Indian Journal of Agricultural Sciences **95** (4): 413–417, April 2025/Article
https://doi.org/10.56093/ijas.v95i4.159102

Deciphering the bio-stimulants induced alterations in the growth of citrus (*Citrus* spp.) rootstocks though multivariate analysis

MANDEEP KOUR[1], PARSHANT BAKSHI[1], KIRAN KOUR[1]*, BRAJESHWAR SINGH[1], BHARAT BHUSHAN[1], SARABDEEP KOUR[1], ASHIMA CHOUDHARY[1], VIKRAMJIT SINGH[2] and PRIYANKA DAHIYA[3]

Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Chatha, Jammu, Jammu and Kashmir 180 009 India

Received: 28 October 2024; Accepted: 31 January 2025

ABSTRACT

The present study was carried out during 2022 and 2023 at Research Farm, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Chatha, Jammu, Jammu and Kashmir to study the correlation and principal component analysis in eight different citrus (*Citrus* spp.) rootstocks. The experiment was laid in factorial completely randomized design (FCRD) with four distinct plant growth-promoting rhizobacteria (PGPRs), viz. P_1 (*Bacillus subtilis*); P_2 (*Pseudomonas fluorescens*); P_3 (consortia-*Bacillus subtilis* + *Pseudomonas fluorescens*); P_4 (control) and eight distinct citrus rootstocks, viz. R_1 (Rough lemon); R_2 (Carrizo citrange); R_3 (Rangpur lime); R_4 (Goutoucheng); R_5 (Sun Chu Sha); R_6 (Swingle citrumelo); R_7 (Troyer citrange) and R_8 (Volkamer lemon) which were replicated thrice. The results of the investigation revealed that seedling height was positively correlated with stem girth (0.5510), root:shoot ratio (0.0399), chlorophyll content (0.5426) and leaf area (0.369) whereas, dry weight of leaves was negatively and significantly correlated with days taken for germination (-0.8693). Germination percentage was positively correlated with fresh (0.5816) and dry weight of shoots (0.5986). Moreover, chlorophyll content was negatively correlated with root: shoot ratio (-0.0912), leaf area (-0.2041) and days taken for germination (-0.1168) while, it was positively correlated with fresh leaf biomass (0.3533) and dry leaf biomass (0.3537). In principal component analysis, days taken for germination, seedling height (90 DAS) and chlorophyll content (90 DAS) showed positive contribution (PC2) whereas, number of secondary roots, dry weight of root and leaf area showed negative contribution.

Keywords: Correlation, *Citrus* spp., PGPRs, Principal component analysis, Rootstock

Citrus (*Citrus* spp.) is a prominent sub-tropical fruit crop believed to have originated in Southeast Asia, particularly China and from a triangle which also include north-eastern Indian region (Wu *et al.* 2018). It belongs to the Rutaceae family and is a vital source of vitamin C and antioxidants. Major citrus producers globally include China, Brazil, India, the USA, and Spain. In India, citrus cultivation spans 10.95 million hectares with a production of 148 million tonnes. (Kumar *et al.* 2023). Major citrus producing states in India are Andhra Pradesh, Maharashtra, Punjab, Haryana, Uttar Pradesh, Rajasthan, Karnataka, Himachal Pradesh, Assam, Meghalaya, Uttarakhand, Arunachal Pradesh, Tripura and Orissa.

Citrus spp. like *Citrus aurantifolia* Swingle and *Citrus limonia* Osbeck propagated by seeds and *Citrus reticulata* B. and *Citrus sinensis* Osbeck propagated by vegetative methods, with T-budding being the most common. Vegetative propagation ensures genetic uniformity, influencing fruit quality and tree productivity. Rootstocks play a vital role in improving growth, reducing the juvenility period and providing resistance to pests and diseases, directly impacting orchard profitability. Rootstock also plays a crucial role for the exclusion of toxic ions, which are important for determining the life of an orchard (Kadam and Patil 1985). Correlation coefficient analysis helps in establishing a relationship between several qualities and aids in identifying the key component characters that can be selected to improve crop yield.

A significant challenge in citrus cultivation is the poor root establishment and low nutrient uptake, leading to high mortality rates. The use of bio-inoculants, such as *Azospirillum, Azotobacter* and *Pseudomonas* has gained importance to overcome stress like salinity, drought and heat. These plant growth-promoting rhizobacteria (PGPR) enhance root development, nutrient uptake and overall plant vitality through mechanisms like nitrogen fixation, phosphate solubilization and antifungal activity.

[1]Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Main Campus, Chatha, Jammu, Jammu and Kashmir; [2]Punjab Agricultural University, Fruit Research Station, Jallowal, Lesriwal, Punjab; [3]University of Georgia, Athens, USA. *Corresponding author email: kiran61@skuastj.org

Among the PGPRs, variants belonging to genera *Bacillus* and *Pseudomonas* are well recognized as the most powerful phosphorus solubilizers as phosphorus uptake is important, it is one of several factors affecting rootstock establishment (Adhyaningtyas *et al.* 2023). The study conducted by Requena *et al.* (1997) suggested that native plant beneficial microbes, such as plant growth-promoting rhizobacteria (PGPR), offers greater advantages to the host plant compared to commercial or introduced forms of such microbes. These are cluster of bacteria that live in the rhizosphere, which is the section of soil surrounding plant roots and enhance plant root structure and vegetative growth through several mechanisms (Herman *et al.* 2008). Hence, this study aimed to understand the bio-stimulants induced alterations in the growth of citrus rootstocks though multivariate analysis.

MATERIALS AND METHODS

The present study was carried out during 2022 and 2023 at Research Farm, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Chatha (32.40°N, 78.48°E, 293 m amsl) Jammu, Jammu and Kashmir. The experimental site experiences subtropical climate with mean annual maximum and minimum temperatures of 28.7°C and 15.7°C, respectively and annual precipitation of approximately 1388 mm.

Experimental design and treatments: The experiment was laid out in a factorial completely randomized design (FCRD) with three replications. The treatments comprised four PGPR levels, P_1, *Bacillus subtilis*; P_2, *Pseudomonas fluorescens*; P_3, Consortia (*B. subtilis* + *P. fluorescens*) and P_4, Control; applied to eight citrus rootstocks, viz. R_1, Rough lemon (*Citrus × jambhiri* Lush.); R_2, Carrizo citrange (*Citrus sinensis* × *Poncirus trifoliata*); R_3, Rangpur lime (*Citrus* × *limonia*); R_4, Goutoucheng (*Citrus aurantium* L. hybrid of sour orange); R_5, Sun Chu Sha (*Citrus reticulata* Blanco); R_6, Swingle citrumelo (*Citrus paradisi* × *Poncirus trifoliata*); R_7, Troyer citrange (× *Citron cirus* spp.); R_8, Volkamer lemon (*Citrus limonia* Osbeck). Each treatment combination had 10 plants/replication, totaling 960 plants.

Isolation and preparation of PGPR inoculants: PGPRs were isolated from rhizosphere soil samples collected from established orchards. Soil samples were pre-treated at 50°C for one hour and processed using serial dilution technique (10–6 to 10–10). Isolates were cultured on King's B medium and incubated at 28 ± 2°C for 48 h. Pure cultures were maintained on nutrient agar slants. For inoculant preparation, isolated strains were grown in nutrient broth (containing peptone 5.0 g, NaCl 5.0 g, beef extract 1.5 g and yeast extract 1.5 g/L) at 32°C for 48 h under constant shaking to achieve a concentration of 108 CFU/ml.

Seed treatment and sowing: Seeds extracted from mature fruits were washed, shade-dried and treated with bavistin (1 g/L) followed by PGPR inoculation. The bio-inoculants were mixed with jaggery solution (2 kg/L) at 15 g/kg seed rate. Treated seeds were sown in polythene bags (12 inch × 9 inch) containing soil, sand and FYM (1:1:1). Rough lemon and Carrizo citrange were sown in June, while other rootstocks were sown in October.

Data collection: Observations were recorded on five randomly selected plants/replication for the following parameters, Germination parameters, Days to germination, germination percentage (30 DAS), and survival percentage (90 DAS); Growth parameters, shoot characteristics, height, stem diameter, fresh and dry weight; Leaf characteristics, number, chlorophyll content (SPAD), fresh and dry weight, leaf area; root characteristics, length, diameter, number of secondary roots, fresh and dry weight, root:shoot ratio. The chlorophyll content was measured using SPAD 502 meter on 25 randomly selected leaves/replication. Leaf area was determined using graph paper method. Fresh and dry weights were recorded after drying samples at 70°C for 72 h.

Statistical analysis: Data were analyzed using factorial completely randomized design (FCRD) with JMP Pro-17 software. Correlation coefficients were calculated following Al-Jibouri *et al.* (1958) method, and principal component analysis was performed using Sneath and Sokal (1973) method. Treatment means were compared using Duncan's multiple range test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Biofertilizers plays an important role in plants to influence various biological mechanisms which influence the uptake and availability of nutrients and further increases the production of growth promoting substances (Bora *et al.* 2016). The results of our correlation study illustrated in Table 1 and Fig. 1 showed that different bio-inoculants produced significant effect on growth and morphological traits of different citrus rootstocks. The seedling height is significantly positively correlated with stem girth (0.5510), root: shoot ratio (0.0399), chlorophyll content (0.5426) and leaf area (0.369) whereas, dry leaf biomass was negatively and significantly correlated with days taken for germination (-0.8693). These results are in conformity with other authors (Williamson *et al.* 2001, Farzana and Radizah 2005 and Majidi 2014) who observed the beneficial effects of bio-inoculants on different crops and stated that morphological responses involve change in root architecture, lateral roots, root:shoot growth and plant height. Yousif *et al.* (2011) observed positive and significant correlation between number of primary branches, number of secondary branches and stem length in snake melon. Shankarappa *et al.* (2018) reported that combination of three biofertilizers- *Azospirillum*, *Pseudomonas fluorescence* and VAM resulted in highest plant height (68.23 cm), stem girth (3.83 cm) and number of leaves (18.33) in grafted mango cv. Alphanso. The survival percentage (90 DAS) showed strong significant correlation between seedling height (0.5801), stem girth (0.5211), fresh weight of shoot (0.6678), dry weight of shoot (0.6535) and dry weight of root (0.5363). These results are in accordance with Singh *et al.* (2017) who reported that 2.5 kg/ha dose of each *Azospirillum* and P-solubilizing biofertilizers resulted in best growth and yield of tomato cv. Arka Vikas. Abdelmoaty *et al.* (2022) observed that *Citrus aurantifolia* plant growth,

Table 1 Pearson correlation coefficient and scattered plot matrix among various parameters in citrus rootstocks

Parameters	DG	G%	S%	SH	SG	FW (shoot)	DW (shoot)	FW (root)	DW (root)	FLB	DLB	LA	R: S	CC	NoL	LR	NoR
DG	-	-0.5094	-0.4819	-0.5883	-0.3071	-0.7584	-0.7377	-0.6433	-0.6466	-0.7559	-0.8693	-0.5085	-0.1635	-0.1168	-0.7286	-0.3267	-0.5544
G%		-	0.4904	0.5557	0.5762	0.5816	0.5986	0.5360	0.4988	0.6870	0.5955	0.3004	0.0727	0.3419	0.3996	0.4238	0.4659
S%			-	0.5801	0.5211	0.6678	0.6535	0.5369	0.5363	0.5549	0.5008	0.3047	0.0944	0.2900	0.5921	0.6465	0.5222
SH				-	0.5510	0.8957	0.7986	0.5746	0.6224	0.8366	0.7454	0.3269	0.0399	0.5426	0.7483	0.6805	0.3842
SG					-	0.5965	0.6140	0.5026	0.4121	0.5804	0.4826	-0.1001	-0.0781	0.7826	0.3474	0.8069	0.4902
FW(shoot)						-	0.8816	0.6994	0.6833	0.9151	0.8529	0.3802	0.0476	0.4309	0.8160	0.6558	0.4905
DW(shoot)							-	0.7743	0.7609	0.9029	0.8480	0.5072	-0.0183	0.4549	0.8582	0.6321	0.5876
FW(root)								-	0.8926	0.7920	0.7171	0.3039	0.4644	0.3050	0.5915	0.6581	0.7497
DW(root)									-	0.7733	0.6988	0.3437	0.6130	0.2892	0.6566	0.5371	0.8088
FLB										-	0.8780	0.3927	0.1234	0.3533	0.7595	0.6112	0.5478
DLB											-	0.4482	0.0707	0.3537	0.7982	0.5650	0.5316
LA												-	-0.0280	-0.2041	0.5101	-0.0808	0.1999
R: S													-	-0.0912	0.0077	0.0964	0.5394
CC														-	0.3642	0.7176	0.3808
NoL															-	0.4514	0.4516
LR																-	0.5137
NoR																	-

DG, Days taken for germination; G%, Germination percentage (%); S%, Survival percentage (90 DAS); SH, Seedling height (90 DAS); SG, Stem girth (90 DAS); FW(shoot), Fresh weight of shoot; DW (shoot), Dry weight of shoot; FW(root), Fresh weight of root; DW(root), Dry weight of root; FLB, Fresh leaf biomass; DLB, Dry leaf biomass; LA, Leaf area; R: S, Root: shoot ratio; CC, Chlorophyll content (90 DAS); NoL, No. of leaves (90 DAS); LR, Root length; NoR, No. of secondary roots; DAS, Days after sowing.

physiology and fruit quality were enhanced by inoculating the soil with 25–50% NPK for both *T. harzianum* and *B. thuringiensis* during transplantation. According to Singh *et al.* (2018) reported that Soil + FYM + Cocopeat (SFC) with Azospirillum (Azo) + AM fungi consortium enhanced the seed germination, seedling growth, chlorophyll, P, K, root growth and root epidermis and moreover, the xylem and phloem diameter in rough lemon and the budding success and growth in Kinnow mandarin saplings were found to be positively correlated with the number of AM spores and AM root colonization. The germination percentage was positively and significantly correlated with fresh leaf biomass (0.6870), dry weight of shoot (0.5986) and fresh weight of shoot (0.5816). Saeed *et al.* (2021) reported that bio-inoculants strongly adheres to the rhizosphere and make colony around the roots which provides better root architecture, seedling and overall plant growth. Chlorophyll content was negatively correlated with root:shoot ratio (0.0912), leaf area (-0.2041) and days taken for germination (-0.1168), while it was positively correlated with fresh leaf biomass (0.3533) and dry leaf biomass (0.3537), whereas number of leaves (90 DAS) was negatively correlated with days taken for germination (-0.7286), respectively which were in close proximity to the results reported by Jain *et al.* (2007) that in greengram bio inoculants increases the presence of unbound nitrogen and phosphorus in the soil which further increases the metabolic activities and consequently enhancement in the morphological characteristics and germination of the seedlings. The leaf area showed positive correlation with dry leaf biomass (0.4482), while negative correlation with number of leaves (-0.0808) and root:shoot ratio (-0.0280). Singla *et al.* (2023) revealed that under laboratory conditions, highest seedling length (20.33 ± 0.83 cm), maximum weight of seedlings (35.06 ± 0.8 mg) and maximum weight of seedlings (159.8 ± 2.7 mg) was recorded on seeds treated with integrated biofertilizer (NPK-1:1:1). Ortas (2012) observed that application of bio-inoculants

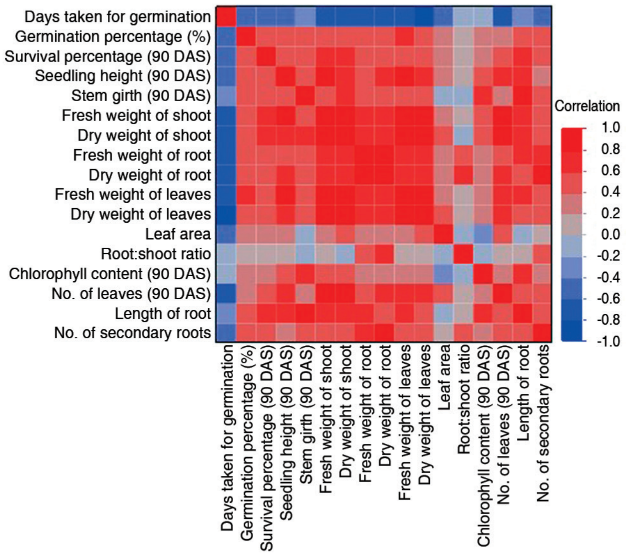

Fig. 1 Colour map on correlation explaining phenotypic characters of citrus rootstock.
DAS, Days after sowing.

Table 2 Eigen value and variance explained in citrus rootstock

Principal component	Eigen value	Percentage of variance
PC_1	9.79	57.6%
PC_2	2.10	12.4%

results in increase in uptake of nitrogen and phosphorus by the plants may be due to the nitrogenase and phosphatase enzymes activity. Singh *et al.* (2018) recorded that soil + FYM + cocopeat (SFC) with *Azospirillum* (Azo) + AM fungi consortium improved the seedling height, chlorophyll content, seed germination, root growth, root epidermis and cortical region thickness, xylem and phloem diameter in rough lemon whereas, budding and growth success was observed in Kinnow mandarin saplings. Mosa *et al.* (2018) concluded that biofertilizers play important role in increasing

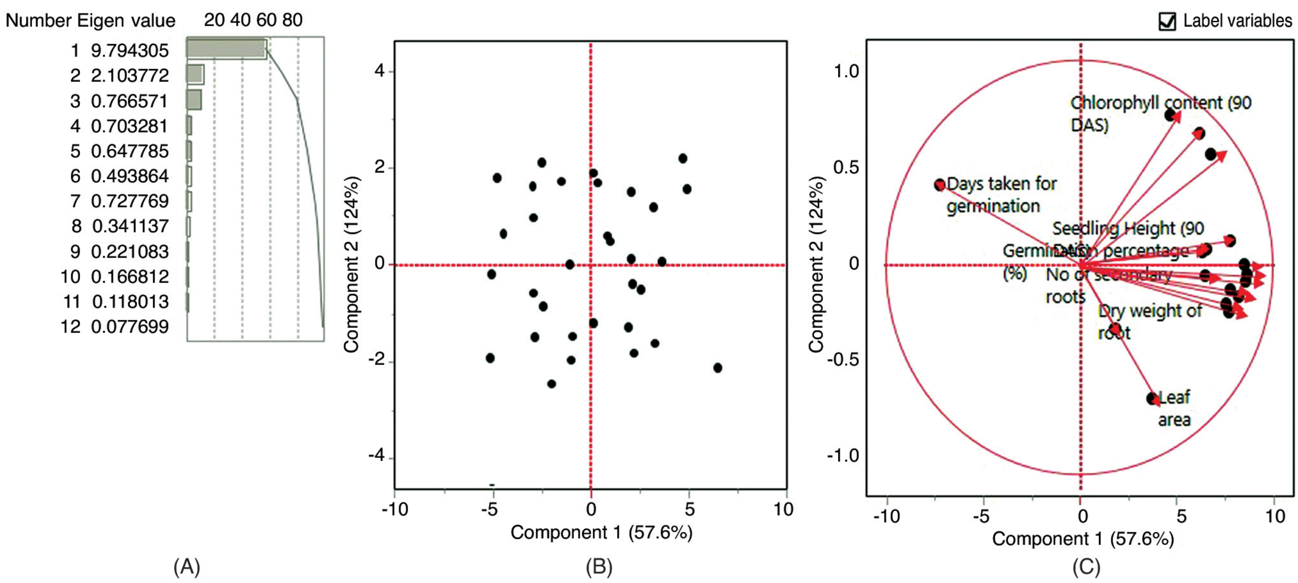

Fig. 2 Principal component analysis (A) Eigen values; (B) Scatter plot of PC_1 and PC_2; (C) A biplot of PC_1 and PC_2.

photosynthetic rate and stomatal conductance compared to NPK in apple might be due to positive effect of humic substances on chlorophyll content in leaves. Intensive crop production in modern days has led to widespread use of chemical fertilizers which are not only costly but causing soil, water and environmental pollution as well. Therefore, the outcomes of our analysis promote the incorporation of bio-inoculants as a pre-sowing treatments in citrus rootstocks, promoting the reduction of chemical fertilizers with the goal of fostering a more environmentally friendly and sustainable agriculture for future.

Principal component analysis (PCA): To better understand the connection between important variables and complex dataset, PCA was performed. Having eigen values exceeding one, the two principal components (PCs 1 and 2) collectively explained 70% of the difference in the original data set. The primary principal component (PC_1) was shown to be responsible for 57.6% of the overall variation seedling height (90 DAS), chlorophyll content (90 DAS) and leaf area had a positive interaction to PC_1 while, days taken for germination had a negative interaction (Table 2). The second component (PC_2) outlined 12.4% of variation with days taken for germination, seedling height (90 DAS), chlorophyll content (90 DAS) had positive contribution, while number of secondary roots, dry weight of root and leaf area showed negative contribution (Fig. 2). The PCA analysis makes this very evident the use of bio-inoculants on citrus rootstock showed varied results. The initial component was vital in differentiating the information gleaned of the phenotypic characters of citrus rootstocks. The vector of characters, viz. chlorophyll length, seedling height and germination percentage showed longer lengths, which suggested a beneficial contribution to both components.

REFERENCES

Abdelmoaty S, Khandaker M, Mahmud K, Majrashi A, Alenazi M and Badaluddin N. 2022. Influence of *Trichoderma harzianum* and *Bacillus thuringiensis* with reducing rates of NPK on growth, physiology, and fruit quality of *Citrus aurantifolia*. *Brazilian Journal of Biology* **82:** 261032.

Al-Jibouri H, Miller P A and Robinson H F. 1958. Genotypic and environmental variances and covariances in an upland cotton cross of interspecific origin. *Agronomy Journal* **50**: 633–36.

Adhyaningtyas R F I, Rahmawati R and Mukarlina M. 2023. Effect of phosphate solubilizing and nitrogen-fixing bacteria on Pontianak siam citrus (*Citrus* × *nobilis* var. microcarpa) seed germination. *Nusantara Bioscience* **15**: 1–2.

Bora L, Tripathi T, Bajeli J, Chaubey A and Chander S. 2016. A review on microbial association: Its potential and future prospects in fruit crops. *Plant Archives* **16**: 1–11.

Farzana Y and Radizah O. 2005. Influence of rhizobacterial inoculation on growth of the sweet potato cultivar. *Journal of Biological Sciences* **1**: 176–79.

Herman M A B, Nault B A and Smart C D. 2008. Effects of plant growth promoting rhizobacteria on bell pepper production and green peach aphid infestations in New York. *Crop Protection* **27**: 996–1002.

Jain A K, Kumar S K and Panwar J D S. 2007. Role of rhizobium, phosphorus and micronutrients on growth and nodulation of green gram [*Vigna radiata* (L.) Wilczek]. *Advances in Plant Sciences* **20**: 337–39.

Kadam B A and Patil V K. 1985. 'Effect of different concentration of chloride, bicarbonate and high ESP in soil on growth and chemical composition of citrus rootstocks'. PhD Thesis, Marathwada Agricultural University, Parbhani, Maharashtra.

Kumar D, Bhattacharyya S and Ghosh D. 2023. Assessing the export potential of Nagpur mandarin: The promising citrus fruit of central India. *Current Science* **1**: 458–60.

Majidi A. 2014. Effect of integrated nutrient management in some quantitative and qualitative characteristics of apple. *Applied Research Soil* **2**: 92–104.

Mosa W, Paszt L S, Frac M, Trzciński P, Treder W and Klamkowski K. 2018. The role of biofertilizers in improving vegetative growth, yield and fruit quality of apple. *Horticultural Science* **45**(4): 173–180.

Ortas I. 2012. Mycorrhiza in citrus: Growth and nutrition. *Advances in Citrus Nutrition*, pp. 333–51.

Requena N, Jimenez I, Toto M and Barea J M. 1997. Interactions between plant growth-promoting rhizobacteria (PGPR) arbuscla mycorrhizal fungi and *Rhizobium* spp. in the rhizosphere of *Anthyllis cystisoides*, a model legume for revegetation in Mediterranean semi-arid ecosystem. *New Phytologist* **136**: 667–77.

Saeed Q, Xiukang W, Haider F U, Kucerik J, Mumtaz M Z, Holatko J, Naseem M, Kintl A, Ejaz M, Naveed M and Brtnicky M. 2021. Rhizosphere bacteria in plant growth promotion, biocontrol and bioremediation of contaminated sites: A comprehensive review of effects and mechanisms. *International Journal of Molecular Sciences* **22**: 10529.

Shankarappa T, Reddy R N, Subramanyam B, Sreenatha A and Reddy N A. 2018. Biofertilizers for growth and establishment of alphonso mango grafts under nursery condition. *International Journal of Current Microbiology and Applied Sciences* **7**: 5205–11

Singh A, Thakur A, Sharma S, Gill P P S and Kalia A. 2018. Bio-inoculants enhance growth, nutrient uptake and buddability of citrus plants under protected nursery conditions. *Communications in Soil Science and Plant Analysis* **49**: 2571–86.

Singh R K, Dixit P S and Singh M K. 2017. Effect of bio fertilizers and organic manures on growth, yield and quality of tomato (*Lycopersicon esculentum* Mill.) cv. Arka Vikas. *Journal of Pharmacognosy and Phytochemistry* **6**(5): 1793-1795.

Singla A, Kumar M, Sharma J R, Lamba M, Dhundwal A, Dhattarwal G and Gavri A. 2023. Effect of bio-inoculants and plant growth regulators on germination and seedling growth of wild ber (*Ziziphus rotundifolia* Lamk.) under *in vitro* conditions.

Sneath P H A and Sokal R R. 1973. *Numerical Taxonomy: The Principles and Practice of Numerical Classification*, pp. 573. WF Freeman and Co., San Francisco.

Williamson L C, Ribrioux S P, Fitter A H and Leyser H O. 2001. Phosphate availability regulates root system architecture in Arabidopsis. *Plant Physiology* **126**: 875–82.

Wu G A, Terol J, Ibanez V, Lopez-Garcia A, Perez-Roman E, Borreda C, Domingo C, Tadeo F R, Carbonell-Caballero J, Alonso R and Curk F. 2018. Genomics of the origin and evolution of citrus. *Nature* **554**: 311–16.

Yousif M T, Elamin T M, Baraka M, Jack A A and Ahmed E A. 2011. Variability and correlation among morphological, vegetative, fruit and yield parameters of snake melon (*Cucumis melo* var. flexuosus). *Cucurbit Genetics Cooperative Report* **33**(35): 32–35.