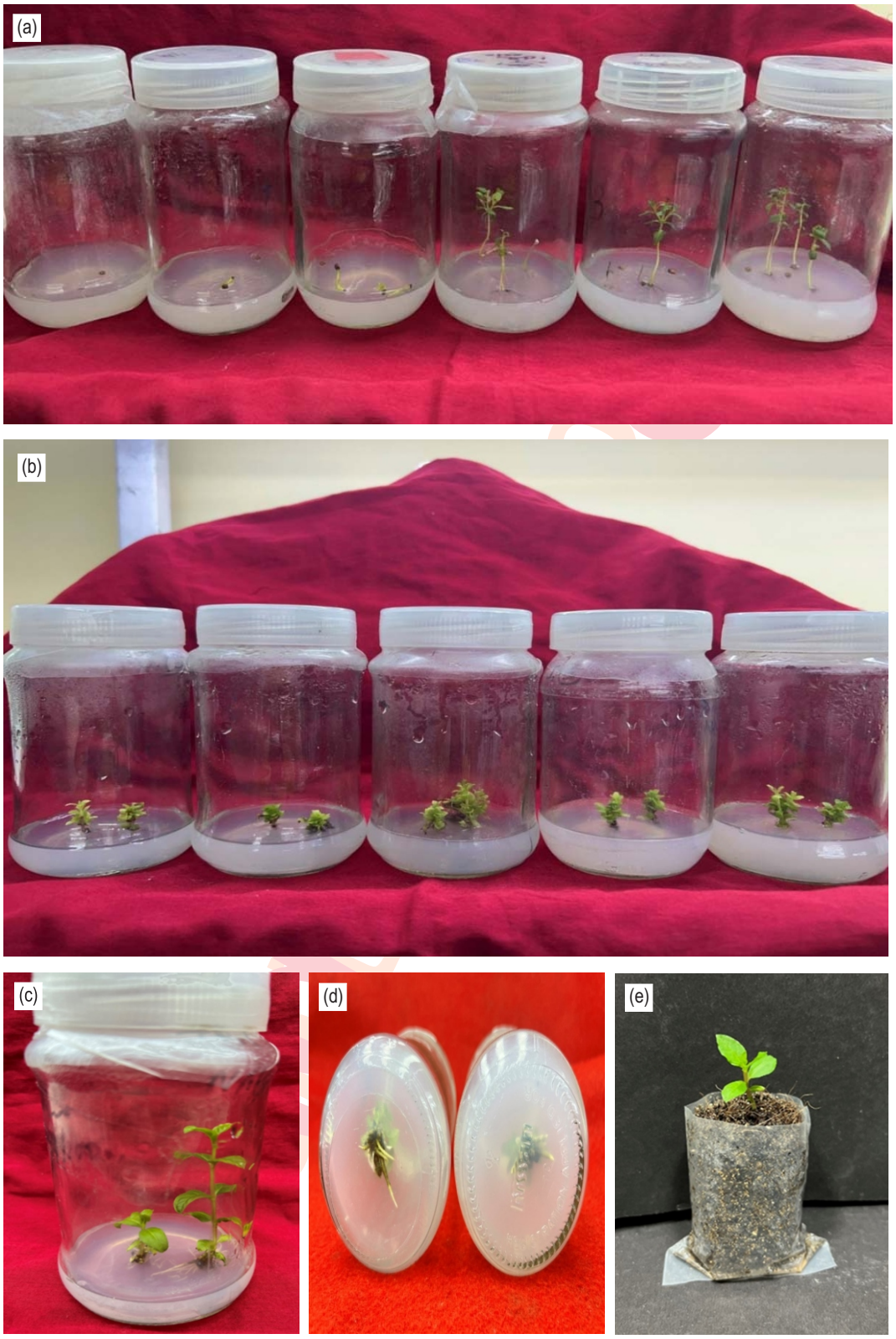


Fig. 2: (a) *In-vitro* germination of guava seeds (b) Production of micro shoots on MS medium enriched with BAP (2.0 mg l^{-1}) and GA_3 (0.5 mg l^{-1}), (c and d) *In-vitro* root induction on MS medium fortified with NAA (0.5 mg l^{-1}) and (e) Hardening of *in-vitro* rooted guava plants in potting mixture containing cocopeat: vermiculite: perlite (2:1:1).

Table 5: Effect of different plant growth regulators on rooting of guava rootstock cv. Sardar under *in-vitro* conditions

Treatments	Days for root induction	Rooting (%)	Average no. of roots/shoot	Average root length (cm)	Acclimatization (%)
Basal MS	-	0.00^{g}	0.00^{f}	0.00^{f}	0.00^{g}
MS + NAA (0.5 mg l^{-1})	22.67^{g}	54.63^{a}	2.94^{a}	1.53^{a}	74.64^{a}
MS + NAA (1.0 mg l^{-1})	29.33^{e}	28.37^{b}	2.77^{ab}	1.29^{b}	48.53^{b}
MS + NAA (1.5 mg l^{-1})	33.00^{cd}	20.43^{d}	2.58^{b}	1.10^{c}	16.11^{d}
MS + NAA (2.0 mg l^{-1})	38.33^{b}	16.07^{e}	2.00^{c}	0.79^{d}	15.00^{d}
MS + IBA (0.5 mg l^{-1})	31.33^{d}	20.50^{d}	1.57^{d}	0.62^{e}	47.43^{b}
MS + IBA (1.0 mg l^{-1})	25.00^{f}	25.15^{c}	2.70^{ab}	1.22^{bc}	28.20^{c}
MS + IBA (1.5 mg l^{-1})	33.33^{c}	15.30^{e}	1.28^{d}	1.10^{c}	12.80^{e}
MS + IBA (2.0 mg l^{-1})	40.67^{a}	10.26^{f}	0.90^{e}	0.58^{f}	10.10^{f}
SEM	2.04	5.02	0.33	0.16	8.01
LSD ($p < 0.05$)	1.59	1.99	0.31	0.16	1.22

Means followed by same letters within each column are not significantly different at $p<0.05$

The impact of various rooting media on the rooting properties of *in-vitro* multiplied shoots of guava rootstock cv. Sardar (L-49) is shown in Table 5 and Fig. 2 ©, d). When only basal MS medium was used without adding rooting hormones, no root induction occurred. The addition of NAA 0.5 mg l^{-1} to the MS medium resulted in highest percentage of successful root formation (54.63%) in the shortest amount of time (22.67 days). Additionally, this concentration of NAA also led to the highest number of roots per shoot (2.94) and the longest root length (1.53 cm), which was statistically greater than other test concentrations of NAA and IBA. Machado *et al.* (2020) found that the highest percentage of root formation in cultures of *Campomanesia xanthocarpa* (a plant from the Myrtaceae family) occurred when the basal medium was enriched with IBA. Our analysis revealed that NAA outperformed IBA as a rooting hormone, resulting in a noteworthy augmentation in the quantity of roots per shoot. However, among the various concentrations of IBA, 1.0 mg l^{-1} led to a higher rooting percentage (25.15%) compared to the rooting seen with IBA at 0.5, 1.5 and 2.0 mg l^{-1}. No survival of microshoots was observed when MS basal medium was utilised alone, resulting in zero acclimatisation percentage which was significantly evident from the data.

The addition of auxins to MS medium is required to stimulate *in-vitro* root formation (Yasmin *et al.,* 2022). Effective acclimatisation can enhance the ability of tissue culture plants to endure outdoor conditions, hence increasing their likelihood of long-term survival and development. The percentage of acclimatized plantlets transferred to greenhouse was highest (74.24%) when they were grown on MS culture medium supplemented with NAA (0.5 mg l^{-1}). Conversely, the addition of IBA (0.5 mg l^{-1}) to MS medium resulted in 47.53% acclimatisation rate. The increase in acclimatisation rate observed in the presence of NAA-supplemented medium was clearly greater than that of the medium supplemented with IBA. This finding indicate that the guava rootstock developed a highly functional rooting system in response to NAA supplementation. In addition, the use of shoot tips and nodal segments containing meristematic cells, together with the addition of a relatively low concentration of plant growth regulators (PGRs), led to the direct stimulation of shoot and root growth. On the contrary, the increased concentrations of both auxins led to decreased rates of acclimatisation success, as seen in Table 4 which might be due to inverse inhibition at extremely high concentrations as reported by Doungous *et al.* (2019). The successful establishment of *in-vitro* raised plantlets under field conditions is a critical criterion for determining the ultimate success of a micropropagation protocol (Mohammed *et al.,* 2023). The use of some growth regulators and elicitors under *in-vitro* conditions can induce stress which can induce alterations in genomic sequence of cultured cells and tissues leading to instability. But a great degree of genetic stability is indicated by the direct development of tissue from meristematic cells in the planting material as these cells are physiologically stable and show high metabolic activity.

Thus, it can be inferred from the results, that this study presents a novel protocol for the micropropagation of guava rootstock seedlings, which has practical applications in the field of applied biotechnology and may be utilised in ongoing breeding and propagation efforts for this species.

Acknowledgments

The authors are grateful to Punjab Agricultural University, Ludhiana for all the facilities, assistance and resources provided to conduct the research work.

Authors' contribution: K. Kaur, G. Kaur: Conceptualization of research; **K. Kaur, G. Singh:** Methodology; **H. Singh, K. Kaur:** Formal analysis and investigation; **K. Kaur:** Writing - original draft preparation; **J.S. Brar:** Writing - revision and editing.

Funding: Not applicable.

Research content: The research content of manuscript is original and has not been published elsewhere.

Ethical approval: This is an observational study and no ethical approval is required.

Conflict of interest: The authors have no competing interests to declare that are relevant to the content of this article. The data supporting the findings of this study are available on request.

Data availability: The data supporting the findings of study are available on request.

Consent to publish: All authors agree to publish the paper in *Journal of Environmental Biology.*

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