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Effect of growth regulators and bio-inoculants on rooting and growth of vanilla stem cuttings

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ABSTRACT

The investigation on rooting and growth of vanilla (*Vanilla planifolia* Andrews) stem cuttings was carried out under 50% shade net to find out the effect of growth regulators and bio-inoculants on rooting and growth of vanilla cuttings. There were ten treatments comprising growth regulators such as IBA and NAA @ 500 and 1000 ppm each and combination of both @ 500 ppm, and different bio-inoculants like *Trichoderma harzianum*, *Bacillus megatherium*, *Pseudomonas fluorescence* and *Azotobacter chroococcum* (each @ 2 g/polybag of size 5"× 3") along with untreated control. Bio-inoculants in general proved to be superior over growth regulators (particularly NAA), but they were on par with IBA in respect of some of the rooting and growth parameters. *Trichoderma harzianum* was considered to be the best treatment which resulted in better sprouting percentage, length of sprout, and number of leaves, root thickness and fresh weight of roots. These results compare well with growth regulators and control treatment. Hence, *Trichoderma harzianum* could be conveniently used for the commercial propagation of vanilla stem cuttings planted under 50 per cent shade net, which would be more economical and eco-friendly.

Key words: *Vanilla planifolia,* stem cuttings, growth regulators, bio-inoculants, *Trichoderma.*

INTRODUCTION

Vanilla (*Vanilla planifolia* Andrews) belongs to the family Orchidaceae, is a climbing terrestrial orchid suitable for the warm humid tropics. It is grown for its pleasantly aromatic beans which are the source of natural vanillin which is extensively used in food industry as a flavouring agent in cakes, chocolates, cookies, baked foods, biscuits, puddings, etc., besides its perfumery and pharmaceuticals application. The demand for vanilla beans by 2010 is expected to be around 14,000 tonnes (Thomas and Rao, 12), which can be met only through large scale cultivation. Availability of healthy genuine planting material at right time is one of the pre-requisite for successful vanilla cultivation on large scale. Conventionally, vanilla is propagated through stem cuttings and occasionally though tissue culture. The information on the use of growth regulators and particularly bio-inoculants on multiplication of vanilla is scanty. Further, vanilla plants in the nursery are frequently affected by soil borne fungal diseases such as root rot causing death. In order to obtain healthy planting material with improved rooting efficiency many workers have tried several bio-inoculants with success in various horticultural plants. Furthermore, application of *Trichoderma harzianum* and *Pseudomonas fluorescence* is known to promote growth and vigour of black pepper cuttings, besides suppressing the soil-borne fungal pathogens (Jisha *et al.*, 5). Hence, the present investigation was undertaken.

MATERIALS AND METHODS

The present investigation was carried out during *kharif* 2006 at the Division of Horticulture, University of Agricultural Sciences, Bengaluru. The experiment was laid out in a factorial complete randomized design (FCRD) with four replications with 25 cuttings in each replication. There were 10 treatments comprising two growth regulators, *viz.,* IBA and NAA (each @ 500 and 1000 ppm and a combination of both @ 500 ppm each) and four bio-inoculants (*Trichoderma harzianum*, *Bacillus megatherium*, *Pseudomonas fluorescence* and *Azotobacter chroococcum* @ 2g/polybag) along with untreated control. Rooting medium was prepared by mixing red earth, FYM and sand (2:1:1). Mixture was filled in black poly bags (5"× 3"). The basal portion of the three node cuttings was dipped in the growth regulator solution for five seconds and then planted in the rooting medium in polythene bag with one basal node inside the rooting media. In control treatment, the cuttings were dipped in distilled water for five seconds and then planted. Bio-inoculants were applied to the planting holes just before placing the cuttings. Then, the polybags with cuttings were kept under 50 per cent shade net (green) and after care such as staking, watering, weeding and plant protection were carried out on need basis. Two sprays of 0.1 per cent Bavistin were given at an interval of one month to

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prevent fungal infection. Observations on the shoot characteristics were taken at regular intervals. At the end of the experiment, five cuttings from each replication were randomly lifted from the polybag and observations on the root characters were recorded. The data collected on various parameters were subjected to statistical analysis as described by Sundararaja *et al*. (9).

RESULTS AND DISCUSSION

The two synthetic auxins *viz.,* IBA and NAA at various concentrations showed significant influence on root parameters of vanilla cuttings (Table 1, Fig. 1) compared to control (except rooting percentage). Cuttings treated with IBA 500 ppm recorded the highest rooting percentage, maximum number of roots per cuttings, whereas all these parameters were minimum in control. Similar rooting pattern were observed in the stem cuttings of *Massdevia tenacissima* (Pandey *et al.,* 6) treated with IBA. However, IBA 1000 ppm recorded maximum length of the longest root (23.4%), fresh weight (168.45%) and dry weight (133.33%) of the root over control. This may be due to better mobilization of primary metabolites for better root formation with the help of growth regulators. Farooqi *et al.* (2) reported that single node cuttings of vanilla dipped in IBA 10,000 ppm followed by NAA 2,000 ppm produced maximum rooting. But the thickness of the longest root was highest in NAA 1000 ppm (66.67%). This might be ascribed to greater metabolic activity and maximum utilization of sugar and starch after hydrolysis in the stem. All the root parameters were lowest in control. Exogenous application of auxins at different concentrations seems to activate sugar metabolism for release of energy and protein which are necessary for cell division and differentiation during adventitious root primordial initiation or development in the rooting zone of shoot cuttings.

Among shoot parameters (Table 2) NAA 500 ppm took minimum number of days to bud sprout (27.5 days), whereas control and NAA + IBA 500 ppm each took longer days to bud sprout (30 days). Earliness in sprouting will make the cuttings less dependent on

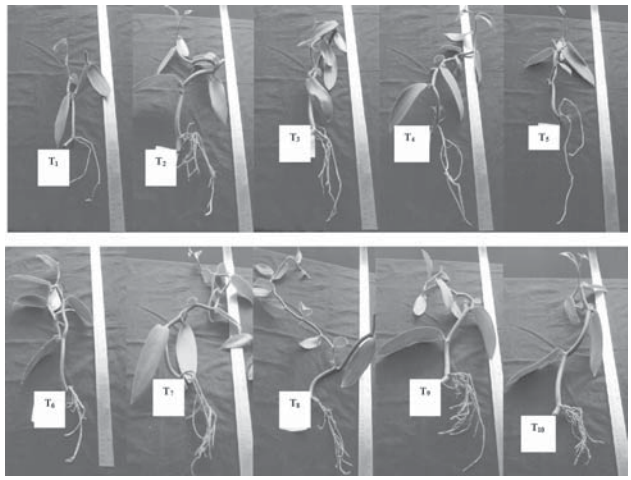

Fig. 1. Rooting in vanilla as influenced by growth regulators and bio-inoculants.

Table 1. Effect of growth regulators and bio-inoculants on rooting of vanilla stem cuttings.

Treatment	Days taken for rooting	Percentage rooting	No. of roots / cutting	Length of longest root (cm)	Thickness of longest root (cm)	Fresh weight of root (g)	Dry weight of root (g)
T_1- Control	19.4	90.0	1.4	23.4	0.30	2.06	0.30
T_2- NAA 500 ppm	19.3	91.0	1.5	27.1	0.37	2.68*	0.29
T_3- NAA1000 ppm	19.3	92.0	1.8	25.4	0.50*	2.23	0.39
T_4- IBA 500 ppm	17.4*	94.25	2.3*	27.1	0.31	5.08*	0.67*
T_5- IBA1000 ppm	17.9*	91.0	1.6	29.0*	0.42*	5.53*	0.70*
T_6- NAA + IBA 500 ppm each	18.6	93.0	1.8	26.1	0.41*	4.31*	0.51
Mean for growth regulators	18.5	92.25	1.8	26.94	0.4	3.97	0.51
T_7- *Trichoderma harzianum*	17.6*	97.0	2.1*	29.3*	0.49*	7.25*	0.64*
T_8- *Bacillus megatherium*	19.3	98.0	1.8	27.6	0.38	4.81*	0.62*
T_9- *Pseudomonas fluorescence*	18.1*	92.0	1.8	26.9	0.37	3.99	0.56
T_{10}- *Azotobacter chroococcum*	18.2*	95.0	2.1*	25.4	0.45*	4.26*	0.54
Mean for bio-inoculants	18.3	95.5	1.95	27.3	0.42	5.08	0.59
Grand mean	18.5	93.33	1.82	26.73	0.4	4.22	0.52
CD at 5%	10.9	12.7	0.6	4.9	0.09	1.97	0.26

*Significant at 5%.

Table 2. Effect of growth regulators and bio-inoculants on shoot parameters of vanilla stem cuttings.

Treatment	Days taken for sprouting	Percentage sprouting	Length of sprouted shoot (cm)	Thickness of sprouted shoot (cm)	Inter-nodal length of sprouted shoot (cm)	No. of leaves on sprouted shoot	Fresh weight of shoot (g)	Dry weight of shoot (g)
T_1- Control	30.0	45.0	22.2	0.68	4.9	8.1	54.3	5.4
T_2- NAA 500 ppm	27.5	59.0	34.6*	0.80*	6.4*	8.6	57.5	8.7*
T_3- NAA1000 ppm	28.0	62.0*	33.4*	0.79*	5.9	8.3	74.3	9.4*
T_4- IBA 500 ppm	28.5	46.0	33.3*	0.84*	5.8	9.3	88.5*	6.5
T_5- IBA 1000 ppm	28.5	74.0*	39.9*	0.88*	5.4	9.2	106.6*	11.6*
T_6- NAA + IBA 500 ppm each	30.0	48.0	31.2	0.73	5.5	8.8	70.7	6.2
Mean for growth regulators	28.5	30.6	6.24	0.15	4.52	8.84	40.5	2.54
T_7- *Trichoderma harzianum*	26.5	83.0*	55.4*	0.76	6.6*	11.3*	105.9*	8.9*
T_8- *Bacillus megatherium*	24.0*	81.0*	53.3*	0.78	5.4	10.7*	93.7*	8.1
T_9- *Pseudomonas fluorescence*	32.0	82.0*	35.8*	0.79*	5.5	9.9*	81.7*	8.6*
T_{10}- *Azotobacter chroococcum*	28.5	73.0*	41.4*	0.79*	5.8	9.0	95.9*	8.0
Mean for bio-inoculants	15.13	38.75	19.3	0.40	2.83	4.73	44.4	4.15
Grand mean	28.3	65.3	38.05	0.79	5.72	9.32	82.91	8.14
CD at 5%	15.7	15.5	9.5	0.10	1.2	1.4	25.1	2.9

*Significant at 5%.

stored food (Sen and Bose, 7). In general, there was a progressive increase in percentage of sprouting over a period of sixty days after planting of the cuttings. IBA 1000 ppm and NAA 1000 ppm showed maximum percentage of bud sprout (74 and 62%, respectively), which differed significantly with control. The better bud sprouting in the cuttings treated with different growth regulators can be related to the synthesis of auxins in the cuttings which helped to promote other shooting and rooting parameters. Maximum thickness of the sprout (29.41%), fresh (96.32%) and dry weight (114.8%) of shoots was recorded in the cuttings treated with IBA 1000 ppm and all these parameters were minimum in control. Sulikeri *et al.* (8) observed better initiation of shoot system, shoot development and more compact root system in the vanilla cuttings treated with IBA and NAA in equal proportion in two concentrations of 1000 and 2000 ppm, which was nearly four times higher than that of the control. Thicker sprouts, fairly higher number of leaves and auxins activated stem and leaf growth might have resulted in elongation of the stems and leaves through cell division and cell elongation accounting for higher fresh and dry weight of new shoot (Sundhari *et al.* 10; Ganesh, 3). Even though, control plants showed better rooting (90%), they recorded meagre bud sprouting (45%). Similarly, use of IBA showed better rooting (94%), but minimum shooting (46%). This may be due to the continuous availability of hormones in bio-agents than just one time availability in externally applied hormones. Moreover, rooting cuttings requires certain hormones and shoot bud emergence requires certain hormones, which influence the rooting and sprouting of stem cuttings in different ways.

Different bio-inoculants showed significant differences for different rooting parameters (Table 1) of vanilla stem cuttings compared to control. *Trichoderma harzianum* recorded the minimum number of days for rooting, maximum number of roots per cutting (50%), length of root (25.21%), thickness of the longest root (63.33%), fresh weight (251.94%) and dry weight of the root (113.33%) which significantly differed from control. Whereas, maximum rooting was in *Bacillus megatherium* (98%) which was on par with *Trichoderma harzianum* (97%) and minimum in control (90%). Higher root fresh weight due to *Trichoderma harzianum* can be related to longer shoot length with longer internodes and maximum number of leaves which are responsible for synthesis of higher amounts of photosynthates and subsequent translocation to the root system. Harman *et al.* (4) opined that *T. harzianum* is capable of increasing the uptake of nutrients by secreting enzymes that solubilize the insoluble nutrients. Thankamani *et al.* (11) obtained similar results in black pepper with the application of *T. harzianum* and *P. fluorescence*.

Application of bio-inoculants to the planting holes before planting of vanilla cuttings resulted in earliest sprouting (24 days) with *Bacillus megatherium* followed by *Trichoderma harzianum* which were at par, but

differed significantly from control (Table 2). However, application of *Pseudomonas fluorescence* resulted in delayed sprouting. Earliness in sprouting will make the cuttings less dependent on stored food (Sen and Bose, 7). Similar observation has been made in respect of long pepper cuttings treated with *Glomus mosseae* and *Trichoderma harzianum* by Ganesh (3). Cuttings treated with *T. harzianum* exhibited maximum sprouting percentage (83%) which is closely followed by *Pseudomonas fluorescence* (82%) and *Bacillus megatherium* (81%) which differed significantly from control. This may be attributed to the production of auxins and other growth promoting substances by microbes resulting in increased cell elongation and cell division ultimately formation of longer shoots in cuttings treated with *T. harzianum*. Further, *Trichoderma harzianum* recorded the maximum length of sprout (149.55%), maximum number of leaves (39.51%), maximum fresh (95.03%) and dry weight of spouted shoots (64.81%). Whereas, fresh and dry weight of shoot was minimum in *Pseudomonas fluorescence* and *Azatobacter chroococcum,* respectively. However, most of the bio-inoculants tried had a significant influence. Thicker sprouts, fairly higher number of leaves and auxin activated shoot length and leaf growth due to increased cell division and cell elongation might have resulted in higher fresh and dry weight of new shoots (Sundhari *et al.,* 10; Ganesh, 3).

Bio-inoculants in general proved superior to growth regulators (particularly NAA), but, they were at par with IBA in respect of some shoot and root parameters. Considering the sprouting percentage, length of sprout, number of leaves, root thickness and fresh weight of roots. *Trichoderma harzianum* was the best bio-agent for rooting of vanilla stem cuttings and these results compare well with growth regulators tried. Considering the economy and easy handling of this bio-agent and high value for the organically grown vanilla, *T. harzianum* could be used conveniently for the commercial propagation of vanilla cuttings. Besides, application of *T. harzianum* will greatly help to reduce the incidence of root rot caused by *Fusarium* spp. in vanilla, thus, paving way for eco-friendly means to control this menace.

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