

Original Research Paper

Morphophysiological characters of *Dendrobium* var. Yellow Splash as influenced by bioinoculants and different levels of benzyladenine

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ABSTRACT

Dendrobium is the most commonly grown tropical orchid species in India and Kerala. They are highly specific about their nutrient requirement. The use of bio-inoculants in crop production of ornamentals has opened up a new possibility of using them for improving the growth and yield of orchids. Hence the objective of study was to evaluate the response of *Dendrobium* cv. Yellow Splash to different kinds of bio-inoculants viz., AMF, *Azospirillum* and a microbial consortia PGPR Mix – 1 developed from KAU, along with 50, 100 and 150 ppm of benzyladenine. The experiment consisted of ten different treatments involving bio-inoculants and benzyladenine. Plant height and number of leaves were maximum in the plants inoculated with AMF along with 100 ppm benzyladenine. Treatment comprising of *Azospirillum* and 100 ppm benzyladenine was superior in terms of other morphological parameters like leaf breadth, leaf area and plant spread. Maximum leaf length and highest number of shoots were observed in plants inoculated with *Azospirillum* and 150 ppm benzyladenine. Considering the floral parameters, both quantitative and qualitative attributes were found to be superior in the treatment consisting of AMF along with 150 ppm benzyladenine. Highest root length was observed under the treatment AMF along with 100 ppm benzyladenine while number of roots and root volume were maximum in the plants inoculated with *Azospirillum* and 150 ppm benzyladenine. Plants inoculated with AMF and 100 ppm benzyladenine had highest chlorophyll content while highest stomatal frequency was observed under the treatment *Azospirillum* and 100 ppm BA. From the study it could be concluded that inoculation of *Dendrobium* orchids with bio-inoculants like AMF and *Azospirillum* can significantly improve the morphological characters of the plants which in turn influence the production of quality spikes.

Keywords: Bio inoculants, benzyl adenine, AMF, *Azospirillum* and PGPR Mix - 1

INTRODUCTION

Orchids are elegant cut flowers that capture the title of ‘highest selling flower’ in Indian cut flower industry due to their unique shape, size, colour and characteristic aroma of certain species. Among the diversified species of orchids, *Dendrobium* is most commonly grown species in India, especially in Kerala. Commercial growers of *Dendrobium* in Kerala are facing many problems including inclement weather, pest and disease attack as well as lack of technical knowhow. They are unaware about the scientific nutrient management practices and their injudicious way of nutrient application leads to a decline in crop yield, higher incidence of pests

and diseases and this will affect the production of export quality flowers. Commercial formulations of bio-inoculants have found to be widely used in crop production programmes of various ornamentals. Application of exogenous growth hormones has also been reported to increase the yield and quality attributes in ornamental crops. There is a need to develop a package which integrates the benefits from all possible sources of organic, inorganic and biologic components which can be recommended to orchid growers. Hence the present study was conducted with the objective of evaluating the response of *Dendrobium* var. Yellow Splash to bio-inoculants and different levels of benzyl adenine.

MATERIAL AND METHODS

The study was conducted in the Department of Floriculture and Landscaping, College of Horticulture, Vellanikkara, KAU. The experiment consisted of ten different treatments with three replications laid out in CRD. The adhoc nutrient recommendation for orchids as per Package of Practices recommendations of KAU is foliar application of N:P, O... :K, O, 3:1:1 during period of vegetative growth and 1:2:2 during flowering period at the rate of 0.2 per cent twice a week. Three bio-inoculants *viz.*, PGPR Mix – 1, AMF and *Azospirillum* and three levels of benzyladenine *viz.*, 50, 100 and 150 ppm were superimposed on this recommendation.

PGPR Mix-1 was applied through root dipping of plants in loose water slurry at the rate of 500g/2.5l of water for 20 minutes prior to planting. AMF was directly applied at the rate of 50g/kg of potting media at the time of planting. Likewise, *Azospirillum* was applied through root dipping of plants in a slurry of 500g of the inoculum in 50 ml of water for 30 minutes prior to planting. Benzyl adenine (BA) was applied as foliar at monthly intervals.

Treatments:

- T - POP + PGPR Mix-1 + BA 50 ppm
- T₁ - POP + PGPR Mix-1 + BA 100 ppm
- T₂ - POP + PGPR Mix-1 + BA 150 ppm
- T₃ - POP + AMF + BA 50 ppm
- T₄ - POP + AMF + BA 100 ppm
- T₅ - POP + AMF + BA 150 ppm
- T₆ - POP + *Azospirillum* + BA 50 ppm
- T₇ - POP + *Azospirillum* + BA 100 ppm
- T₈ - POP + *Azospirillum* + BA 150 ppm
- T₉ - Control (KAU POP recommendation for orchids)

RESULTS AND DISCUSSION

Morphological parameters

Vegetative characters

A significant improvement in vegetative characters were observed in treatments consisted of bio-inoculants and benzyladenine when compared to control (Table 1). Treatments consisted of AMF along with different concentrations of BA were superior in terms of plant height and maximum plant height was observed in T₄ (POP + AMF + BA 100 ppm). Increased plant height of AMF inoculated plants may be due to efficient endomycorrhizal association of the fungus with roots of *Dendrobium* orchids which might have helped in better uptake and mobilization of nutrients. The mycorrhizal extrametrical hyphal strands extend into the medium, absorb water and minerals effectively and make it available to the plants. Better availability of nutrients along with the production of phytohormones and antibiotics might be contributed to the morphological and physiological changes resulting a significant improvement of plant height in plants inoculated with AMF. An improvement of plant height as a result of AMF inoculation has been reported in various flower crops (Varshney *et al.*, 2002; Prabhat *et al.*, 2003; Bhatia and Gupta, 2007)

A perusal of the data with respect to other vegetative characters like plant spread, number of leaves, leaf length, leaf breadth, leaf area and number of shoots per plant revealed that there was a significant improvement of all these parameters in plants inoculated with *Azospirillum* along with different levels of BA and maximum values for all these parameters were observed in T₈ (POP + AMF + BA 150 ppm). Beneficial effect of *Azospirillum* might be the result of versatile mechanisms like increased nutrient uptake, enhanced stress resistance, vitamin production, siderophores and biocontrol, operating simultaneously or sequentially in *Azospirillum* inoculated plants (Cohen *et al.*, 2015). *Azospirillum* species are having the capacity of self-production of phytohormones like auxin, gibberlins, cytokinins and nitric oxide and inducing the synthesis of these compounds in plant tissues (Gadagi, 1999). Cytokinin regulates several processes such as cell division, leaf expansion, shoot and root morphogenesis. Gibberlins are responsible for cell division and elongation of plant cells. *Azospirillum* present in the rhizosphere are able to produce

Table 1. Influence of bioinoculants and benzyl adenine on morphological characters

Treatments	Plant height (cm)	Plant spread (cm)	Leaf length (cm)	Leaf breadth (cm)	Leaf area (cm ²)	Number of pseudobulbs/plant	Number of roots	Root length (cm)	Root volume (cm ³)
T ₁ (POP + PGPR Mix-1 + BA 50ppm)	27.75	20.5	13.47	5.13	49.49	4.00	47.00	20.80	15.45
T ₂ (POP + PGPR Mix-1 + BA 100ppm)	25.88	21.17	13.52	5.12	49.46	3.50	31.00	18.75	15.40
T ₃ (POP + PGPR Mix-1 + BA 150ppm)	24.33	22	12.43	5.05	46.49	3.67	36.67	16.00	20.33
T ₄ (POP + AMF + BA 50ppm)	28.92	20.83	13.43	5.03	48.53	4.33	46.33	23.67	22.90
T ₅ (POP + AMF + BA 100ppm)	33.09	22.29	13.33	4.68	45.18	3.83	37.00	24.18	15.43
T ₆ (POP + AMF + BA 150ppm)	30.43	22.08	13.73	5.25	51.12	3.33	44.00	18.50	20.20
T ₇ (POP + Azospirillum + BA 50ppm)	22.25	19.58	14.30	5.23	52.21	3.33	35.67	17.77	5.73
T ₈ (POP + Azospirillum + BA 100ppm)	25.45	27.10	13.23	6.13	57.94	4.83	44.67	24.07	19.73
T ₉ (POP + Azospirillum + BA 150ppm)	25.38	27.25	15.78	5.45	57.39	5.00	48.33	23.85	30.25
T ₁₀ (POP)	21.93	21.17	12.55	4.43	41.23	3.00	39.00	22.00	10.30
CD (0.05)	5.389	4.087	1.313	0.657	6.933	0.713	10.808	4.835	9.29

Table 2. Influence of bioinoculants and benzyl adenine on floral characters

Treatment	Days to flower	Spike length (cm)	Stalk length (cm)	Number of floret per spike	Internodal length (cm)	Floret size (cm ²)	Longevity in the field (days)
T ₁ (POP + PGPR Mix-1 + BA 50ppm)	289.00	17.27	7.33	6.00	5.07	17.00	63.33
T ₂ (POP + PGPR Mix-1 + BA 100ppm)	308.67	21.33	10.23	6.00	4.10	18.389	60.33
T ₃ (POP + PGPR Mix-1 + BA 150ppm)	262.67	14.00	6.40	7.00	5.63	18.43	50.33
T ₄ (POP + AMF + BA 50ppm)	264.33	22.03	10.63	9.00	5.83	20.51	56.33
T ₅ (POP + AMF + BA 100ppm)	274.00	20.43	12.97	8.67	5.17	20.35	57.67
T ₆ (POP + AMF + BA 150ppm)	283.33	29.40	16.27	9.33	7.17	27.41	72.67
T ₇ (POP + Azospirillum + BA 50ppm)	296.67	20.43	13.83	5.00	5.13	17.72	63.67
T ₈ (POP + Azospirillum + BA 100ppm)	256.00	20.30	12.23	6.67	5.43	19.02	66.00
T ₉ (POP + Azospirillum + BA 150ppm)	304.33	21.80	12.37	7.33	5.03	21.35	64.33
T ₁₀ (POP)	300.33	17.80	10.00	5.33	3.67	17.33	47.67
CD (0.05)	NS	2.41	2.84	2.13	1.44	3.11	7.04

metabolites which act as signals for the production of phytohormones in the plant system. Action of phytohormones like cytokinins and gibberlins coupled with increased nutrient status of *Azospirillum* inoculated plants might have contributed to the improvement of vegetative characters like plant spread, leaf length, breadth and leaf area. These results are in conformity with findings of Bhaskar *et al.* (2002) in marigold, Anon. (2003) in *Dendrobium*, Srinivasa (2006), Chaudhary *et al.* (2013) in gladiolous, Hoda and Mona (2014) in petunia and Yadav *et al.* (2013) in gerbera, who reported an improvement in vegetative growth as a result of *Azospirillum* inoculation.

In both the cases of inoculation with AMF and *Azospirillum*, optimistic results were obtained when the plants were supplied with benzyladenine along with these bio-inoculants. Benzyladenine is characterised as a synthetic cytokinin which highly helps in the cell division, cell elongation, organ formation and regeneration and also they play an important role in translocation of assimilates to growing cells. Application of exogenous cytokinin has been reported to increase the synthesis of endogenous cytokinin in plants (Latham, 1994). In the present study the exogenous application of benzyladenine might have increased the level of cytokinin in the plants. Elevated cytokinin level and morphophysiological changes due to bio-inoculant treatments might have resulted an improvement of vegetative growth in plants under these treatments.

Root parameters

From the results obtained, it could be clearly observed that, maximum root length was exhibited by the plants inoculated with AMF. This result might be mainly attributed to higher phosphorous uptake in the plants by extra radical mycelium leading to higher shoot and root growth in inoculated plants (Smith and Read, 1997). AMF was reported to produce metabolites that can alter the plant's ability to produce roots and to alter root regeneration and root morphology resulting an increased absorptive surface area and feeder root longevity (Linderman, 1988). A diffusible symbiotic signal produced by AMF which is recently identified as lipochito oligosaccharides (LCOs), designated as *My factors* which helps in root growth and branching (Maillet *et al.*, 2011) could be considered as another reason for the high rate of root growth in AMF inoculated plants. This result is in accordance with the findings of Martin and Stutz (2004); Perner *et al.* (2007).

In the case of number of roots and root volume, higher number of roots and maximum root volume were produced by the plants under the treatment T%_o (POP + *Azospirillum* + 150 ppm BA). *Azospirillum* is a gram negative diazotrophic rhizobacteria which exhibits chemotactic response to plant exudates, i.e., the ability to sense and navigate towards the most favourable niches for growth (Rodriguez *et al.*, 2009). When plants are inoculated with *Azospirillum*, the signalling molecules will be produced by the bacteria and the plant will produce lateral roots and root hairs which are the source of exudates to maintain bacterial population in rhizosphere (Gadagiet *et al.*, 2004). Significant changes in the root volume and number of roots were reported as a result *Azospirillum* inoculation in different cereals (Wani, 1990 and Okon and Itzigsohn, 1995). Production and secretion of phytohormones like auxin, cytokinin and gibberlins by *Azospirillum* itself might be the reason for more number of root formations in the inoculated plants. Similar findings were reported by Molla *et al.* (2001) and Remans *et al.* (2008)

In different root parameters considered, bio-inoculants along with 100 and 150 ppm benzyladenine exhibited maximum values for all the root characters. Even though benzyladenine has least effect on root growth, sometimes through auto inductive cytokinin regulation, this effect may reverse, favouring better root production in the plants. So here in addition to the phytohormone produced inside the plant due to *Azospirillum* inoculation, exogenous application of BA might have elevated the level of cytokinin concentration within the plants and higher root production might have occurred due to autoinductive cytokinin regulation. Similar findings were also reported by Wroblewska (2013).

Floral characters

An improvement in the parameters like length of the spike, stalk length, number of flowers per spike, flower size and field life of spikes were observed in the treatments consisting of AMF in combination with different levels of benzyl adenine (Table 2). However the treatment T† (POP + AMF + BA 150 ppm) was found to be the best among all the treatments. Even though epiphytic orchids like *Dendrobium* are characterised by the presence of aerial roots covered with velamen tissue, it can be function as absorbing roots, once it gets attached to a substrate (Dycus *et*

al., 1957). Hence in addition to the nutrients supplied through foliar spray, there might have been an additional exploration of nutrients, especially phosphorous, from the growing substrate also. The positive influence of AMF inoculated plants in floral characters might be due to this enhanced absorption and availability of nutrients to the plants combined with efficient translocation of phytohormones resulting in early flower initiation and production of quality spikes.

Benzyladenine, a synthetic reported to be one of the multifactorial components that functions as floral stimulus and exogenous application of cytokinin was reported to hasten the rate of flower initiation and flower production due to an increase in endogenous level of cytokinin in plant system (Blanchard and Runkle, 2008). The result of the study is in conformity with the findings of Perner *et al.*, (2007) and Barman *et al.*, (2014) who reported a positive influence of benzyladenine on floral characters in various flower crops.

Physiological parameters

Chlorophyll content

Regarding chlorophyll content, maximum chl a, chl b and total chlorophyll content were observed in the treatment T... (POP + AMF + 100 ppm benzyladenine) **Fig. 1**. This might be due to an elevated level of stomatal conductance, transpiration, photosynthesis and plant growth. Formation of larger and more number of bundle sheath chloroplast could be another reason for maximum production of chloroplast in inoculated plants (Arumugan *et al.*, 2010).

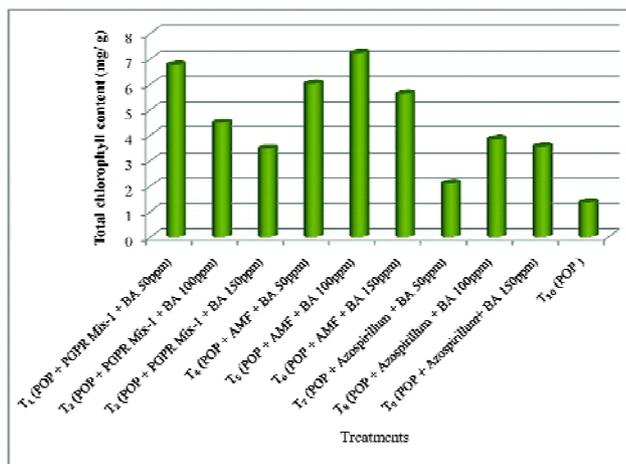


Fig 1. Influence of bioinoculants and benzyl adenine on total chlorophyll content

In addition to the effect of AMF, benzyladenine also carries an important role for higher chlorophyll production in the plants under this treatment (T...). Cytokinin encourage the protease activity and results in the release of cations like Ca²⁺, Mg²⁺ and Zn²⁺ from their nature bound or complexed state (Fletcher *et al.*, 1971) and the release of these ions may also be the possible reason for higher chlorophyll production in this treatment. The result of the present study is found to be in accordance with the findings of Fletcher and McCullagh (1971).

Stomatal characters

Considering the stomatal frequency, maximum number of stomata was obtained in the treatment consisted of *Azospirillum* and 100 ppm benzyladenine applied along with the recommended dose of NPK and cow dung slurry (POP)(**Fig.2**). Since the maximum leaf area was observed in the same treatment, it could be the reason for more number of stomata compared to other plants.

CONCLUSION

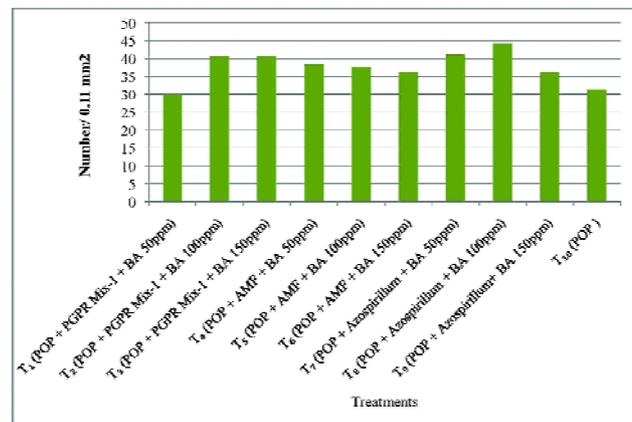


Fig 2. Influence of bioinoculants and benzyl adenine on stomatal density

From the study it could be observed that there was a significant improvement in the vegetative as well as root parameters in the treatment consisting of *Azospirillum* along with 100 and 150 ppm benzyladenine. However the treatment T† (POP + AMF along with 150 ppm benzyladenine) was found to be superior in both qualitative and quantitative floral attributes. Since the orchids are grown for their high value cut spikes, the treatment T₆ (POP + AMF + 150 ppm BA) can be recommended for production of quality spikes in *Dendrobium* orchids.

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