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In Vitro Asymbiotic Seed Germination and Propagation of Polystachya concreta (Jacq) Garay & H.R Sweet, An Important Medicinal Orchid

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Abstract: ***This is the study of in vitro asymbiotic seed germination and efficient mass production of polystachya concreta. Asymbiotic germination of counterfeit seed through protocorms gives a valuable method to restore plants in the wild for germplasm conservation just as for business engendering. Overall KC medium containing 1.0 mg_L–1 NAA, 10% CW, and 0.1% AC gave the highest percent germination and The most suitable medium for callus induction was KC with 1.0 mg_L–1 2,4-D, which resulted in 71.3% callus induction. KC medium supplemented with 0.1%AC and 1.0 mg_L–1 NAA with or without 50 mg_L–1 banana homogenate was most suitable for shoot formation. Callus could be induced on KC medium with 0.1 to 2.0 mg_L–1 2, 4-D or a high concentration of BAP (2.0 mg_L–1). The most suitable medium for callus induction was KC with 1.0 mg_L–1 2, 4-D, which resulted in 71.3% callus induction. PLBs were induced on KC medium with 0.1 to 2.0 mg_L–1 BAP. The most suitable medium for PLB induction was KC medium with 1.0 mg_L–1 BAP, which resulted in 57.0% PLB induction. KC medium supplemented with 0.5 or 1.0 mg_L–1NAA and 50 g_L–1 banana homogenate most favored for plantlet growth plant regeneration. In the plant acclimatization worked in different media but On all media, plantlet survival percentage was significantly higher at 30 days after transplanting compared with 60 days.***

I. INTDRODUCTION

Orchids are the second largest group of flowering plants covering about 788 genera and 18,500 species. The family Orchidaceae have nearly 22,500 species belonging under 779 genera. They are distributed all over the world, except the hot desert and Antarctica. In India, the family is addressed by more than 131 genera and 956 species, gathered mostly in the Himalayas, north-East area and peninsular India(Linthoingambi L et al 2013). Orchids are considered as headstrong plants in in vitro propagation. Due to the absences of appropriate micropropagation techniques for mass production and mutilation to their ecological distribution stood by local gatherers these species are threatened with extinction. Orchid seeds are very small. For this reason, in nature they need a fungal association during germination which provides them nutrients required for growth and development. This method is known as "symbiotic germination" and until 1922 was the solitary known strategy for seed based proliferation of orchids. That year, Lewis Knudson distributed an article depicting a counterfeit technique to develop orchids without the participation of a fungus.This process, known as asymbiotic propagation, makes use of micropropagation techniques to achieve the germination and development of plants in an artificial culture medium under sterile conditions. Right now, the medium known as "Knudson culture medium" keeps being utilized all throughout the planet to grow orchids quick and effectively. Knudson originally published his work on asymbiotic germination in a Spanish language journal which was little known at the time, and he thus republished the next year in English in a journal with wide circulation. Polystachya one of the genus in orchid that commonly known as yellowspike orchid. In Asia, three types of Polystachya are perceived, in view of definite morphological examinations. They happen in southern Asia, from Tamil Nadu State in southern India and Yunnan Region in southeastern China to Sumbawa Island in southern Indonesia. As encircled here, two Asiatic species are endemic to southern India separated from Polystachya concreta. One species from southeastern India (Kalrayan Hills) is newly described: Polystachya seidenfadeniana Dendrobium parvum Seidenf. is reduced to a synonym of Polystachya concreta (Jacq.) Garay & H.R. Sweet.(Ejsmont JM et al 2010) All three Asiatic Polystachya species represent the typical section of the genus. In vitro germination strategies have brought about more solid germination and spread of numerous orchid taxa, and addresses an ideal framework for considering the development and improvement of orchid seeds and seedling .Orchids are of immense horticultural as well as medicinal importance which fetches a very high price in the international market. They additionally assume a helpful part to adjust the woodland environments. Interest for superior grade of orchids has been expanding step by step because of their prevalence in horticulture industry. A single orchid capsule contains millions of seeds, which paralysis also chronic illness. The whole plant of Polystachya concreta are also attracts the floriculture market because of its long-lasting highly attractive beautiful flowers.

Because of its different uses, individuals evacuated this plant from wild and subsequently reach to the compromised classification. Thus, an attempt was made to produce artificial seeds in orchid, their short term storage and mass propagation within a short span of time by using tissue culture technique. Asymbiotic germination of counterfeit seed through protocorms gives a valuable method to restore plants in the wild for germplasm conservation just as for business engendering. absents of any metabolic machinery and do not have any endosperm. In spite of a very large number of seeds produced, only few seeds germinate in nature as they require specific fungus (Magaray MM et al 2017). Currently, wild orchid population was very less due to illegal trade and consumption by local people. Therefore, the development of an artificial propagation is needed to reduce collection pressures on wild population.

II. MATERIALS AND METHODS

A. Source of Plant Material

Undehisced green capsules of *polystachya concreta* were collected from the kolli Hill, Tamil Nadu, which is located at 11.2° N and 78.33° E along the hill sides of the Eastern Ghats. An authentic sample was identified by Dr. B. Balaguru Taxonomist and Assistant Professor Department of Botany JAMAL MOHAMED COLLEGE (Autonomous).

B. Explant and Mode of Sterilization

The capsules of *polystachya concreta* were harvested from actively growing healthy plants and washed thoroughly under running tap water followed by 1% detergent solution of 'Teepol' for 5 min. The explants were then rinsed repeatedly with sterile distilled water and then treated with a fungicide Bavistin (1% w/v) for 10 min and washed thoroughly with sterile distilled water. The explants were then surface sterilized with 0.01% (w/v) $HgCl_2$ solution for about 5 min followed by five to six repeated washes with sterilized double-distilled water in order to remove traces of sterilant. Then the capsules were dipped in 80% ethanol for 1 min and flamed and aseptically inoculated in to pre cooled autoclaved media.

C. Asymbiotic Germination of Seed

Number of seeds from each capsule were subjected to a tetrazolium (TZ) viability test (Lakon, 1949). To determine the influence of basal medium on seed germination, the disinfected capsules were cut open vertically with a sterile scalpel, and the seeds were placed on four basal sowing media without plant growth regulators: Murashige and Skoog (MS), (Murashige and Skoog, 1962), half-strength MS (half-strength MS macro- and micronutrients), KC (Knudson, 1946), Vacin and Went VW (Vacin andWent, 1949). NAA, coconut water (CW), and activated charcoal (AC) were added to media to improve orchid seed germination (Hong et al., 2008). After initial trials, KC medium was shown to be the most appropriate basal medium for seed germination of polystachya concreta. Thus, we prepare KC medium containing NAA (0.5, 1.0, or 2.0 mg_L–1), coconut water (5%, 10%, or 20%), and activated charcoal (0.05%, 0.1%, or 0.2%) were tested for improving seed germination. All media were supplemented with 30 g_L–1 sucrose and 6 g_L–1 agar. The CW used in these experiments was obtained from 1 month-old green coconuts from Calicut, Kerala. The water was filtered through a of filter paper. The pH value of all media was adjusted to 5.8 with 1 N KOH or 1 N HCl before autoclaving at 121 _C for 15 min. For every treatment, 50seeds were cultured in a 250-mL flat-bottomed culture flask containing 90 mL of medium. All experiments contained three independent replicates. All cultures were incubated in a growth chamber at 25 ± 2 _C under a 16-h photoperiod provided by fluorescent lamps. Seed germination was estimated for each treatment by two parameters:

1) The time taken for germination.
2) Percentage of seed germination.

The two parameters were recorded after 30 days of culture. The seed germination rate was determined as the quantity of sprouted seeds out of the complete number of refined seeds in a conical flask

D. Expansion of Callus and Protocorm-like Bodies

Callus and PLB induction, development, and subculture from seed-derived protocorms were studied as possible methods for the large-scale propagation of polystachya concreta. Seed-derived protocorms were cultured on KC medium for inducing callus or PLBs by supplementing the medium with 10% CW and thidiazuron (TDZ) at 0.01, 0.1, 0.5, or 1.0 mg_L–1, 6-benzylaminopurine (BAP) at 0.1, 0.5, 1.0, or 2.0 mg_L–1, or 2,4- dichlorophenoxyacetic acid (2,4-D) at 0.1, 0.5, 1.0, or 2.0 mg_L–1 singly and some in combinations. The level of plantlets that framed, the measure of callus or number of PLBs that were initiated, and the quantity of inert or dead protocorms were determined following 50 days of culture. Ten protocorms were cultured per flask, and each experiment was repeated three times.

E. Regeneration of Plant and Shoot Formation

Shoot formation percentage from protocorm or PLBs and subsequent plantlet growth status was assessed on KC media containing 0.1, 0.5, 1.0, or 2.0 mg_L–1 NAA, 0.1% AC, and 50 or 100 g_L–1 banana homogenate. All experiments consisted of three independent replicates per replicate with 10 protocorms or PLBs in each flask.

F. Acclimatization of Plants

The impacts of two relocating strategies and two planting media on ex-vitro plantlet acclimatization were examined. Plantlets with roots 2 cm or longer were enveloped by Chilean sphagnum greenery and become joined to fir bark hinders or relocated into pots with Chilean sphagnum greenery. The transplanted plantlets were grown in a greenhouse under no more than 800 mmol_m–2_s 1 natural light. Plantlets were watered at 1- to 2- days intervals. After two week of acclimatization, plantlets were fertilized weekly with 150 mg_L–1 20N–20P–20K fertilizer. Average temperatures ranged from 15 to 30 _C and humidity levels ranged from 70% to 98%. The percentage plantlet survival was recorded at 30 and 60 days after transplanting. Each experiment consisted of three independent replicates.

III. DATA ANALYSIS

All trials were set up in a totally randomized plan.. Percentage data were converted to relative proportions, and then analyzed for significant differences. The data were analyzed with Windows 10 (Microsoft Corp., Office Excel).

IV. RESULT

A. Asymbiotic germination of seed

Tetrazolium testing indicated that seeds were 85.5% viable. After 4 weeks of culture, seeds germinated on all five tested basal media, although the period required for germination and the germination percentage differed (Table 1). The seed germination percentage on KC media were significantly higher than on MS, VW, and half-strength MS media. Seed germination percentage on VW and half-strength MS media. The seed germination period on KC media was significantly shorter than on MS or half-strength MS media. Therefore, KC basal media was the most appropriate basal medium for seed germination of *polystachya concreta* among all media tested.

Basal medium	Germination period (Days)	Germination Percentage (%)
KC	35	40.3
VW	37	29
Half- strength MS	38	24.7
MS	40	15.7

(Table 1.Effect of basal medium on the period and percentage of seed germination)

KC medium supplemented with NAA (0.5, 1.0, or 2.0 mg_L–1), with or without AC and CW, significantly shortened the germination period and increased germination percentage compared with KC basal medium, except for KC medium supplemented with 2.0 mg_L–1 NAA, which did not significantly increase germination percentage and KC medium supplemented with 1.0 mg_L–1 NAA, 10% CW, and 0.2% AC, which did not significantly shorten the germination period (Table 2). KC medium enhanced with CW just (5%, 10%, or 20%) essentially expanded germination rate, however just KC medium enhanced with 10% CW altogether abbreviated the germination period contrasted and KC basal medium alone (Table 2). KC medium supplemented with AC only (0.05%, 0.1%, and 0.2%) did not significantly shorten the germination period and increased germination percentage compared with KC basal medium except for a high concentration of AC (0.2%), which significantly prolonged the germination period (Table 2). The KC media tried with mixes of NAA, CW, and AC fundamentally expanded germination rate and essentially abbreviated the germination period aside from KC medium to which 1.0 mg_L–1 NAA, 10% CW, and 0.2% AC were added, which didn't altogether abbreviate the germination time frame contrasted and KC medium alone. KC medium containing 1.0 mg_L–1 NAA, 10% CW, and 0.1% AC gave the highest percent germination, significantly higher than other media tested except for KC medium containing 0.5 mg_L–1 NAA, 10% CW, and 0.1% AC.

NAA(mg.L^{-1})	Coconut water (%)	Activated charcoal (%)	Germination period (d)	Germination Percentage (%)
0	0	0	35	40.3
0.5	0	0	25	48.3
1.0	0	0	28	46.0
2.0	0	0	28	43.0
0	5	0	36	49.3
0	10	0	28	55.0
0	20	0	35	50.3
0	0	0.05	33	38.7
0	0	0.1	32	41.0
0	0	0.2	36	36.3
0.5	10	0	27	58.0
0.5	10	0.05	29	56.0
0.5	10	0.1	30	60.3
1.0	10	0.1	27	64.7
1.0	10	0.2	32	50.0

Table 2. Effect of NAA, coconut water, and activated charcoal on period and percentage of *polystachya concreta* seed germination.

B. Expansion of Callus and Protocorm-like Bodies

In enlistment culture (Table 3), some protocorms shaped plantlets with roots, some framed callus or PLBs, though others kicked the bucket or didn't react (protocorms framed were kept up uncertainly minus any additional development). PLBs and callus were effectively separated, in light of the fact that PLBs were round, single, and simple to strip off from PLB bunches, though callus was irregularity like and difficult to strip off and furthermore callus is comparable to scaled down PLB groups, which are indistinguishable somatic embryos. No callus or PLBs formed, 91.7% protocorms formed seedlings, whereas only 5.7% of protocorms died on KC medium without plant growth regulators and with 10%CW. Callus could be induced on KC medium with 0.1 to 2.0 mg_L–1 2,4-D or a high concentration of BAP (2.0 mg_L–1). The most suitable medium for callus induction was KC with 1.0 mg_L–1 2,4-D, which resulted in 71.3% callus induction. PLBs were induced on KC medium with 0.1 to 2.0 mg_L–1 BAP. The most suitable medium for PLB induction was KC medium with 1.0 mg_L–1 BAP, which resulted in 57.0% PLB induction. Callus and PLBs were induced synchronously on the same KC medium with 0.01 to 1.0 mg_L–1 TDZ or with a high concentration (2.0mg_L–1)of BAP. The higher percentage of protocorm death occurred on KC medium with a high concentration of TDZ (1.0 mg_L–1), BAP (2.0 mg_L–1), or 2,4-D (2.0 mg_L–1).

Plant growth regulators (mg.L^{-1})			Seedling formation (%)	Callus induction (%)	PLB induction (%)	Protocorn with no response (%)	Protorn death (%)
TDZ	BAP	2,4-D					
0	0	0	91.7	0	0	2.7	5.7
0	0.01	0	82.3	1.0	4.7	4.7	7.3
0.1	0	0	57.7	7.0	20.3	0	15.0
0.5	0	0	33.0	9.7	36.3	0	21.0
1.0	0	0	20.3	9.7	38.0	0	32.0
0	0.1	0	86.3	0	5.0	4.7	4.0
0	0.5	0	75.3	0	19.7	0	5.0
0	1.0	0	31.3	0	57.0	0	14.0
0	2.0	0	15.7	5.3	54.0	0	25.0
0	0	0.1	60.7	30.3	0	3.7	5.7
0	0	o.5	39.3	50.0	0	0	10.7
0	0	1.0	15.7	71.3	0	0	13.0
0	0	2.0	4.7	62.0	0	0	33.3

Table 3. Effect of TDZ, BAP, and 2,4-D on induction of callus and PLB from *polystachya concreta* protocorms.

C. Regeneration of Plant and SHOOT Formation

KC medium supplemented with 1.0 mg_L–1 NAA with or without 50 mg_L–1 banana homogenate was most suitable for plantlet formation among the tested media, and 89.3% or 95.3% of PLBs are formed plantlets in these media (Table 4). Some PLBs died on KC media supplemented with 0.5 or 1.0 mg_L–1 NAA and 100 g_L–1 banana homogenate or with 2.0 mg_L–1 NAA without banana homogenate. KC medium supplemented with 0.5 or 1.0 mg_L–1NAA and 50 g_L–1 banana homogenate favored plantlet growth most (Table 4).

NAA ($mg.L^{-1}$)	Banana homogenate ($g.L^{-1}$)	Plantlet formation (%)
0	0	74.3
0.5	0	79.3
1.0	0	89.3
2.0	0	73.7
0.5	50	78.0
0.5	100	76.3
1.0	50	95.3
1.0	100	85.7

Table 4. Effect of NAA and banana homogenate concentration on plantlet formation from PLBs derived from the subculture of *polystachya concreta*

D. Acclimatization of Plants

Plantlets grew vigorously after 30 days transplanting with high plantlet survival percentages that were not significantly different between media. After growth in a greenhouse for 60 days, plantlet survival rate on fir bark blocks was significantly higher than on Chilean sphagnum moss or on the sand/peat/fir bark mixture (Table 5). On all media, plantlet survival percentage was significantly higher at 30 days after transplanting compared with 60 days.

Transplanting Conditions	Survival rate after 30 d of transplanting (%)	Survival rate after 60 d of transplanting (%)
Fixed on fir bark blocks	90.0	69.3
Chilean sphagnum moss	92.3	56.0
Mixture media	85.7	45.3

Table 5. Survival rate of *polystachya concreta* seedlings grown on three different supporting mixtures after 30 and 60 d.

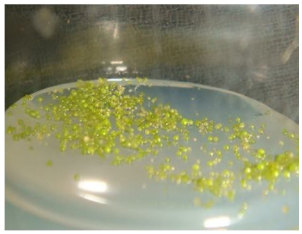
V. CONCLUSION

To the best of our knowledge this is the first study of an efficient mass production of *polystachya concreta.* Overall KC medium containing 1.0 mg_L–1 NAA, 10% CW, and 0.1% AC gave the highest percent germination and The most suitable medium for callus induction was KC with 1.0 mg_L–1 2,4-D, which resulted in 71.3% callus induction. KC medium supplemented with 0.1%AC and 1.0 mg_L–1 NAA with or without 50 mg_L–1 banana homogenate was most suitable for shoot formation.

In conclusion, this study reports a procedure for asymbiotic germination, in vitro seedling culture, and regeneration system through PLBs and callus as well as greenhouse acclimatization of Polystachya concreta that can be used for conservation and commercial production. Conservation of endangered of threatened orchid species can benefit from germination and acclimatization protocols that focus on propagating orchid seedlings for reintroduction or maintaining protocorms and seedlings in vitro culture.

REFERENCES

[1] Linthoingambi L,Das AK, Singh P. K. and Ghosh S. K,(2013). Medicinal uses of orchid by tribes in India: A review , International Journal of Current Research,2796-2798
[2] Ejsmont JM, Baranow P, (2010)Taxonomic study of Polystachya Hook. (Orchidaceae) from Asia, Springer, 290:57–63
[3] Magray MM, Wani KP, Chatto MA and Ummyiah HM (2017), Synthetic Seed Technology, International Journal of Current Microbiology and Applied Sciences, 6(11): 662-674 662.
[4] Lakon G, (1949) The topographical tetrazolium method for determining the germination capacity of seeds, Plant Physiology. 24:389–394.
[5] Murashige T, Skoog F, (1962)Arevised medium for rapid growth and bio-assays with tobacco tissue cultures. Physiology Plant. 15:473–497.
[6] Knudson L, (1946) A new nutrient solution for the germination of orchid seeds, Amer Orchid Society Bull. 15:214–217.
[7] Vacin EF, Went FW, (1949). Some pH changes in nutrient solutions, Botanical Gaz, 110:605–613
[8] Hong PL, Chen JT, Chang WC (2008). Plant regeneration via protocorm-like body formation and shoot multiplication from seed derived callus of a maudiae type slipper orchid. Acta Physiology of Plant, 30:755–759.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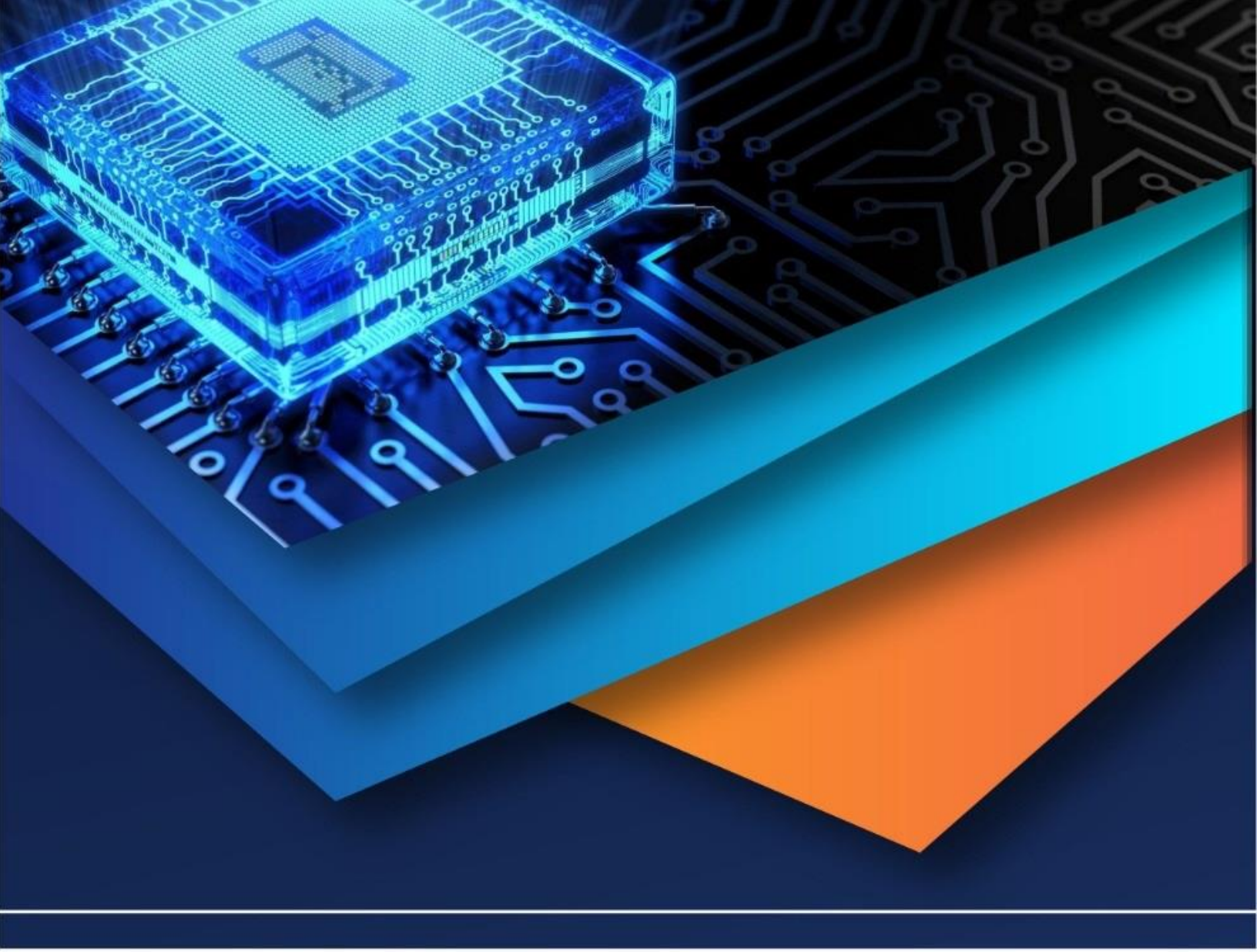
A. Habit with inflorescence
B. Imbibed seeds
C. Protocorm with shoot apex
D. Protocorm with developing leaf
E. Protocorm with developing leaf
F. Protocorm with evident root
G. Well-developed leaf with evident root.
H. Hardening



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