



## **Response of *Phaius tankervilleae* Bl. to Various Nutrient Concentration Levels**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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### **ABSTRACT**

To ensure the healthy growth and development of vegetative and reproductive tissues, all plants must obtain a specific amount of inorganic mineral elements from their surroundings. One of the most crucial elements that regulates the growth and development of floral crops is the availability of mineral nutrients in sufficient quantities. The current study was carried out at the Assam Agricultural University's Horticulture Experimental Farm in Jorhat, Assam, during the 2020–2021 academic year utilising a completely randomised design with seven different treatment combinations and three replications. The findings showed that mineral nutrients had a substantial impact on all growth features. The treatment T5 (RDF+ Ca 500 ppm) was the most effective in boosting all growth characteristics, including plant height (56.27 cm), leaf count (12.33), leaf length (53.07cm), leaf width (9.67cm), leaf area (469.23cm<sup>2</sup>), stem diameter (7.07cm), and number of pseudobulbs (2.80). T2 (RDF+ Zn 750ppm) exhibited the shortest days for inflorescence visibility (50.87), bud development (142.80), days to half bloom (146.30), and days to full bloom (148.77), as well as the greatest value for spike length (66.73cm), floret diameter (9.67cm), stalk girth (3.67cm), number of florets per spike (12.93), number of spikes per plant (2. (22.80g). For Physiological characters, treatment T2 (RDF+Zn(750ppm) was best for increasing total chlorophyll content (1.55 mg g<sup>-1</sup> fw), stomatal number (37.13), and stomatal size (5.80 μm<sup>2</sup>).

**Keywords:** Mineral; nutrients; growth characters; flower characters; physiological characters.

## 1. INTRODUCTION

Orchids are the world's most fascinating and beautiful flowers, they are one of the most innumerable flowering plant families [1] and are globally distributed. Orchids are members of the monocotyledon family orchidaceae. Orchidaceae, with 30,000-35,000 species, one of the world's largest flowering plant families belonging to 800 genera [2].

Orchidaceae is one of the best known plant families in the global horticulture and cut flower trades and they are harvested, grown, and traded for a variety of reasons, including ornamental plants, medicinal products and food [3,4]. Orchids are the most popular tropical cut flower in the world, and they are the eighth most popular cut flower in the world. In India, commercial orchid farming is still in its infancy and they are only farmed on a modest scale in Kerala, Tamil Nadu, Karnataka, Darjeeling, West Bengal and Arunachal Pradesh in the North Eastern Region.

Orchids are herbaceous perennial plants which are classified as monopodial (single-footed orchids) or sympodial (two-footed orchids/many footed orchid). Orchids are classified into four types based on their cultural requirements: epiphytic, terrestrial, lithophytic and saprophytic. Terrestrial orchids include *Phaius tankervilleae* Bl. *Phaius tankervilleae* Bl., also known as the nun orchid, has silvery brown blooms. There are approximately thirty species, which are grown from East Africa to tropical Asia and the Pacific Islands. The leaves are about 90cm long and dark green in colour. Inflorescence grows from the base of the pseudobulb, reaching a height of 150cm and bearing 10-20 flowers. Flowers are large, fragrant, heavy textured and long lived, making them ideal for bed planting, potted plants and floral arrangements. *Phaius tankervilleae* Bl. is considered as indigenous orchid species of the North-East region. In order to commercialize and for quality production of *Phaius tankervilleae* Bl. there is a need to develop proper growing techniques including standardization of nutrient supplements. Thus, the present experiment was carried out to check the response of *Phaius tankervilleae* Bl. to selected nutrient concentrations. For high yield and high quality products, a balanced fertilisation programme that incorporates macro and micronutrients in plant nutrition is essential [5].

Micronutrients are necessary in small amounts for satisfactory plant development and production; nonetheless, their deficiency causes an extreme aggravation of the plant's physiological and metabolic cycles [6]. Plants take nutrients from soils through their roots, however foliar sprays can act as fertiliser by providing nutrients to plants. To compensate for their scarcity, microelements like as Fe, Zn, Mn and Cu are added to foliar fertilisers all over the world, particularly in arid and semi-arid areas [7]. The macro elements includes nitrogen, phosphorus, carbon, oxygen, potassium, calcium, magnesium and sulphur and the micro or minor or trace elements which includes molybdenum, zinc, copper, manganese, iron, boron and chlorine are essential nutrients for plant growth. These elements have a wide range of beneficial effects on flower production. Though micronutrients are only required in trace amounts, they are critical for the majority of flowering plants [8]. The use of minerals in commercial orchid flowers can aid in the production of world-class orchid flowers

The main functions of calcium involve its ability to form coordination bonds and its ability to establish stable but reversible intermolecular and intramolecular bonds, especially in the cell wall and on the surface. membrane surface Calcium plays an important role in the excitatory-response coordination involved in signal transduction pathways, because it is released from intracellular pools that activate various protein kinases, phosphatases or phospholipases, target molecules that then regulate many cellular functions [9].

Zinc is absorbed mainly as the divalent cation, and it exists only in the oxidation state when complexed with macromolecules. The ability of zinc to form tetrahedral complexes with N, O, and S ligands influences both the tertiary structure of protein (e.g., through the formation of zinc fingers) and enzyme catalytic activity [10]. Alcohol dehydrogenase, carbonic anhydrase, superoxide dismutase, and RNA polymerase are all zinc-containing enzymes.

In light of the aforementioned, a field trial was carried out to see how zinc and calcium affect on growth and flowering of *Phaius tankervilleae* Bl.

## 2. MATERIALS AND METHODS

The experimental site was located at 26°47'N latitude, 94°12'E longitude at an elevation of

86.8m above mean sea level. The topography of the land was uniform. Jorhat falls within Upper Brahmaputra Valley Agro Climatic Zone of Assam.

The experiment was laid out in completely randomized block design with 7 numbers of treatments T1 RDF+Zn (500ppm): T2 RDF+Zn (750ppm): T3 RDF+Zn (1000ppm): T4 RDF+Ca (250ppm): T5 RDF+Ca (500ppm): T6 RDF+Ca (750ppm): T7 (Control : RDF) and three numbers of replications. The micronutrients which were used in experiment were Zinc sulphate and Calcium Nitrate.

The orchids were grown in partially controlled atmosphere by reducing light intensity and effective heat with 50 per cent shade net house. The orchids were planted in raised beds measuring 1.2m x 1.5m, at a 40 x 40 spacing. The space between one bed and the next was kept at 30 cm. As a foliar spray, RDF: NPK-19:19:19 fertiliser mixture @ 1g/L was applied twice a week. The mineral treatments T1, T2, T3, T4, T5 and T6 were administered as foliar sprays at 15-day intervals. Five sampled plants from each of the plot were selected randomly according to the area sampling technique for recording the observations. The selected plants were tagged 1-5 in each plot for facilitating correct measurement. From planting till flowering, all growth characteristics and flowering characters were documented.

All the physiological data were recorded during the flowering stage. Chlorophyll estimation was done from fresh leaf samples. The absorbance of the extract was measured at 645 and 663 nm wavelength filters in UV-Vis spectrophotometer for the determination of total chlorophyll content according to the method developed by Anderson and Boardman (1964):

$$\text{Total chlorophyll in mg/g tissue} = \frac{20.25 (D.645) + 8.02 \times (D.663) \times V}{1000 \times W}$$

where, V = final volume of the extract (ml)  
W = weight of the sample taken (g)  
D.645 = O.D. at 645  
D.663 = O.D. at 663

For stomatal character, fully expanded leaf samples at flowering stage were collected and the epidermis was peeled off a strip from ad axial side. Then it is stained and mounted on a slide and a cover slip was put on it and then it was observed under high power magnification. The number of stomata was counted in the area 1.85 mm<sup>2</sup> microscopic field using 100X magnification [11]. Stomatal size was estimated by measuring guard cell length and breadth of five stomata per sample using 100X magnification [11]. Calibration was done with the help of stage and ocular micrometer.

The experimental data obtained from various observations were statistically analysed by using Fisher's method of analysis of variance in Randomized Block Design as described by Panse and Sukhatme [12]. Significance or non significance of the variance due to different treatment effects was determined by finding 'F' values.

### 3. RESULTS AND DISCUSSION

There was significant differences in the mineral nutrient treatments for all the growth characters. Of all the treatments, T5 (RDF + Ca 500ppm) exhibited the highest mean performance for all the growth characters in *Phaius tankervilleae* Bl. as depicted in Table 1.

**Table 1. Growth Characters recorded for *Phaius tankervilleae* Bl**

Treatment	Plant height (cm)	No. of leaves	Leaf length (cm)	Leaf width (cm)	Leaf area (cm <sup>2</sup> )	Stem Diameter (cm <sup>2</sup> )	No. of pseud-oulbs
T <sub>1</sub>	52.7	9.73	49.47	8.07	393.7	5.07	1.80
T <sub>2</sub>	55.73	10.40	51.37	9.60	463.4	6.43	2.37
T <sub>3</sub>	51.20	9.50	48.73	7.73	343.7	5.10	1.67
T <sub>4</sub>	52.10	9.87	49.33	8.70	402.1	5.20	2.00
T <sub>5</sub>	56.27	12.33	53.07	9.67	469.2	7.07	2.80
T <sub>6</sub>	51.50	10.20	47.43	7.60	337.3	5.23	1.63
T <sub>7</sub>	49.80	6.87	39.80	6.33	243.2	4.43	1.20
S. Ed. (±)	0.69	0.26	0.42	0.29	1.18	0.07	0.15
CD <sub>0.05</sub>	1.52	0.58	0.93	0.64	2.61	0.21	0.32

Table 2. Flowering Characters recorded for *Phaius tankervilleae* Bl

Treatment	Days to inflorescence visibility	Days to bud development	Days to half bloom	Days to full bloom	Spike length (cm)	Floret diameter (cm)	No. of florets per spike	No. of spikes per plant	Self life (days)	Fresh weight of spike at harvest (g)	Weight of spike at senescence (g)	Vase life of spike (days)
T <sub>1</sub>	56.70	147.33	153.0	155.3	65.5	9.33	10.73	1.13	15.7	54.83	19.77	8.3
T <sub>2</sub>	50.87	142.80	146.3	148.7	66.7	9.67	12.93	2.50	17.8	61.67	22.80	10.4
T <sub>3</sub>	54.57	147.33	150.8	154.1	65.7	9.11	11.27	1.33	13.8	50.90	20.33	7.67
T <sub>4</sub>	60.77	148.27	152.9	157.6	63.6	9.13	11.40	1.33	14.6	49.57	19.90	7.83
T <sub>5</sub>	51.00	145.03	148.6	150.8	65.40	9.41	12.20	2.13	15.9	58.67	20.97	9.80
T <sub>6</sub>	62.00	149.40	151.9	156.8	62.63	8.91	11.50	1.33	14.2	55.40	18.70	7.90
T <sub>7</sub>	65.67	153.27	155.4	159.2	52.60	8.40	8.50	1.00	10.5	45.90	15.83	5.60
S. Ed. (±)	1.29	0.96	1.31	0.76	0.21	0.09	0.29	0.07	0.19	0.41	0.14	0.13
CD <sub>0.05</sub>	2.74	2.11	2.12	1.67	0.45	0.21	0.63	0.17	0.43	0.90	0.32	0.28

Table 3. Physiological characters recorded for *Phaius tankervilleae* Bl.

Treatment	Total chlorophyll content (mg g <sup>-1</sup> fw)	Stomata number	Stomata size (µm <sup>2</sup> )
T <sub>1</sub>	1.34	35.13	5.53
T <sub>2</sub>	1.55	37.13	5.80
T <sub>3</sub>	1.27	33.76	5.30
T <sub>4</sub>	0.90	30.33	4.60
T <sub>5</sub>	1.04	28.43	4.30
T <sub>6</sub>	0.85	27.90	4.17
T <sub>7</sub>	0.72	26.70	3.97
S. Ed. (±)	0.04	0.72	0.08
CD <sub>0.05</sub>	0.09	1.60	0.18

The highest mean values were recorded for T5 (RDF +Ca 500 ppm) viz: plant height (56.27cm), number of leaves (12.33), leaf length (53.07cm), leaf width (9.67cm), leaf area (469.23cm<sup>2</sup>), stem diameter (7.07cm,) and number of pseudobulbs (2.80). Plant height is a central part of plant ecological strategy. It is strongly correlated with life span and time to maturity. The increase in plant height could be due to a major role of calcium in cell metabolism being an integral part of the cell wall and being involved in cross-linkage of pectic molecules. Calcium is involved in various processes that preserve the structural and functional integrity of plant membranes, stabilize cell wall structures, regulate ion transport and selectivity, and control ion-exchange behaviour as well as cell wall enzyme activities as reported by Marschner (1955). Calcium have played indirect role in vegetative growth leading to the improvement in intake of nitrogen and other trace elements. Similar results were reported by Naik et al. [13] in Cymbidium hybrid. The increase in leaf length may be due to the fact that calcium is one of the main components in plant cell wall which causes plant cell wall solidity and elongation [14]. The highest leaf area was recorded in T5 (RDF +Ca 500), i.e 469.23 cm<sup>2</sup> ,Calcium regulates the uptake and movement of other nutrients within the plant by activating protein pumps resulting higher photosynthesis rate and causes increase in leaf area. Similar observations were reported by Poovaiah [15]. The highest stem diameter recorded was 7.07 in T5 (RDF +Ca 500), Calcium play a role in mitosis, cytoplasmic streaming, phytochrome responses, and the activity of cytokines that promote correct cell elongation, among other growth responses. The highest number of pseudobulbs. was 2.80 recorded in T5 (RDF +Ca 500), the increased number of pseudobulbs could be attributed to the role of calcium in vegetative growth, improvements in N metabolism, and plant absorption of other trace elements, all of which resulted in an increase in the number of pseudobulbs. This finding is consistent with the findings of Jeeva Jothi et al. [16].

The time required for inflorescence visibility and flower harvesting are important plant characteristics that determine a plant's early yield potential. The findings in Table 2. revealed that the minimum days required for inflorescence visibility (50.87 days), bud development(142.80 days), half bloom (146.30 days) and full bloom (148.77 days) were recorded in T2 (RDF + Zn 750ppm). This could be because zinc promotes

proper hormonal levels in plants [17], which promotes early maturity and, as a result, early flowering [18]. According to Khosa et al. [19], foliar application of micronutrients such as Zn, B, Fe and Mn in combination with macronutrients influenced the days to flower emergence in *Gerbera jamesonii*. In Orchids, spike length is one of the most essential grading characteristics. In terms of spike length, there was a substantial variation between the mineral nutrients. The maximal height observed was 66.73cm, this could be because zinc activates enzymes and is involved in a variety of physiological functions, and it aids in the accumulation of more assimilates, which are required for improving the spike and increasing the stem's girth. This finding is consistent with that of Ganga et al. [20] in *Dendrobium* cv. Sonia 17. In T2 (RDF + Zn 750ppm), the flower diameter was found to be considerably greater, 9.67cm, this could be due to zinc's role in the photosynthesis process, which results in increased chlorophyll concentration and faster vegetative and floral growth. The maximum number of florets recorded in was 12.93 in T2 (RDF + Zn 750ppm), Zinc is also suggested to be important in a variety of enzymatic activities, including the formation of IAA, which helps to increase the number of florets. Ganga et al. [20] reported similar results in *Dendrobium* cv. Sonia 17. Self life was higher in T2 (RDF + Zn 750ppm) ,this could be due to zinc's active role in slowing the formation of the abscission layer in the flower pedicel, thereby preventing flower drop. Post-harvest longevity is a critical factor in determining marketability of a plant. Maximum vase. was found to be 10.43 days in T2 (RDF + Zn 750ppm. The increased vase life of the flower in response to zinc foliar spraying could be attributed to the presence of increased photosynthates in the flower part as growth parameters increased. Moreover, zinc is an activator of enzymes that may regulate antioxidative activity. As a result, it has the potential to increase vase life by scavenging Reactive Oxygen Species (ROS) and protecting membrane integrity from oxidative damage. This findings are in associated with Saikia et al. [21] in *Rhyncostylis retusa*. Increase in fresh weight of flowers is an important character of flowers in terms of quality, freshness and longevity of the flowers. The results revealed the fresh weight and the weight at senescence were higher in T2 (RDF + Zn 750ppm) compared to the control.. This may be due to involvement of zinc in photosynthesis [22] as evident from enhancement of chlorophyll and protein, activating several enzymes and accumulating

high amount of assimilates needed for improvement of spike, which resulted maximum fresh weight of spikes.

Chlorophyll is an important plant component because it traps solar energy and aids in the process of photosynthesis, which is a vital plant process. Photosynthesis is required for plant survival. The results in Table 3. revealed that the chlorophyll content in T<sub>2</sub> (RDF + Zn 750ppm) was higher (1.55 mg g<sup>-1</sup>). This could be because zinc is involved in increasing photochemical reduction rates (Kumar et al., 1988), chloroplast structure, photosynthetic electron transfer, and photosynthesis [23]. Moreover Zn acts as metal component of various enzymes and also are associated with saccharide metabolism, photosynthesis, and protein synthesis and chlorophyll content. This findings are in association with Nasiri et al. [24] in chamomile. One of the most important characteristic for plant growth and development is stomatal character. Stomata are small adjustable pores found on both the upper and lower surfaces of plant aerial tissues. These pores open to allow carbon dioxide uptake and close to prevent water loss. The current study revealed a significant difference between stomatal number and stomatal size as influenced by mineral nutrients (Table 3). The highest stomatal number and stomatal size recorded for *Phaius tankervilleae* Bl. were 37.13 and 5.80 μm<sup>2</sup> respectively,

The maximum stomatal number and stomatal size were recorded in T<sub>2</sub> (RDF + Zn 750ppm). This could be due to zinc's involvement in stomatal opening, possibly as a component of the enzyme carbonic anhydrase and as a factor in membrane integrity and K<sup>+</sup> uptake [25-40].

#### 4. CONCLUSION

From the findings, it can be concluded that application of adequate amount of mineral nutrients favourably contributed towards growth and flowering of *Phaius tankervilleae* Bl. Treatment T<sub>5</sub>(RDF + Ca 500ppm) and T<sub>2</sub> (RDF + Zn 750ppm) significantly increased the growth and flower characters, physiological characters [41-69].

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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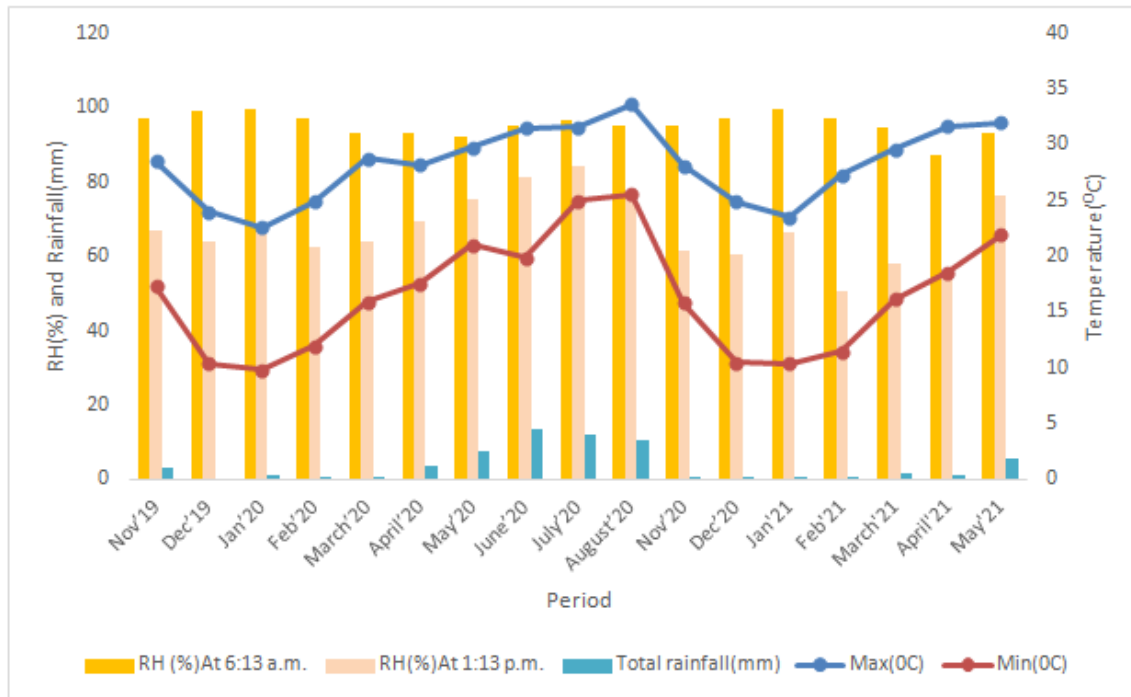
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**APPENDIX**



**Fig. 1. Metereological data observed during the experimental periods (2020-2021)**

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