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Effect of Growth Regulators and Time on *In vitro* Pollen Germination in three Ornamental Tropical Tree Species

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ABSTRACT

***In vitro* pollen germination of three tropical tree species, viz. *Spathodea campanulata, Bauhinia purperia* and *B. racemosa* was done to know the effect of growth regulators and time on pollen germination. Three concentrations, i.e. 100, 200 and 300 ppm of four growth hormones (IAA, IBA, GA_3 and Kinetin) and sucrose (5 and 10%) alone were used as germination medium. The results revealed that pollen germination under control condition is very low and oscillating between 4.6±1.2 and 17.8±3.2%. The growth hormones and sucrose was found effective inducing pollen germination. IAA and IBA were found effective for both species of *Bauhinia* whereas GA_3 and kinetin were found suitable for *Spathodea campanulata*. Maximum germination was recorded in the initial 24h of setting experiment, which further declined in 48h and was recorded very less and even 0.0% after 72 hours of treatment. There was significant (< 0.0001) effect of time, hormone and species on pollen germination. Sucrose has shown good response (43 to 64%) in all selected tropical tree species. All the three tree species are cross pollinated, which depend on the variety services of pollinators. Low % *in vitro* pollen germination in control condition in *Spathoda campanulata* and *Bauhinia purpurea* reflects that both species are prone to pollination and fertilization failure if appropriate pollinators and receptive stigmas are unavailable to them early after anther dehiscence.**

Keywords: Gibberellic Acid, Pollen Production, Pollen viability, *Spathodea*, *Bauhinia*.

INTRODUCTION

In vitro pollen germination is of practical importance as it can unravel the physiological and biochemical conditions required for the successful pollen germination and pollen tube development. Pollen conditions such as reserved food material, conditions of membrane and rate of conversion could be judged by *in vitro* pollen germination (Heslop-Harrison, 1979). Pollen viability has been correlated with *in vitro* pollen germination (Schori *et al*., 1992; Shivanna *et al*., 1991) and in many species it revealed good correlation with fruit and seed setting. Pollen germination varies with respect to species, composition of medium, temperature, relative humidity and time (Stanley and Liskens, 1974), pollen longevity and sucrose level (Pacini, 1996). Pollen grains develop inside the anther then they dehisce in the environment at the time of anther maturity in a dehydrated and metabolically inactive state. In cross pollinating tree species the pollen needs to be transferred on compatible, receptive stigma via pollinating agents within a limited period of time until pollen grain has a sufficient level of viability. *In vivo* pollen germination on stigmatic surface is an integrated complex process; therefore to attempt a study on such complex situation

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with respect to physiological requirement of pollen is rather difficult but also technologically demanding. Henceforth, *in vitro* pollen germination of tree species is an important method to know the differential physiological requirements for pollen germination and its development at least to a certain degree. *In vitro* pollen germination would be a precisely accurate and comparatively easy protocol if optimum conditions were standardized (Taylor and Helper, 1997). Though *in vitro* pollen germination may be lower or higher compared to *in vivo* germination under various physical and chemical stimuli but still indicates its biological potential of pollen for pollination and fertilization. *In vitro* pollen germination is a better technique than vital staining to assess the pollen viability.

Pollen germination is also regulated by water, amino acids, sugars, boron (Johri and Vasil, 1961), calcium (Pfahler, 1967) and growth regulators such as gibberellins (Singh *et al*., 2002), auxins and kinetin. Soluble sugars such as sucrose, glucose, fructose and acidic polysaccharides are major constituents of stigmatic exudates in many plant species (McConchie and Knox, 1989). Gibberellins (GAs) are vital endogenous plant growth hormones that play a crucial role in plant growth and development such as seed germination, stem and leaf elongation, floral induction, anther development and fruit and seed development. Several studies have cited the effect of GAs on *in vivo* and *in vitro* pollen germination and GAs have also been traced in developing pollen grain after anthesis (Singh *et al*., 2002). Indole-3-Acetic Acid (IAA) plays an essential role in sexual reproduction of plants viz., regulating development of gynoecia, ovary, stamen and egg cells (Nemhauser *et al*., 2000). IAA promotes the possibility of pollen tube growth as concentration of IAA increased in pistil after pollination (Aloni *et al*., 2006). In *Torenia fournieri*, IAA has been found to stimulate pollen tube growth and mediates the modification of its wall composition and structure in *in vitro* culture system (Wu *et al*., 2008).

The important aspects of reproductive biology of tropical tree species are pollination, pollen production and pollen germination. Pollen production and germination is often correlated with fruit and seed setting which reflects the species' reproductive potential. Quantitative attributes such as total pollen production per tree and qualitative attributes such as pollen germination under differential physical and chemical stimulus and their response shall bring new insights in understanding the reproductive ability and adaptability of tropical tree species. Pollen germination, viability and longevity are matters of concern and research particularly in cross pollinated tree species because their pollen is dispersed by a variety of abiotic and biotic agents in space and time. So, retention of pollen germination ability and viability with respect to time and what physical and chemical factors are governing these shall help in understanding the developmental perspectives of pollen grain and tree species. In tropical tree species few studies have been reported on pollen germination compared to their proportion and diversity, which is very high in tropics. Therefore, the present study was conducted in three important ornamental tropical tree species namely *Spathodea campanulata, Bauhinia purpurea* and *B. variegata* of Indo-Burma hot-spot region to know the effect of growth hormones and time on pollen germination which will be helpful to formulate the breeding strategy on the aforesaid species to increase their ornamental value. Furthermore, the study would augment the knowledge of pollen physiology of investigated tree species with respect to growth and development.

MATERIALS AND METHODS

This study was conducted during 2009 on three tropical species, *viz. Spathodea campanulata, Bauhinia purpurea* and

Bauhinia variegata, of Mizoram, the North Eastern Hill region (NEH) of India. The details of the study species are as follows:

1. Spathodea campanulata *P. Beauv.* (Family: Bignoniaceae, Common Name: African tulip tree), is a medium size ornamental evergreen tree species native of tropical Africa. The flowers are large and bell shaped with a brilliant orange flame colour, bisexual, zygomorphic and hypogynous. S. campanulata is bird pollinated (Faegri and van der Pijl, 1979) and also insect pollinated. Flowering period in India varies from January to March with peak flowering in mid February (Nalawadi et al, 1980). In the study sites (Aiawl, Mizoram), this species blooms during November to December. The fruit is capsule and dehiscent type.

2. *Bauhinia purpurea* L. (Family: Caesalpiniaceae, Common Name: Butterfly tree): It is a moderate size ornamental deciduous tree preferred mostly on road side plantation. Flowers are conspicuous, scented, coloured rose-purple, bisexual and zygomorphic. Leaves are shallowly cordate. Flowering and fruiting period ranges from September to April (Singh *et al*., 2002). Style is long with capitated stigma. It is pollinated by insects and birds (Ali, 1933). Pods are oblanceolate-subfalcate. Locally, wood is used for firewood and charcoal making. Flower-buds and fruit are eaten as vegetable and the leaves are used as cattle fodder (Sawmliana, 2003). Flowering in this species was observed in October and November in the study region.

3. *Bauhinia variegata* L. (Family: Caesalpiniaceae, Common Name: Kachnar): It is a medium size deciduous tree planted as street or shade trees. Flowers are large, white or purple colour, bisexual and zygomorphic. Leaves are broadly ovate-suborbicular, deeply cordate, glabrous and dull green. Flowering and fruiting period ranges from February to September (Singh *et al*., 2002). However, flowering was recorded in February and March in the study site. It is pollinated by birds and insects. Pods are dark brown, flat, subfalcate, lignose and glabrous. Tender fruits, leaves, flower buds are locally used as a vegetable. Decoction of the bark and leaves are useful in diarrhoea. The leaves are broadly used as cattle fodder (Sawmliana, 2003).

Methods

The sampling of the analyzed species was done at the time of peak flowering *viz. Spathodea campanulata* (mid November), *Bauhinia purpurea* (October) and *Bauhinia variegate* (early March). Five trees of each species were selected and five flowers from each tree with a total of twenty five flowers for each species were harvested in order to estimate the number of pollen grains per flower. The number of pollen grains per anther was estimated on five anthers from different flowers of the selected tree of each species. The anthers were obtained from closed flowers prior to anthesis, placed in a small vial containing five drops of glycerine 50%, smacked, and the pollen grains were suspended. From this concentrate, five 10 µL droplets were removed and pollen grains were counted under the microscope. Production of pollen grains per flower was estimated by multiplying the number of pollen grains per anther by the number of anthers per flower. The mode of anther dehiscence was observed by using hand lens (X20).

The effect of growth hormones on pollen germination was done on four different hormones viz., Indole-3-Acetic Acid (IAA), Indole-3-Butyric Acid (IBA), Gibberellic Acid (GA_3), and Kinetin. For each hormone the concentration was selected as 100, 200, and 300 mg L^{-1}. The *in vitro* pollen germination was done by taking pollen from freshly dehisced anthers from different trees in each species. Pollen grains from these anthers were filtered by a 100 micron Sieve and were germinated in optimized media composition. Basically the method used was that of Brewbaker and Kwack (1963). A factorial experimental design (Tuinstra and Wedel, 2000) was used to evaluate the effects

of sucrose, IAA, IBA, GA_3, and Kinetin against the control (distilled water) on pollen germination. Sucrose has been proved to be the key substrate for pollen germination in other species (Raina *et al*., 2003). Sucrose was tested at 5 and 10%. The experiment was blocked in time with five replications in a randomized complete block design. Bulk of pollen was distributed onto germination media in cavity slides and placed at a room condition. Room temperature was measured with thermo-hygrometer and the average temperature was recorded as 18±1.14°C. The progress of germination was recorded at the interval of 24, 48, and 72 hours after planting the pollen grains. Germination was quantified as the evaluated percentage of germinated pollen grains per 100. Pollen grains were considered germinated when the pollen tube length was greater than the diameter of the pollen grain (Tuinstra and Wedel, 2000).

The effect of time (24, 48 and 72 hours), growth hormones, sucrose and species on the germination of pollen was examined using ANOVA, with time, growth hormones and species as fixed effects – independent variables. ANOVA was performed using the SPSS package. Counts were log-transformed in order to improve normality of residuals and to reduce heteroscedasticity (Sokal and Rohlf, 1995).

RESULTS

The number of pollen grains per anther in each species varied considerably. The maximum number was observed in *Bauhinia purpurea* (2,0416±1,590 pollen/anther) and minimum in *Spathodea campanulata* (1,3280 pollen/anther). The mode of anther dehiscence in all species was Longitudinal slit (Table 1). It is evident from the results of pollen grain germination of selected tropical tree species under various growth regulators that the sucrose and distilled water (control) show a differential response with respect to varied concentration of growth regulators and sucrose in a time frame. All three species viz., *Spathodea campanulata*, *Bauhinia variegata* and *Bauhinia purpurea* show very less percentage (%) of pollen germination under control (4.6, 17.8 and 6.0, respectively) in the first 24 hours (Figure 1),

Table 1. Pollen outlay in three tropical species.[a]

Species	Pollen/Anther	Anther/ Flower	Pollen/Flower	Mode of anther dehiscence
Spathodea campanulata	13280±1040	4	53053.33±2529.01	Longitudinal slit
Bauhinia purpurea	20416±1590	3	60140.4±3042.2	Longitudinal slit
Bauhinia variegata	17246±1296	5	85830.7±2809.4	Longitudinal slit

[a] Results are shown as Mean±SEM.

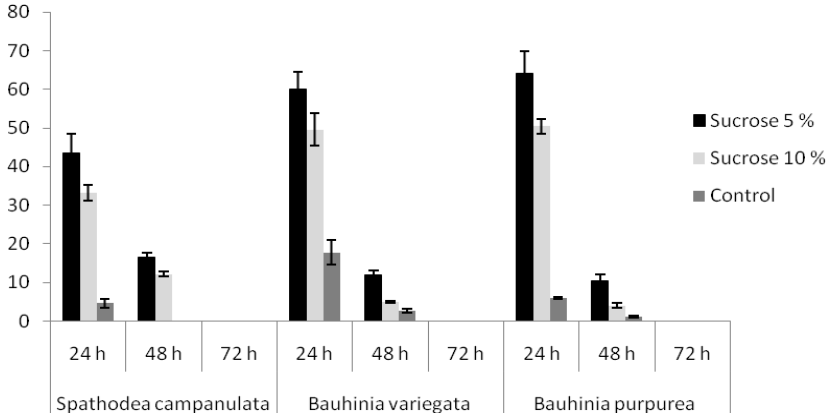

Figure 1. Effect of sucrose concentration on *In-vitro* pollen germination in three tropical species.

which indicates low germination ability and viability among them. All selected species showed a comparatively better *in vitro* pollen germination in 5% sucrose than 10% sucrose in the first 24 hours, furthermore it declines with time i.e., at 48 hours (Figure 1). It may be attributed that sucrose is a respiratory substrate vital for induction of pollen germination in all analysed species. *Bauhinia variegata* and *Bauhinia purpurea* have shown better *% in vitro* pollen germination in 100, 200, and 300 mg L^{-1} IAA, the value was 45.2, 49.0and 34.4%, respectively in *B. variegata* and 59.0, 78.6 and 68.8%, respectively in *B. purpurea* (Table 2). However, *Spathodea campanulata* has shown only 11.8, 6.2, and 2.2% germination in 100, 200, and 300 mg L^{-1} of IAA, respectively, which is very low compared to *B. variegata* and *B. purpurea*. Pollen grains of *Spathodea campanulata* have reflected poor germination response to IBA in the first 24 hours, which was comparatively high in *B. variegata* and *B. purpurea* (Table 2). *Spathodea campanulata* proclaimed the highest percentage of *in vitro* pollen germination of 61.8, 80.6, and 52.0, respectively in 100, 200, and 300 mg L^{-1} of GA_3 in the first 24h, which was followed by Kinetin (43.8, 53.0, and 34.0, respectively). *B. variegata* has shown a fair response of pollen germination percentage to GA_3 in 100, 200, and 300 mg L^{-1} with a value of 46.8, 39.8, and 22.4 respectively. Both *Bauhinia* species have been found to have ineffective responses to kinetin. All selected tropical tree species have shown a general trend of decrease in the percentage of *in vitro* pollen germination with time. Maximum germination was recorded in the initial 24 hours, which further declined in 48h and was recorded very less and even 0.0% after 72 hours of treatment. There was significant (< 0.0001) effect of time, hormone and species on pollen germination (Table 3). In short, sucrose has shown good response in all selected tropical tree species, Auxin has a better response to *Bauhinia variegata* and *Bauhinia purpurea* and GA_3

Table-2. Effect of growth hormones on *In-vitro* pollen germination in three tropical species.[a]

Hormones	Concentrations	Pollen germination (%)							
		Spathodea campanulata			*Bauhinia variegata*			*Bauhinia purpurea*	
		24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
IAA	100 mg L^{-1}	11.8± 0.46	8.2± 0.86	2.2± 0.10	45.2± 4.2	9.0± 0.84	1.0± 0.20	59.0± 6.2	3.2± 0.62	2.0± 0.20
	200 mg L^{-1}	6.2± 0.21	6.4± 0.40	1.8± 0.12	49.0±5.6	14.6± 1.2	2.2± 0.24	78.6± 8.6	8.8± 0.78	4.2± 0.52
	300 mg L^{-1}	2.2± 0.12	1.8± 0.32	0.8± 0.06	34.4±3.2	4.6± 0.56	0.4± 0.02	68.8± 5.2	3.5± 0.32	1.2± 0.16
IBA	100 mg L^{-1}	5.6± 0.75	10.2± 0.96	3.8± 0.18	33.2±2.5	7.2± 0.60	3.8± 0.36	26.2± 1.8	18.0± 1.4	8.2± 0.84
	200 mg L^{-1}	15.0± 1.2	10.6± 0.64	4.4± 0.28	24.6± 1.6	8.6± 0.84	2.8± 0.40	21.8± 1.2	13.6± 1.2	9.1± 0.64
	300 mg L^{-1}	4.4± 0.26	5.6± 0.42	2.2± 0.12	18.4± 1.0	8.0± 0.78	0.8± 0.01	12.4± 0.86	12.4± 1.8	8.6± 0.98
GA_3	100 mg L^{-1}	61.8± 5.6	23.6± 2.0	2.5± 0.52	46.8± 3.6	18.4± 1.2	3.1± 0.32	14.4±1.6	11.5± 1.2	1.0± 0.12
	200 mg L^{-1}	80.6± 6.8	34.4± 2.4	2.8± 0.65	39.6± 2.0	17.2± 0.80	3.7± 0.28	23.4± 1.8	5.6± 1.0	1.6± 0.12
	300 mg L^{-1}	52.0± 3.4	22.5± 1.6	1.2± 0.56	22.4± 1.4	12.8± 1.4	2.5± 0.36	16.2±1.0	1.4± 0.21	0.8± 0.06
Kinetin	100 mg L^{-1}	43.8 ±3.4	12.0± 1.0	3.2± 0.56	19.5± 1.0	4.4± 0.56	0.0	4.0± 0.54	0.0	0.0
	200 mg L^{-1}	53.0± 5.0	12.8± 1.2	2.0± 0.23	9.0± 0.86	3.8± 0.23	0.0	1.0± 0.02	0.0	0.0
	300 mg L^{-1}	34± 3.0	6.4± 0.86	1.8± 0.24	9.0± 0.42	1.6± 0.20	0.0	0.0	0.0	0.0

[a] Results are shown as Mean±SEM.

Table 3. ANOVA of the effect of time, hormones and species on pollen germination.

Response variable	*df*	*MS*	*F*	*P*
Time	2	32.45	3.42	< 0.0001
Hormones	3	42.60	5.26	< 0.0001
Species	2	28.60	3.56	< 0.0001

and kinetin have shown considerable effect on *in vitro* pollen germination of *Spathodea campanulata* in the initial 24 hours of treatment.

DISCUSSION

The very low percentage of *in vitro* pollen germination in control condition in *Spathodea campanulata* and *Bauhinia purpurea* (4.6 and 6.0%, respectively) reflects that both species are prone to pollination and fertilization failure if appropriate pollinators and receptive stigmas are unavailable to them early after anther dehiscence. *In vitro* Pollen germination under sucrose (5 and 10%) has a fairly good response to all three analyzed tropical tree species. Stigmatic soluble sugars such as sucrose, glucose and fructose are major fractions of stigmatic exudates in many plant species. Sugar in stigmatic exudates acts as a source of carbon for pollen tube growth and also acts as an osmoprotectant (Kroh *et al.*, 1970). Though, pollen has endogenous limited reserve of carbohydrates but it will only suffice for initiation of pollen germination after hydration. In later stages of pollen germination, pollen requires exogenous source of soluble carbohydrate for its growth and development as in case of *in vivo* conditions it is supplied by stigmatic exudates. Sugar, particularly sucrose is an important regulatory factor for pollen germination and pollen tube growth in *in vitro* conditions in many plant species. Plant growth regulators are involved to regulate a variety of plant developmental processes. Differential response of pollen germination in selected tropical tree species towards different plant growth regulators exhibits physiological variability among them. IAA has stimulated better *in vitro* pollen germination in *B. variegata* and *B. purpurea* compared to *S. campanulata*. IAA promotes cell elongation in several plant species by H^+-ATPase activity via transport of H^+ into the cell wall, which results in acidification and loosening of wall leading to expansion of cell. IAA promotes and regulates pollen tube growth in *Torenia fournieri* by the aforesaid mechanism (Wu *et al*., 2008). IAA is also reported to promote pollen tube in *Pinus roxburghii* (Konar, 1958) and vital for pollen germination in *Pinus austrica* (Smith, 1939). Gibberellic Acid (GA_3) has induced high *in vitro* pollen germination in *Spathodea campanualata* compared to *B. variegata* and *B. purpurea*. Gibberellins (GAs) induce germination via promotion of amylase activity. GAs plays a crucial role in several plant growth and developmental processes, particularly flower induction, leaf and stem elongation and fruit and seed development (Sun, 2004). It is also reported to induce pollen tube growth under *in vitro* conditions in *Pistacia vera* (Acar *et al*., 2010). GAs plays a role in pollen viability and pollen tube growth in *Arabidiopsis* and rice (Singh *et al*., 2002; Chhun *et al.*, 2007). IBA and kinetin were found to enhance pollen tube growth in *Calotropis procera*, (Malik, 1977), as these hormones play an important role in activating the catalytic activity of peroxidase, an enzyme essential for pollen germination and pollen tube growth (Parui *et al.,* 1998). Kinetin was reported to promote pollen germination and the elongation of the pollen tube length in *Pinus roxburghii* (Konar, 1958). Both hormones, however, were less effective to all three analyzed species in this study. Application of kinetin at low concentrations has been reported to improve pollen germination and pollen tube lengths in

Prunus armeniaca¸ however, the high concentrations reduce pollen tube enlargement (Bolat and Pirlak, 1999). In *Prunus dulcis*, GA_3 has shown the highest pollen germination and pollen tube growth and the least was observed with Kinetin (Sotomayor *et al.,* 2012). Gibberellins have been reported to have a high impact on pollen viability and pollen tube growth (Ye *et al*., 2010). GA_3 was also found effective to increase the root yield of *Angelica dahurica* (Hou *et al.,* 2013) and plant growth (Emam and Moaied, 2000).

CONCLUSIONS

Different hormones under study showed different responses to *in vitro* pollen germination of analyzed tropical tree species. Selection of proper growth regulating chemicals with appropriate concentration could be vital for *in-vitro* pollen germination studies. Fruit set and fruit growth are synchronized by the corresponding action of hormones formed in the ovary after pollination or fertilization. Pollination and consequent fertilization lead to a sturdy alter in the stability of phytohormones and development of the ovule. Furthermore, the result of this study might be valuable in optimization of conditions for pollen storage of these species. Since all three tree species are cross pollinated, depending on the services of variety of pollinators for pollination success. The germination of stored pollen can be enhanced by the use of proper growth regulators in appropriate concentrations. This would be utilized for making crosses between similar species, when they flower in different periods, to develop new species of more attractable ornamental trees.

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REFERENCES

1. Acar, I., Ak, B. E. and Sarpkaya, K. 2010. Effect of Boron and Gibberellic Acid on *In vitro* Pollen Germination of Pistachio (*Pistacia vera* L.). *African J. Biotech.,* **9(32):** 5126-5130.
2. Ali, S. 1933. Flowers-birds and Bird-flowers in India. J. Bombay Natural History Society, Oxford University Press, Bombay.
3. Aloni, R., Aloni, E., Langhans, M, and Ullrich, C. I. 2006. Role of Auxin in Regulating *Arabidopsis* Flower Development. *Planta*, **223:** 315–328.
4. Bolat I. and Pirlak, L. 1999. Effects of some Chemical Substances on Pollen Germination and Tube growth in Apricot. *Acta Horticul*., **488:** 341–4.
5. Brewbaker, J. C. and Kwack, B. H. 1963. The Essential Role of Calcium Ion in Pollen Germination and Pollen Tube Growth. *Am. J. Bot*., **50:** 859-865.
6. Chhun,T., Aya, K., Asaw, K., Yamamoto, E., Morinaka, Y., Watanbe, M., Kitano, H., Ashikari, M., Matsuka, M. and Ueguchi-Tanaka, M. 2007. Gibberellin Regulates Pollen Viability and Pollen Tube Growth in Rice. *Plant Cell*, **19:** 3876-3888.
7. Emam, Y. and Moaied, G. R. 2000. Effect of Planting Density and Chlormequat Chloride on Morphological and Physiological Characteristics of Winter Barley (*Hordeum vulgare* L.) Cultivar "Valfajr". *J. Agr. Sci. Tech*., **2(2)**: 75–83.
8. Faegri, K. and van der Pijl, L. 1979. *The Principles of Pollination Ecology*. Pergamon Press, Oxford.
9. Heslop-Harrison, J. 1979. An Interpretation of Hydrodynamics of Pollen. *Am. J. Bot*., **66:** 737-743.
10. Hou, H., Chen, J. W., Li, J.Y., Shen, H., Chen, L. and Wu, W. 2013. Effect of Gibberellic Acid and Chlormequat Chloride on Growth, Coumarin Content and Root

Yield of *Angelica dahurica* var. Formosana. *J. Agr. Sci. Tech.,* **15:** *1415-1423.*

11. Johri, B. M. and Vasil, I. K. 1961. Physiology of Pollen. *Bot. Rev*., **27(3):** 325-381.
12. Konar, R. N. 1958. Effect of IAA and Kinetin on Pollen Tubes of *Pinus rouxburghii*. *Sar. Curr. Sci*., **6:** 216-217.
13. Kroh, M., Miki-Hirosige, H., Rosen, W. and Loewus, F. 1970. Incorporation of Label into Pollen Tube Walls from *Myo*-inositol Labeled *Lilium longiflorum* Pistils. *Plant Physiol.*, **45:** 92–94.
14. Malik, C. P. 1977. Enzymes in Pollen Development and Pollen Tube Growth. In: "Adv*ances in Pollen-Spore Research*", (Ed.): Nair, P. K. K.. Today and Tomorrow's Prints Publs., New Delhi, **II**: 30-43.
15. McConchie, C. A. and Knox, R. B. 1989. Pollination and Reproductive Biology of Seagrasses. In: "*Biology of Seagrasses. A Treatise on the Biology of Seagrasses with Special Reference to the Australian Region*", (Eds.): Larkum, A. W. D., McComb, A. J. and Shepherd, S. A.. Elsevier, Amsterdam, PP. 74-111.
16. Nalawadi., U. G. Gowda, J. V. N. and Sulladmath, U. V. 1980. Varied Season of Flowering of *Spathodea campanulata* (Beauv.) under Bangalore Conditions. *Curr. Rese.,* **9(4):** 58-59
17. Nemhauser., J. L., Feldman, L. J. and Zambryski, P. C. 2000. Auxin and ETTIN in *Arabidopsis* Gynoecium Morphogenesis. *Development*, **127:** 3877–3888.
18. Pacini, E. 1996. Types and Meaning of Pollen Carbohydrates Reserves. *Sex. Pl. Rep*., **9:** 362-366
19. Parui, S., Mondal, A. K. and Mandal, S. 1998. Peroxidase Isozyme Profiles of Immature and Mature Pollen of Seven Tropical Plants from Eastern India. *Grana*, **37(4):** 228-232.
20. Pfahler, P. L. 1967. Fertilization Ability of Maize Pollen Grains. II. Pollen Genotype Female Sporophyte and Pollen Storage Interactions. *Genet.,* **57:** 513–521.
21. Raina, R., Behera, M. C., Chand, R. and Sharma, Y. 2003. Reproductive Biology of *Gentiana kurroo* Royle. *Curr. Sci*., **85:** 667-670.
22. Sawmliana, M. 2003. *The Book of Mizoram Plants*. Zakhuma, P. Aizawl, Mizoram, India.
23. Schori, Y., Goren, T. and Ben-Jacov, J. 1992. Pollen Germination and Storage in *Banksia* and some other Proteaceae Plants. *Acta Hort*., **316:** 19-20
24. Shivanna., K. R. Linskens, H. F. and Cresti, M. 1991. Pollen Viability and Pollen Vigor. *Theor. Appl. Genet*., **81:** 38-42.
25. Singh, D. P., Jermakow, A. M. and Swain, S. M .2002. Gibberellins are required for Seed Development and Pollen Tube Growth in *Arabidopsis*. *Plant Cell*., **14:** 3133-3147.
26. Singh, N. P. Singh, K. P. and Singh, D. P. 2002. *Flora of Mizoram*. Volume I, Botanical Survey of India, Kolkata, India.
27. Smith, P. F. 1939. The Influence of Indole-3 Acetic Acid on Pollen Germination. *Sci.,* **90:** 163-164.
28. Sokal, R.R. and Rohlf, F.J. 1995. *Biometry,* 3rd Edition, WH Freeman, San Francisco.
29. Sotomayor, C., Castro, J., Velasco, N. and Toro, R. 2012. Influence of Seven Growth Regulators on Fruit Set, Pollen Germination and Pollen Tube Growth of Almonds. *J. Agr. Sci. Tech*., **B 2:** 1051-1056.
30. Stanley, R. G. and Linskens, H. F. 1974. *Pollen: Biology, Biochemistry and Management.* Springer, New York.
31. Sun, T. P. 2004. Gibberellin Signal Transduction in Stem Elongation and Leaf Growth. In: "*Plant Hormones: Biosynthesis, Signal Transduction, Action*", (Ed.): Davies. P. J.. Kluwer Academic Publishers, Dordrecht, The Netherlands, PP. 304–320.
32. Taylor, L. P. and Helper, P. K. 1997. Pollen Germination and Tube Growth. *Annu. Rev. Plant Physiol. Plant Mol. Biol*., **48:** 461–491.
33. Tuinstra, M. R. and Wedel, J. 2000. Estimation of Pollen Viability in Grain Sorghum. *Crop Sci.,* **40:** 968-970.
34. Wu, J.Z., Yi, L., Xue-Lian, Z., Dai-Wen, P., Jie, Z. 2008. IAA Stimulates Pollen Tube Growth and Mediates the Modification of Its Wall Composition and Structure in *Torenia fournieri*. *J. Exp. Bot.,* **59(9):** 2529–2543.
35. Ye, Q., Zhu, W., Li, L., Zhang, S., Yin, Y. and Ma, H. 2010. Brassinosteroids Control Male Fertility by Regulating the Expression of Key Genes involved in Arabidopsis Anther and Pollen Development. *PNAS,* **107:** 6100–6105.

اثر تنظیم کننده های رشد و زمان در محیط آزمایشگاهی بر جوانه زنی دانه گرده سه گونه درخت زینتی گرمسیری

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چکیده

در محیط آزمایشگاهی جوانه زنی دانه گرده سه گونه درخت زینتی گرمسیری با نامهای علمی*Spathodea campanulata*، *Bauhinia purperia* و *B. racemosa* به منظور شناخت تاثیر تنظیم کننده های رشد و زمان بر جوانه زنی دانه گرده انجام گرفت. سه غلظت برای نمونه های ۱۰۰، ۲۰۰ و ۳۰۰ ppm از جهار هورمون رشد (IAA, IBA, GA_3 and Kinetin) و ساکاروز ۵ و ۱۰ درصد به عنوان وسیله ی جوانه زنی استفاده شدند. نتایج نشان دادند که جوانی زنی دانه گرده در شرایط کنترل شده بسیار پایین است، که بین 4.6±1.2 و 17.8±3.2 درصد در نوسان بود. هورمون های رشد و ساکاروز (قند) در تحریک جوانه زنی دانه گرده مؤثر بودند. IAA و IBA برای هر دو گونه ی *Bauhinia* مؤثر بودند، در حالی که GA_3 و Kinetin برای *Spathodea campanulata* مناسب شناخته شد. بیشترین جوانه زنی در ۲۴ ساعت اولیه آزمایش ثبت شد، که در ۴۸ ساعت بعد کاهش یافته و حنی بعد از ۷۲ ساعت به صفر درصد هم رسید. زمان، هورمون ها و گونه ها تأثیر قابل ملاحظه ای (0.0001>) بر جوانه زنی دانه گرده داشتند. ساکاروز پاسخ مناسبی (۴۳ تا ۶۴ درصد) در تمامی گونه های درختان گرمسیری نشان داد. هر سه گونه ی درختان گرده افشانی مصنوعی دارند که به تنوع گرده افشان ها بستگی داشت. درصد پایین جوانه زنی دانه گرده در شرایط کنترل شده در *Spathoda campanulata* و *Bauhinia purpurea* نشان میدهد که این دو گئنه در معرض شکست در گرده افشانی و لقاح هستند، اگر گرده افشان مناسب و یا کلاله ی پذیرنده بلافاصله بعد از باز شدن بساک در دسترس نباشد.