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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2018; 7(7): 441-446

www.thepharmajournal.com
Received: 06-05-2018
Accepted: 10-06-2018

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Studies on induction of early flowering in orchids (*Phalaenopsis* hybrid) cv. fuller's sunset

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Abstract
The experiment was conducted as a pot culture trial in the shade net house located at the Biotechnology-cum-Tissue Culture Centre, College of Agriculture, Orissa University of Agriculture and Technology, Bhubaneswar during 2016-17, with three replications and seventeen treatments treated in Completely Randomized Design. The treatments consisted of foliar application of different plant growth regulators such as GA_3, BAP, Kinetin and IAA; each growth regulator in four different concentrations (100 ppm, 200 ppm, 300 ppm and 400 ppm) and a control (without any growth regulators). The results of the study revealed that foliar application of plant growth regulators at various concentrations exhibited significant variation with respect to growth and flowering parameters. Among these seventeen treatments, the induction of early flowering was observed in the plants treated with kinetin @100ppm. Among the different concentration of plant growth regulators, best result with respect to most of the flowering parameters such as number of flowers per inflorescence, diameter of flower and flowering duration were recorded in plants treated with kinetin @200ppm. Considering the satisfactorily performance of plant growth regulators (especially Kinetin and Benzyl Amino Purine) under Bhubaneswar agro-climatic condition, the flower growers may be advised to take up cultivation of *Phalaenopsis* hybrids on a commercial scale in and around Bhubaneswar to cater the need of local as well as outside market.

Keywords: *Phalaenopsis*, orchid, early flowering, PGRs, GA_3, BAP, Kinetin, IAA

Introduction
Orchids with their bewildering range of flowers in terms of exquisite colour combinations provide a source of profound aesthetic pleasure. Taxonomically, orchids represent the most highly evolved family "Orchidaceae" among monocotyledons. With advancement in technology, the orchids have become most important segment in floriculture trade. *Phalaenopsis,* commonly known as moth orchids, is one of the most popular orchids in the trade, having approximately 60 species.

Phalaenopsis do not tolerate excessive amounts of high bright light or direct sunshine, rather do well in intermediate to cool environments; however, there are varieties like Fuller's Sunset tolerates intermediate to warm temperatures. Under controlled condition, the *Phalaenopsis* can be grown in specially designed 'orchidaria' with RH should not be less than 30% at night and 80% during day time, in any kind of pot/container, which can hold medium and provide aeration is suitable. Possessing neither pseudobulbs nor rhizome, *Phalaenopsis* shows a monopodial growth habit. *Phalaenopsis* hybrid Fuller's Sunset have gorgeous light yellow color flower. Petals and sepals are rounded. The triangulated lip is a distinctive feature of this orchid. It blooms in the fall-spring and the flowers are long lasting. *Phalaenopsis* shows a juvenile phase, in which plants must reach a certain stage of growth to attain the capacity to flower. The ability of *Phalaenopsis* to spike and bloom under inductive environmental condition is highly correlated with its 'leaf size' (Lee, 1991) [11]. The juvenile phase of *Phalaenopsis* is relatively long and greatly varies among varieties and hybrids, ranging from 6 to over 24 months from the transplanting of young micro-propagated plants.

Orchid flower initiation is usually associated with light intensity, (Kataoka *et al*., 2004) [9], temperature and photoperiod (Vaz *et al*., 2004) [21] or hormonal changes (Campos and Kerbauy, 2004) [21]. Plant hormones are natural chemicals produced in minute quantities in one part of a plant and have a physiological effect when moved to another part of the plant; a chemical messenger.

As no such work has been done on this species under the climatic conditions of Odisha, farmers or nurserymen interested in cultivation of orchids will be benefited from the outcome of the experiment.

Keeping the above facts in view, the present investigation has been carried out with the objectives to achieve early flowering and to know the effect of growth regulators on different growth and flowering parameters.

Materials and Methods

The experiment was carried out in the shade net house of the Biotechnology-cum-Tissue Culture Centre, College of Agriculture, Orissa University of Agriculture and Technology, Bhubaneswar during the year 2016-17. The experimental site is situated 63 km away from the Bay of Bengal at an altitude of 25.5 meters above mean sea level; 20^0 – 15' north latitude and 85^0 – 50' east latitude. The mean annual precipitation is 1646.43 mm, average maximum temperature ranges from 35 ^{0}C to 39 ^{0}C during May and June, while the minimum temperature varies from 13^0C to 15^0C during December to January, relative humidity varies between 50% in summer and 90% in rainy season.

One month old, healthy, uniform tissue culture plantlets of *Phalaenopsis* hybrid cv. Fuller's Sunset were used in this experiment. Utmost care was taken during the transportation to avoid any damage to the roots and growing tip of the plant. These were planted in 6" earthen pots containing charcoal and coconut fiber in equal proportion and pots were kept on 2.5ft high iron benches inside a shade net house (50% shade). Initially, the plants were sprayed with water soluble fertilizer (NPK : 19:19:19) for two months to acclimatize the young plantlets to the new environment before exposing them to any treatments.

Depending upon moisture content of media and prevailing weather conditions the pots were irrigated regularly to keep the media moderately moist. Usually, the plants were not watered after 3PM. Weed population was very less in the pots and they were removed manually, as and when noticed. The plants were sprayed with plant protection chemicals as and when any insect or disease attack was observed. The water soluble fertilizer containing 3 major macronutrients NPK in the proportion 19:19:19 @ 0.2% and micronutrients named as 'Multiplex'@ 1ml/l are used as common spray. The fertilizer was sprayed twice a week and the micronutrient once in a month with the help of a fine hand sprayer, till the whole plant get drenched. The growth regulators were applied to the plants starting from 60 DAP, in fortnight intervals. The biometric observations were recorded from 3 plants in each treatment. Data obtained on various growth and flowering characters were recorded and were analyzed statistically by using analysis of variance (ANOVA) table. The treatment effects were tested by using "F" test at 5% level.

Results and Discussion

Effect of plant growth regulators on vegetative growth parameters

Application of plant growth regulators on *Phalaenopsis* hybrid cv. Fuller's Sunset significantly influenced different growth parameters compared to the untreated plants (Control). The plants treated with gibberelic acid were taller than the plants treated with other plant growth regulators. Tallest plants (27.50 cm) were observed in the plants treated with GA_3 @ 300 ppm. Similar findings were reported by Barman *et al*. (2014) [1], who observed increased plant height in *Dendrobium* hybrid "Thongchai Gold" with application of GA_3 @ 200 ppm. Increase in plant height with application of Gibberellic Acid (GA_3) might be due to enhanced cell division, cell enlargement, increased plasticity of cell, promotion of protein synthesis coupled with higher apical dominance. The positive effect of gibberellins on growth may be due to increase in the auxin level of tissue or enhance the conversion of tryptophan to IAA which causes cell division and cell elongation (Kuraishi and Muir, 1964) [10]. The increase in plant height by application of GA_3 was also reported in other crops by Girwani *et al*. (1990) [8] and Mishra P (2017) [15] in marigold. Gibberelic acid, not only increased the plant height but also, increased the leaf length. Longest leaf (21.66 cm) was observed in the treatment T_3 (GA_3 @ 300 ppm) which was statistically at par with the treatments T_4 (GA_3 @ 400 ppm) and T_1 (GA_3 @ 100 ppm). This result relates to the findings of Cardoso *et al.* (2012) [7] in *Phalaenopsis* hybrid, who reported that the increase in leaf length was from 10.9 cm (control) to 18.1 cm in a treatment using 125 ppm GA_3. In relation to the best treatment (125 ppm of GA_3), the higher doses of GA_3 showed a slight decrease in the length of the leaves by increasing the concentrations used. Similar findings were also reported by Vichiato *et al.* (2007) [22] in *Dendrobium nobile*.

Application of Benzyl amino-purine increased the number of leaves per plant. Maximum number of leaves per plant (6.66) were obtained in the treatments T_5 (BAP @ 100 ppm), T_6 (BAP @ 200 ppm) and T_{10} (Kinetin @ 200 ppm). Similar findings were reported by Nambiar *et al.* (2012) [17] in *Dendrobium* hybrid. They reported that application of BAP (150-200 ppm) increased the number of leaves in *Dendrobium hybrid*.

A remarkable increase in leaf width was noticed in case of plants treated with Kinetin and Indole-3-Acetic Acid (IAA) as compared to treatment with other plant growth regulators. The maximum width of leaf (5.96 cm) was recorded in the treatment T_{10} (Kinetin @ 200 ppm), which was statistically at par with the treatments T_9 (Kinetin @ 100 ppm) and T_{13} (IAA @ 100 ppm). This result was supported by the findings of Liang *et al.* (2010) [12] who reported that the kinetin regulates the leaf development. This was also supported by the findings of Nandhini and Chezhiyan, (2001) [18] in *Dendrobium* cv. Sonia-17. In Gibberellic Acid (GA_3) treated plants, width of leaf decreased with increase in concentration and narrowest leaf was observed in the treatment T_4 (GA_3 @ 400 ppm). This result is similar to the findings of Vichiato *et al.* (2007) [22] in *Dendrobium nobile*, who also reported that the width of the leaves were reduced with increase in concentrations of GA_3. Treatment T_{10} (Kinetin @ 200 ppm) showed maximum leaf area (31.08 cm^2) and was found statistically at par with T_9 (Kinetin @ 100 ppm) (30.00 cm^2). This result was similar to the findings of Yadav (2013) [24] in marigold who reported that more leaf area at 150 ppm Kinetin.

Indole-3-acetic acid helped in root development in *Phalaenopsis* hybrid. Maximum number of roots (12.33) were observed in the treatment T_{13} (IAA @ 100 ppm) and it was statistically at par with T_{14} (IAA @ 200 ppm) (12.00). Similar finding was reported by Bhattacharjee and Islam (2014) [2] in *in-vitro* condition. They observed highest number of roots and longest roots with application of IAA in *Vanda tessellate*.

In case of plants treated with Benzyl Amino Purine, development of new shoots from the base of the plant was observed which is a rare phenomenon in *Phalaenopsis spp.*(Fig.1), this being a monopodial species. Similar finding was reported by Blanchard and Runkle (2010) [19] who observed development of new shoots with application of Benzyl Amino Purine (BAP) in *Zygopetalum* Redvale.

Fig 1: Shoot induction in *Phalaenopsis* hybrid cv. Fuller's Sunset with application of Benzyl Amino Purine (BAP)]

Fig 2: Growth of *Phalaenopsis* hybrid cv. Fuller's Sunset influenced by different hormones

Table 1: Effect of foliar application of plant growth regulators on various growth parameters in *Phalaenopsis* hybrid cv. Fuller's Sunset

Treatments width	Plant Height (cm)*	Number of leaves per plant*	Leaf Length (cm) *	Leaf Area (cm)*	Leaf Area (cm^2)*	Number of roots per plant*
GA_3 100ppm (T_1)	23.16	5.66	19.16	2.06	21.66	11.00
GA_3 200ppm (T_2)	22.40	4.33	19.00	2.03	21.40	10.66
GA_3 300ppm (T_3)	27.50	5.33	21.66	1.90	22.91	11.00
GA_3 400ppm (T_4)	23.50	4.66	20.16	1.63	20.33	10.66
BAP 100ppm (T_5)	12.00	6.66	9.90	3.80	19.66	10.66
BAP 200ppm (T_6)	8.93	6.66	7.40	3.90	18.58	10.33
BAP 300ppm (T_7)	8.36	5.33	6.96	3.30	18.00	10.00
BAP 400ppm (T_8)	8.20	5.33	6.83	3.33	15.83	9.33
Kinetin 100ppm (T_9)	15.50	6.33	13.53	5.60	30.00	11.33
Kinetin 200ppm (T_{10})	15.93	6.66	14.56	5.96	31.08	11.00
Kinetin 300ppm (T_{11})	13.50	5.33	11.86	4.83	24.58	11.00
Kinetin 400ppm (T_{12})	13.06	4.66	11.16	4.50	22.91	10.66
IAA 100ppm (T_{13})	15.10	6.33	13.16	5.33	25.41	12.33
IAA 200ppm (T_{14})	14.00	6.00	12.83	4.96	25.16	12.00
IAA 300ppm (T_{15})	13.76	5.66	12.00	4.66	24.33	11.33
IAA 400ppm (T_{16})	13.16	4.66	11.76	4.26	23.00	11.33
Control (T_{17})	12.16	4.33	10.33	4.16	20.66	10.00
SE(m) ±	1.10	0.42	0.86	0.29	1.59	0.51
CD at 5%	3.17	1.21	2.50	0.85	4.59	1.47

* significant at 5%

Effect of plant growth regulators on flowering parameters in *Phalaenopsis* hybrid cv. Fuller's Sunset

Spraying of plant growth regulators on *Phalaenopsis* hybrid cv. Fuller's Sunset significantly influenced different flowering parameters compared to the untreated plants (Control).

The results revealed that there was no significant difference in number of spike per plant by the application of different plant growth regulators on *Phalaenopsis* hybrid cv. Fuller's Sunset as only one spike was produced in each treatment.

Significantly the earliest spike emergence (283 DAP) was observed in the treatment T_9 (Kinetin @ 100 ppm) followed by treatment T_5 (BAP 100 @ ppm (294 DAP), T_6 (BAP @ 200 ppm) (294.66 DAP), T_{10} (Kinetin @ 200 ppm) (294.66 DAP). This result was similar to the findings of Blanchard and Runkle (2008) [5] who reported that *Doritaenpsis* and *Phalaenopsis* orchid clones treated with BAP alone at 200 ppm had a visible spike 3-9 days earlier than non-treated plants and Nambiar *et al.* (2012) [17] stated that application of 200 ppm BAP induced early flowering in *Dendrobium* hybrid.

Fig 3: Early flowering in *Phalaenopsis* hybrid cv. Fuller's Sunset with application of Kinetin @ 100 ppm

Longest spikes (45.80 cm) were observed in treatment T_1 (GA_3 @ 100 ppm), which was statistically at par with T_3 (GA_3 @ 300 ppm) (44.63 cm). This might be due to the fact that gibberellic acid promotes cell division and cell elongation resulting in longer stalks. This result was supported with the findings of Runkle (2010) [19], who reported that exogenous application of gibberellic acid (GA_3) increased spike length, but more commonly, chemicals that inhibit the biosynthesis of active GA_3 are used to suppress spike elongation. This result was also supported with the findings of Biswas *et al*. (1983) [3] and Mishra *et al*. (1993) [16] and Blanchard and Runkle (2008) [5] in *Doritaenpsis* and *Phalaenopsis*; Barman *et al*. (2014) [1] in *Dendrobium* hybrid. Although the length of spike in GA_3 treated plants were more, it was not acceptable as the spikes were thin and lanky. It may be due to the cell growth promoted by the gibberellins, through the activation of hydrolytic enzymes, increases the length of the cells compared to their diameter, making tissues and organs such as leaves, stems and inflorescence, longer and thinner Taiz & Zeiger (2009) [20]. Diameter of spike was maximum (0.39 cm) in the treatment T_9 (Kinetin @ 100 ppm) which was statistically at par with the treatments T_{10} (Kinetin @ 200 ppm), T_6 (BAP @ 200 ppm) and T_5 (BAP @ 100 ppm).

Maximum number of flowers per spike (3.00) was observed in treatments T_5 (BAP @ 100 ppm), T_6 (BAP @ 200 ppm) and T_{10} (Kinetin @ 200 ppm). These treatments were statistically at par with T_1 (GA_3 @ 100 ppm), T_3 (GA_3 @ 300 ppm), T_9 (Kinetin @ 100 ppm), T_{11} (Kinetin @ 300 ppm), T_{13} (IAA @ 100 ppm) and T_{14} (IAA @ 200 ppm). Similar results were obtained with application of 50 to 150 ppm BAP on whole plant by Lin (1994) [13] in *Phalaenopsis amabilis* and *Phalaenopsis taisuco*; Blanchard and Runkle (2008) [5] in *Phalaenopsis* hybrid and Nambiar *et al.* (2012) [17] in *Dendrobium* hybrid. Foliar application of kinetin (200 ppm) significantly increased number of flowers per plant in *Phalaenopsis* (Wu and Chang, 2011).When different concentrations of plant growth regulators were applied, the least number of flowers per spike (1.66) was observed T_7 (BAP @ 300 ppm), T_8 (BAP @ 400 ppm) and T_{12} (Kinetin @ 400 ppm). It is similar to the findings of Lorteau *et al.* (2001) [14] who reported that with increase in concentration of BAP, flower count gradually decreased per spike due to yellowing and withering of bud. This could be because of ethylene secretion in plants that were treated with higher concentrations of BAP.

Table 2: Effect of plant growth regulators on number of spike per plant, time taken for emergence of spike, length of spike, diameter of spike and number of flowers per spike on *Phalaenopsis* hybrid cv. Fuller's Sunset

Treatments Diameter	Number of spike(s) per plant	Time taken for spike emergence (DAP)*	Length of spike (cm)*	Diameter of spike (cm)*	No. of Flowers per spike*
GA_3 100ppm (T_1)	1	305.00	45.80	0.25	2.66
GA_3 300ppm (T_3)	1	308.00	44.63	0.23	2.66
BAP 100ppm (T_5)	1	294.00	21.63	0.37	3.00
BAP 200ppm (T_6)	1	294.66	20.96	0.38	3.00
BAP 300ppm (T_7)	1	308.66	18.40	0.34	1.66
BAP 400ppm (T_8)	1	325.66	14.33	0.34	1.66
Kinetin 100ppm (T_9)	1	283.00	27.63	0.39	2.33
Kinetin 200ppm (T_{10})	1	294.66	27.30	0.38	3.00
Kinetin 300ppm (T_{11})	1	310.00	25.66	0.34	2.33
Kinetin 400ppm (T_{12})	1	315.66	20.93	0.25	1.66
IAA 100ppm (T_{13})	1	295.00	22.10	0.36	2.66
IAA 200ppm (T_{14})	1	298.66	21.70	0.35	2.33
SE(m) ±	NS	3.34	1.10	0.01	0.28
CD at 5%		9.81	3.23	0.02	0.84

*Significant at 5%

The diameter of flower was maximum (7.63 cm) in treatment T_{10} (Kinetin @ 200 ppm) which was statistically at par with the treatments T_9 (Kinetin @ 100 ppm) and T_{13} (IAA @ 100 ppm). It was similar to the findings of Chen *et al.* (1997) who hypothesized that cytokinin may be effective in promoting the lateral expansion of *Phalaenopsi*s sepals and petals. The flower having lowest diameter (5.66cm) was produced in T_8 (BAP @ 400 ppm) and it resembles to the findings of Nambiar *et al.* (2012) [17] in *Dendrobium* hybrid who reported that the application of Benzyl Amino Purine (BAP) did not significantly influence the size of the flowers. Among the treatments of plant growth regulators on *Phalaenopsis* hybrid, maximum length of peduncle (3.96 cm) and pedicel (4.40cm) was observed in the treatment T_1 (GA_3 @ 100 ppm). This result is similar to the findings of Barman *et al.* (2014) [1] in *Dendrobium* hybrid. The minimum length on peduncle (2.10 cm) and pedicel (2.70 cm) was found in treatment T_8 (BAP@ 400 ppm) and it was similar to the findings of Chen *et al.* (1997) in *Phalaenopsis* hybrid, who reported that application of BAP on flowering shoots shortened internodal length between the florets as compared to the application of GA_3 alone.

The longevity of flowers in plants was maximum (46.66 days) in treatment T_{10} (Kinetin @ 200 ppm) which was statistically at par with T_1 (GA_3 @ 100 ppm) (43.66 days). Similar observations were recorded by Padaganur *et al.* (2005) in tuberose and they reported that flowering duration of spike is more due to more number of florets per spike. Yadav (2013) [24] reported that duration of flowering was affected with the application of 150 ppm of kinetin and longer flower longevity recorded with the application of 200 ppm of kinetin in marigold. The study revealed that the vase life of flower spike did not vary significantly between the treatments in *Phalaenopsis* hybrid cv. Fuller's Sunset.

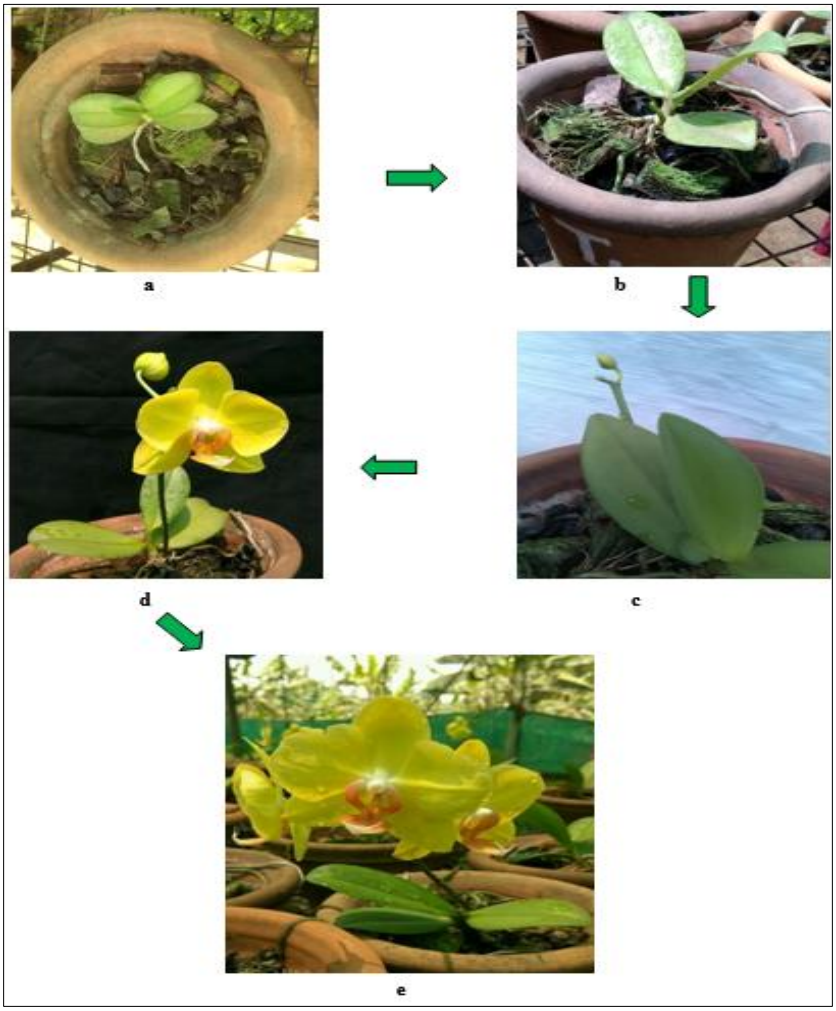
Table 3: Effect of plant growth regulators on diameter of flower, internodal length on peduncle, length of pedicel, flowering duration and vase life on *Phalaenopsis* hybrid cv. Fuller's Sunset

Treatments	Diameter of flower (cm)*	Internodal length on peduncle(cm)*	Length of Pedicel (cm)*	Flowering Duration (days)*	Vase life (days)
GA_3 100ppm (T_1)	6.16	3.96	4.40	43.66	26.33
GA_3 300ppm (T_3)	6.06	3.56	3.76	33.00	26.00
BAP 100ppm (T_5)	6.90	2.56	3.50	27.33	26.66
BAP 200ppm (T_6)	6.33	2.43	3.36	24.33	26.33
BAP 300ppm (T_7)	6.03	2.33	3.10	22.00	25.33
BAP 400ppm (T_8)	5.66	2.10	2.70	15.66	25.33
Kinetin 100ppm (T_9)	7.53	3.13	3.53	41.33	26.66
Kinetin 200ppm (T_{10})	7.63	3.06	3.60	46.66	26.66
Kinetin 300ppm (T_{11})	7.16	2.40	3.23	31.00	25.66
Kinetin 400ppm (T_{12})	6.90	2.20	3.03	28.66	25.66
IAA 100ppm (T_{13})	7.40	2.70	3.70	40.66	25.33
IAA 200ppm (T_{14})	7.13	2.50	3.66	39.00	25.33
SE(m) ±	0.09	0.10	0.13	1.23	**NS**
CD at 5%	0.26	0.29	0.39	3.61	

*Significant at 5%

Conclusion

Based on the result of the present study it is concluded that application of plant growth regulators (especially Kinetin and Benzyl Amino Purine) on *Phalaenopsis* hybrid cv. Fuller's sunset can help in early induction of flowering. Considering the satisfactorily performance of plant growth regulators under Bhubaneswar agro-climatic condition, the flower growers may be advised to take up cultivation of *Phalaenopsis* hybrids on a commercial scale in and around Bhubaneswar to cater the need of local as well as outside market. It is therefore suggested that, farmers can go for foliar application of different plant growth regulators, like Kinetin @ 100 ppm or BAP @100 ppm, in *Phalaenopsis* hybrid cv. Fuller's Sunset, to induce early flowering and obtain better quality spike.



Developmental stages of *Phalaenopsis* hybrid cv. Fuller's Sunset: (a) Vegetative stage, (b) emergence of flower stalk, (c) appearance of visible flower buds, (d) opening of the first flower and (e) the inflorescence.

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