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# Improving seed germination and seedling growth of dragon fruit (*Hylocereus undatus*) by PGRs

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ABSTRACT

Dragon fruit [*Hylocereus undatus* (Haworth) Britton and Rose] is an exotic super fruit introduced in India. It can be naturally propagated through seeds and stem cuttings. Present study was carried out during 2018 to 2021 at Main Horticultural Research and Extension Centre (MHREC), College of Horticulture, University of Horticultural Sciences, Bagalkote, Karnataka to improve the efficiency of seed germination and seedling growth in dragon fruit using PGRs. The results showed that, there were significant differences among various treatments. Treatment 100 ppm $GA_3$ recorded as the best treatment with respect to the parameters, viz. days taken for germination (10.60 days), germination percentage (87.00%), shoot length (10.27 cm), root length (8.06 cm), number of roots (6.20), seedling length (18.33 cm), seedling fresh weight (3.26 g), seedling dry weight (1.22 g) respectively at 90 days after sowing (DAS) and survival percentage (48%) than other treatments.

**Keywords**: Benzyl adenine, Dragon fruit, Gibberellic acid, Seed germination, Seedling growth

Dragon fruit [*Hylocereus undatus* (Haworth) Britton and Rose] is an introduced fruit crop belongs to family Cactaceae, attaining popularity in India owing to its organoleptic characteristics, nutraceutical properties, antioxidants, vitamins and minerals, earliness in bearing, easy agronomical practices and maintenance, and less incidence of pest and diseases (Mercado-Silva 2018, Le *et al*. 2021). In India, it is growing widely in Gujarat, Maharashtra, Karnataka, Kerala, Tamil Nadu, Odisha, West Bengal, Andhra Pradesh, Telangana and Andaman and Nicobar Islands (Nangare *et al*. 2020)

The dragon fruit seeds have better adaptability and less possibility of disease transmission (Elobeidy 2006) and enables to obtain different genetic information which can be used in the selection of genotypes with desirable characteristics. Molecular and genomics research on dragon fruit is lagging behind that on staple crop and there is no improved variety for commercial cultivation. So, there is an acute need of developing varieties in dragon fruit. The seeds are highly recalcitrant, and due to the unique structure of the ovule, the seeds stored under low temperature at 10°C for a week itself shows delayed germination and lower germination percentage (Sheng *et al*. 2016, Vishnupriya *et al*. 2019).

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Gibberellins ($GA_3$) and Benzyl adenine (BA) are used in earlier germination studies in dragon fruit (Dhurve 2016, Sheng *et al*. 2016). Gibberellins are used to speed up the germination of seeds, stem and internode elongation, enzyme production during germination and fruit setting (Davies 1995). Even though BA is mostly used in tissue culture for seed germination and callus development, it can improve seed performance under field conditions also. The previous studies attempted to improve the seed germination of dragon fruit by *in vitro* culture system (Sheng *et al*. 2016, Vishnupriya *et al*. 2019) by using BA and auxins and there is currently no widely available information on a systematic set of protocol for the establishment of direct seeding methods for enhancing seed germination of dragon fruit. Hence, to achieve this objective, it appears, there is a need for developing protocol for improving the seed germination efficiency by using plant growth regulators.

## MATERIALS AND METHODS

The experiments were conducted during 2018 to 2021 under the shade house of Fruit nursery at Main Horticultural Research and Extension Centre (MHREC), College of Horticulture, University of Horticultural Sciences, Bagalkote, Karnataka.

*Plant material source and extraction of seed*: White fleshed, red skin healthy fruits were acquired from farmer field of Bagalkot, Karnataka during september 2019. The average maximum and minimum temperature during this period was 33.4°C and 21.5°C respectively with an average

relative humidity of 56.8%. Cut opened the fresh ripe fruit into two half, scooped the pulp and extracted the seeds. The seeds were handled carefully as they are fragile. Rinsed until the water runs pretty well clear of any colour (Dahanayake and Ranawake 2012). When the seeds got dried, they began to loosen up from the plate. It took about 5 days for complete drying. When thoroughly dried, packed the seeds in an envelope and stored in a dry area.

*Preparation of gibberellic acid ($GA_3$) solution*: A stock solution of 100 ppm gibberellic acid was prepared by dissolving the required amount of HiMedia gibberellic acid (Assay≥90%) in distilled water. The five concentrations (10, 25, 50, 75 and 100 ppm) gibberellic acid solution were prepared by serial dilution using distilled water.

*Preparation of benzyl adenine (BA) solution*: A stock solution of 20 ppm benzyl adenine (6-Benzyl amino purine) was prepared by dissolving the required amount of HiMedia benzyl adenine (Assay≥98%) in distilled water. The four concentrations (1, 5, 10 and 15 ppm) of benzyl adenine solutions were prepared by serial dilution using distilled water. The required quantity of stock solution was taken out from the test tube using a pipette and diluted with distilled water to the required concentration.

*Seed treatment and sowing*: 50 medium-sized pots were filled with coco peat and sand (1:1 ratio) and drenched with 2 g/L of Bavistin to prevent infections. Before sowing seeds were treated with growth regulators ($GA_3$ and BAP) for about 15 min. Treated seeds were shade dried at nursery for 10 min. Then 50 seeds were broadcasted in each pot and covered with a thin layer of soil and watered the pots lightly with rose can. After sowing pots were covered with cardboard for two days for dark incubation. Watering was done at alternate days. Shoot parameters were recorded at monthly interval for three months.

*Days taken for germination*: The number of days taken from the day of sowing to the day of appearance of seedling above the media.

*Germination percentage (%)*: The number of seeds germinated out of the total number (50) of seeds sown expressed in percent.

$$\text{Germination percentage (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

*Shoot length (cm)*: Shoot height was measured from the tip of the shoot to the collar region of the seedling.

*Seedling length (cm)*: Seedling height was measured from tip of the shoot to tip of the root.

*Root length (cm)*: Length of root was measured from the collar region of the roots to the tip of the longest root.

*Number of roots per seedling*: The total number of roots was counted for each seedling and the mean of the five plants per replication is considered for analysis.

*Fresh weight of seedling (g)*: Fresh weight of the seedling was recorded immediately after uprooting the seedling.

*Seedling dry weight (g)*: The dry weight of the seedling was taken after drying in hot air oven at 55°C.

*Survival percentage (%)*:

$$\text{Survival percentage (\%)} = \frac{\text{Number of seedlings survived}}{\text{Number of seed germinated}} \times 100$$

*Statistical analysis:* The data obtained from all the above experiments were tabulated in 3 replications and subjected to statistical analysis (ANOVA) with completely randomized design (CRD) and results were tested at a level of 5% significant level using Fischer's method of analysis of variance. The significance of treatments was worked out by comparing the difference between two treatments mean using CD at 5% level of significance.

## RESULTS AND DISCUSSION

*Days taken for germination*: The earliest germination (10.60 days) was revealed in the treatment with $GA_3$ 100 ppm and it was statistically on par with $GA_3$ 75 and 50 ppm and BA (15 ppm) treatments (Table 1). This may be owing to the fact that the involvement of $GA_3$ in the triggering of cytological enzymes along with an increase in cell wall plasticity and boosted the water absorption in the seeds and $GA_3$ acts directly on embryo by encouraging protein synthesis and elongation of coleoptiles (Stewart and Freebairn 1969). Similarly, earlier study by Lima *et al*. (2020), presence of $GA_3$ reduced the mean germination time and the coefficient of variation of germination time in cactus (*Pilosocereus gounellei* susbp gounellei) seeds. There was a good result in the seeds treated with BA 15 ppm which also showed early germination (11.60 days) compared to control (22.20 days). This might be owing to the involvement of BA in the metabolic activities for the enhancement of dragon fruit seed germination and seedling vigour (Dissanayaka *et al*. 2015). This finding was supported by Vishnupriya *et al.* (2019) who conducted *in vitro* seed germination study in dragon fruit and revealed that BA at 2 ppm induced early germination (6.99 days) compared to control.

*Seed germination percentage (%)*: The $GA_3$ was found highly significant among various concentrations of $GA_3$ and BA and the top most germination percentage was recorded in seeds treated with $GA_3$ at a concentration of 100 ppm (87.00%) (Table 1). Further, as the concentration increases the germination percentage was found increases. It might be attributed to the stimulation of initial enzyme and by the activation of reserve food by gibberellins which have also been used to encourage germination and induce early seedling emergence and growth (Nabil *et al*. 2007). Our results were in accordance with Gurung *et al*. (2014), who found that maximum germination percentage in passion fruit with $GA_3$ 100 ppm (74.00%) and the minimum germination per cent in control (30.00%). There was an enhanced seed germination in the seeds treated with BA and the highest (84.20%) of it was at 15 ppm under polyhouse using cocopeat and sand (1:1) as BA help in the breakage of seed dormancy. This study was supported by the findings of Vishnupriya *et al.* (2019) who revealed enhanced germination of dragon fruit seeds with 2 ppm BA, however it is under *in vitro* condition.

Table 1 Effect of different concentrations of $GA_3$ and BA on days taken for germination, germination percentage, shoot length and seedling length of dragon fruit seedlings

| Treatment | Days taken for germination | Germination percentage (%) | Shoot length (cm) | | | Seedling length (cm) |
|---|---|---|---|---|---|---|
| | | | 30 days | 60 days | 90 days | |
| $T_1$, 0 ppm $GA_3$ | 22.20$^{a}$ | 68.00$^{g}$ | 0.45$^{e}$ | 1.91$^{i}$ | 3.18$^{g}$ | 8.85$^{g}$ |
| $T_2$, 10 ppm $GA_3$ | 19.80$^{b}$ | 70.80$^{fg}$ | 0.95$^{e}$ | 3.60$^{gh}$ | 5.23$^{f}$ | 8.92$^{g}$ |
| $T_3$, 25 ppm $GA_3$ | 14.00$^{d}$ | 74.00$^{cde}$ | 2.24$^{cd}$ | 4.67$^{d}$ | 8.11$^{d}$ | 13.08$^{e}$ |
| $T_4$, 50 ppm $GA_3$ | 11.80$^{e}$ | 76.20$^{bcd}$ | 2.76$^{bc}$ | 5.09$^{c}$ | 8.85$^{c}$ | 14.45$^{d}$ |
| $T_5$, 75 ppm $GA_3$ | 10.80$^{f}$ | 78.20$^{b}$ | 2.81$^{ab}$ | 5.56$^{b}$ | 9.90$^{b}$ | 17.57$^{b}$ |
| $T_6$, 100 ppm $GA_3$ | 10.60$^{f}$ | 87.00$^{a}$ | 3.55$^{a}$ | 6.20$^{a}$ | 10.27$^{a}$ | 18.33$^{a}$ |
| $T_7$, 1 ppm BA | 17.60$^{c}$ | 71.60$^{ef}$ | 1.65$^{bcd}$ | 3.45$^{h}$ | 6.87$^{e}$ | 11.00$^{f}$ |
| $T_8$, 5 ppm BA | 13.60$^{d}$ | 73.40$^{def}$ | 1.74$^{d}$ | 3.65$^{fg}$ | 7.04$^{e}$ | 9.26$^{g}$ |
| $T_9$, 10 ppm BA | 13.60$^{d}$ | 76.40$^{bc}$ | 1.84$^{cd}$ | 3.81$^{f}$ | 7.13$^{e}$ | 11.39$^{f}$ |
| $T_{10}$, 15 ppm BA | 11.60$^{e}$ | 84.20$^{a}$ | 2.54$^{bcd}$ | 4.45$^{e}$ | 8.32$^{d}$ | 16.10$^{c}$ |
| SEm± | 0.57 | 1.71 | 0.15 | 0.22 | 0.29 | 0.33 |
| CD ($P$=0.05) | 1.63 | 4.89 | 0.43 | 0.62 | 0.85 | 0.94 |

*Common letters are non-significant according to Duncan's multiple range test where $P$<0.05.

*Shoot length*: The maximum shoot length was measured with $GA_3$ 100 ppm at 30, 60, and 90 DAS with 3.55, 6.20 and 10.27 cm, respectively and the minimum shoot length was in control (0.45, 1.91 and 3.18 cm) at 30, 60 and 90 DAS (Table 1 and Fig. 1). The increased shoot length in the dragon fruit seedling might be owing to the fact that this hormone enhanced osmotic absorption of nutrients, which triggered cell elongation and thus fastening the height of the seedling (Sen *et al*. 1990). The findings of the present study are in line with the study conducted by Bishwas *et al.* (2018) who found better seedling vigour index and root shoot ratio in kiwifruit using $GA_3$. Compared to control, BA was recorded better shoot length and highest (2.54, 4.45 and 8.32 cm at 30, 60 and 90 DAS, respectively) of it was at 15 ppm owing to the enhanced metabolic activities in the seeds treated with BA which result in the enhanced seedling vigour (Dissanayaka *et al*. 2015).

*Seedling length*: In this study, the maximum seedling length (18.33 cm) was observed in $GA_3$ at 100 ppm concentration which was on par with 75 ppm $GA_3$ and minimum seedling length (8.85 cm) was measured with the control at 90 DAS (Table 2 and Fig. 1). The increased

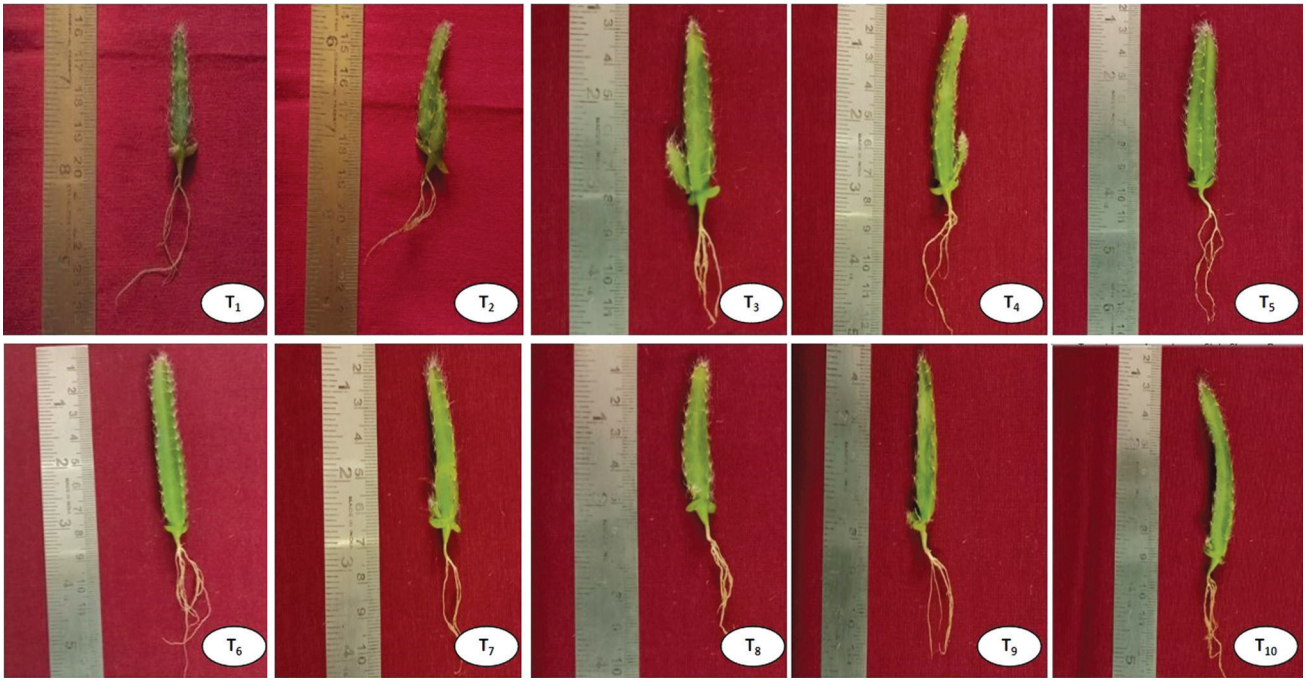


Fig. 1 Effect of different concentration of PGRs on seedling growth of dragon fruit. Treatment details are given under Materials and Methods.

Table 2 Effect of different concentration of $GA_3$ and BA on root parameters, fresh weight, dry weight and survival percentage of dragon fruit seedlings

| Treatment | Root length (cm) | Number of roots per seedling | Fresh weight of seedling (g) | Seedling dry weight (g) | Survival percentage (%) |
|---|---|---|---|---|---|
| $T_1$, 0 ppm $GA_3$ | $4.20^{g}$ | $3.10^{h}$ | $1.28^{h}$ | $0.30^{h}$ | $35.00^{gh}$ |
| $T_2$, 10 ppm $GA_3$ | $4.22^{g}$ | $3.50^{f}$ | $1.54^{g}$ | $0.44^{g}$ | $36.00^{fgh}$ |
| $T_3$, 25 ppm $GA_3$ | $5.60^{d}$ | $4.10^{e}$ | $2.33^{d}$ | $0.69^{e}$ | $38.00^{ef}$ |
| $T_4$, 50 ppm $GA_3$ | $5.78^{d}$ | $4.30^{d}$ | $2.68^{c}$ | $0.82^{c}$ | $39.00^{de}$ |
| $T_5$, 75 ppm $GA_3$ | $7.54^{c}$ | $5.40^{b}$ | $2.72^{c}$ | $0.78^{d}$ | $43.00^{bc}$ |
| $T_6$, 100 ppm $GA_3$ | $8.06^{a}$ | $6.20^{a}$ | $3.26^{a}$ | $1.22^{a}$ | $48.00^{a}$ |
| $T_7$, 1 ppm BA | $4.34^{fg}$ | $3.40^{fg}$ | $1.65^{f}$ | $0.42^{g}$ | $34.00^{h}$ |
| $T_8$, 5 ppm BA | $4.52^{f}$ | $3.30^{g}$ | $1.76^{e}$ | $0.58^{f}$ | $37.00^{efg}$ |
| $T_9$, 10 ppm BA | $5.28^{e}$ | $4.40^{d}$ | $2.26^{d}$ | $0.70^{e}$ | $41.00^{cd}$ |
| $T_{10}$, 15ppm BA | $7.78^{b}$ | $4.70^{c}$ | $3.00^{b}$ | $1.02^{b}$ | $44.00^{b}$ |
| SEm± | 0.23 | 0.28 | 0.10 | 0.06 | 0.87 |
| CD ($P$=0.05) | 0.65 | 0.81 | 0.31 | 0.18 | 2.5 |

*Common letters are non-significant according to Duncan's multiple range test where $P<0.05$.

seedling length might be owing to the increased shoot and root length by the increase of both cell division and internodes elongation. $GA_3$ apparently activating the metabolic processes or neutralizing the effect of an inhibitor on the growth of seedling (Ratan and Reddy 2004). In case of BA treatment, maximum seedling length (16.10 cm) was recorded in seeds treated with 15 ppm which might be attributed to the early germination and raised seedling vigour (Nawaz *et al*. 2013).

*Root length*: The maximum root length (8.06 cm) at 90 DAS was measured in the seeds treated with treatment 100 ppm $GA_3$ and the minimum (4.20 cm) of it was in the control (Table 2). The increase in root length might be owing to the exogenous treatment of $GA_3$, which induced the development of gluconeogenic enzymes in the early stages of seed germination, and this might be the explanation for improved germination and vigour characteristics reflected in the increase in root length (Hota *et al*. 2018). Our findings were supported by Ramteke *et al.* (2015) who revealed that seeds treated with $GA_3$ at 200 ppm concentration of papaya variety Coorge Honeydew showed maximum tap root length and Gurung *et al*. (2014) also found maximum root length with $GA_3$ 500 ppm in papaya. The maximum root length (7.78 cm) in the BA treatment was at 15 ppm concentration compared to control. It might be owing to the rapid germination and enhanced growth rate of seedling further resulted in the increased root length (Nawaz *et al*. 2013).

*Number of roots per seedling*: In this study, the highest number of the root (6.20) was recorded in $GA_3$ at 100 ppm and the lowest (3.10) of it was recorded in control at 90 DAS (Table 2). The vigorous root growth of seedling is owing to the $GA_3$ treatment which might have helped for the synthesis of more quantities of photosynthate and their active translocation to the root zone through the phloem. Similarly, Wagh *et al*. (1998) reported that 400 ppm $GA_3$ resulted in the highest germination percentage and a maximum number of roots in aonla seedling and Hota *et al*. (2018) revealed that the maximum number of roots was counted in jamun seedling at $GA_3$ 450 ppm. Further, the seeds treated with BA 15 ppm also recorded good number of roots compared to control which was might be owing to the early germination and seedling vigour (Nawaz *et al*. 2013).

*Seedling fresh weight*: The maximum fresh weight (3.26 g) was observed with $GA_3$ at 100 ppm concentration and lowest of it (1.28 g) was noted with the control (Table 2). The higher fresh weight is owing to the impact of $GA_3$ on various plant parts growth, which may be due to its role in stimulating cell division, cell elongation, auxin metabolism, cell wall plasticity, and cell membrane permeability leading to increased growth. This explanation for the better fresh weight at higher concentration of $GA_3$ was in accordance with the findings of Hota *et al.* (2018) in jamun seedlings which showed maximum fresh weight at 450 ppm $GA_3$. It may also be due to the effect of mobilizing water and nutrients transported at a higher rate, which could have increased the production of photosynthetic products and translocated them to different parts of the plant, which could have resulted in better growth of seedlings and hence recorded maximum fresh weight (Dilip *et al.* 2017). Among the different concentration of BA, highest seedling weight (3.00 g) was recorded at 15 ppm. It might be due to the early germination and increased growth rate of seedling (Nawaz *et al*. 2013).

*Seedling dry weight*: The highest dry weight (1.22 g) was recorded with the treatment $GA_3$ 100 ppm followed by 15 ppm BA (1.02 g), and the lowest dry weight was noted with the control (0.30 g) (Table 2). The explanation behind the highest dry weight of seedling is correlated with the fresh weight of the seedling and the seedlings which recorded the highest fresh weight could record the highest dry weight also. Our study was supported by Dilip *et al.*

(2017), who conducted seed treatment with $GA_3$ in Kagzi lime and revealed the highest dry weight at 80 ppm $GA_3$ for 12 h.

*Survival percentage:* The highest survival percentage (48.00%) was recorded in seeds treated with $GA_3$ 100 ppm and the lowest of it was catalogued in control (35.00%) (Table 1). The maximum survivability of seedlings from seeds treated with gibberellic acid at higher concentrations (100 ppm) might be attributed to early germination and favourable growing conditions under $GA_3$ treatment which was aided by the findings of Manekar *et al*. (2011) in aonla which showed maximum survival percentage at 200 ppm $GA_3$. The survival of seeds with BA was highest (44.00%) in the 15 ppm in the present study. It might be owing to the early germination good number and length of roots and vigorous seedling which was helped for better survival of seedling.

It can be concluded that $GA_3$ at 100 ppm pre-treatment of dragon fruit seeds before sowing enhance early germination with maximum germination percentage and to enhance various shoot and root parameters and for getting maximum survived seedlings and also added that BA (Benzyl adenine) can also be used as a plant growth hormone to promote seed germination as it can perform seedling growth at a lower concentration as compared to $GA_3$ but the performance is poor and found maximum seed germination at 15 ppm concentration of BA.

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