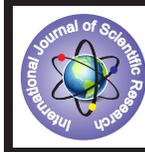


## In Vitro Pollen Germination of *Datura Metel L.* ( 5 ) Effect of Hormones



### Botany

**KEYWORDS :** Hormones, Pollen germination, Pollen tube growth, *Datura metel*

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### ABSTRACT

*In vitro* germination of pollen is simple and fully quantitative test for assaying pollen viability and also can be exploited for study of pollen physiology. A nutrient medium (*in vitro*) is supplemented with some essential compounds necessary for pollen germination and tube growth. During *in vitro* pollen germination and tube growth not only enzyme activity but also the effect of Carbohydrates, boron, calcium, Potassium nitrate, hormones, light and other factors have been studied for different plants. Here *Datura metel L.* was selected. In order to get maximum germination and tube growth in this taxon, modification of the basal medium of Brewbaker and Kwack was used. *Datura metel L.* pollen gave good percent germination in  $10^{-5}$  M Gibberellic acid,  $10^{-5}$  M Indole-3-acetic acid,  $10^{-5}$  M Kinetin, 50 ppm Salicylic acid, 50 ppm Maleic hydrazide. As major component of pollen tube reserves hormones have a vital role to play in its germination and growth.

### INTRODUCTION:

Pollen grains which behave as single cell structure provide a unique system for *in vitro* studies. The pollen tubes are considered as the most rapidly growing cells in the plant world since they are capable of attaining considerable length in a short duration under optimum conditions [Malik, 1977]. A large numbers of pollens have been successfully germinated under laboratory conditions on relatively simple media [Stanley and Linskens, 1974]. The composition of nutrient medium for *in vitro* pollen germination suggested by Brewbaker and Kwack, 1963 is normally modified for optimum pollen germination because germination requirement of pollen grains varies from species to species.

Many investigators have tested the effect of hormones on pollen grain and germination. Promotory effect of  $GA_3$  is reported. There are clear evidences that exogenous levels of hormones do affect endogenous levels of other hormones *in vitro*. The role of various growth substances and some antimetabolites in pollen germination and pollen tube elongation is well established [Malik and Mehan, 1975; Mascarenhas, 1975; Malik and Singh, 1975; Malik and Chhabra, 1976, 1978]. Hormones, vitamins, carotenoids, antibiotics and several other inorganic salts help to improve germination in many cases. Pollen grains are rich in hormones, vitamins and amino acids [Lunden, 1956; Stanley and Linskens, 1974].

As many as six classes of plant growth regulators (auxins, gibberellins, kinins, brassins, ethylene and inhibitors) are reported from pollen and have been shown to potentially influence their germination and pollen tube growth. However not all six classes are reported from a single pollen. Evidence from the application of individual has shown that germination and pollen tube growth are regulated by separate growth substances [Malik, 1985]. Pine (*Pinus roxburghii*) pollen grown in suspension cultures was used to study the effect of growth regulators and light condition on germination and pollen tube growth. Indole-3-acetic acid, gibberellic acid, ethylene, abscisic acid and cyclic AMP (cAMP) at low concentration ( $1-10 \text{ mg l}^{-1}$ ) promoted germination and tube growth. In *Petunia* [Kovaleva *et al.*, 2004] the mature pollen grains contain the complete set of hormones essential for pollen germination and pollen tube growth. ABA, GA and IAA together with cytokinins control the process of pollen hydration, germination and pollen tube growth.

### MATERIAL AND METHODS:

*Datura metel L.* flower buds were selected for the experiments. The pollen was germinated *in-vitro* on modified medium by sitting drop method using watch glasses. Media constituents were standardized so as to obtain optimum germination.

Effects of  $10^{-3}$  M  $GA_3$ ,  $10^{-4}$  M  $GA_3$ ,  $10^{-5}$  M  $GA_3$ ,  $10^{-3}$  M IAA,  $10^{-4}$  M IAA,  $10^{-5}$  M IAA,  $10^{-3}$  M Kinetin,  $10^{-4}$  M Kinetin,  $10^{-5}$  M Kinetin, 50ppm salicylic acid, 100ppm salicylic acid, 200ppm salicylic acid, and 50ppm Maleic hydrazide, 100ppm maleic hydrazide, 200ppm Maleic hydrazide, 400ppm Maleic hydrazide, 600ppm Maleic hydrazide were noted. For those 2-3 drops of their stock solution was added in each 10ml of standard media. Some amount (approx. 2ml) was taken in the watch glass and pollen grains were suspended in it. Results were observed after every 15 minutes for 2 hours to know the effect on germination.

### RESULTS AND DISCUSSION:

Pollen grains of *Datura metel L.* are round in shape with smooth surface. Each pollen grain possesses three germ pores. During germination one, two or three pollen tubes emerges but only one grows and increases in length. Tubes mostly grow straight. Pollen germination and pollen tube growth responded differently to exogenously applied plant growth substances. Effects of exogenous hormones have been studied in number of taxon. The results are highly variable.

Effect of  $GA_3$ , Kinetin, IAA, Salicylic acid and Maleic Hydrazide on pollen germination was studied. While  $GA_3$ , Kinetin and IAA proved to be effective, addition of Salicylic acid and Maleic hydrazide to the media proved to be lethal for *Datura metel L.* pollen grains.  $GA_3$ , Kinetin and IAA influenced the growth of the tube vigorously. The tubes grown were healthy i.e they were thick in diameter at the end of 2 hrs, observed to infinite pollen tube length. Table: 1,2.

Stimulation of pollen tube growth by IAA, Kinetin, and  $GA_3$  is reported in *Calotropis* [Shukla and Tiwari, 1973], *Annona* [Wee and Rao, 1979], and *Arachis* [Malik and Chhabra, 1978]. However, IAA, 2,4-D and Kinetin had no significant effect either on germination or on pollen tube growth.

**TABLE - 1: EFFECT OF DIFFERENT PLANT HORMONES ON % GERMINATION OF *Datura metel L.* POLLEN GRAIN**

Time in minute	$GA_3 10^{-3}M$		$GA_3 10^{-4}M$		$GA_3 10^{-5}M$		IAA $10^{-3}M$		IAA $10^{-4}M$		IAA $10^{-5}M$	
	%G	%B	%G	%B	%G	%B	%G	%B	%G	%B	%G	%B
30	35.41	60.94	36.44	59.70	38.64	57.86	37.89	60.42	66.25	31.49	72.02	24.97
60	34.83	65.13	34.09	64.23	35.36	61.71	34.31	62.59	64.48	32.82	70.81	25.37
90	32.04	67.09	32.87	66.87	34.96	63.87	31.40	65.17	63.00	34.11	69.93	26.82
120	28.17	69.71	29.33	67.36	32.41	65.02	30.87	67.58	61.46	35.50	68.90	27.53

**TABLE - 2: EFFECT OF DIFFERENT PLANT HORMONES ON % GERMINATION OF *Datura metel* L.POLLEN GRAIN**

Time in minute	Kn 10 <sup>-3</sup> M		Kn 10 <sup>-4</sup> M		Kn 10 <sup>-5</sup> M		SA 50 ppm		SA 100 ppm		SA 200 ppm	
	%G	%B	%G	%B	%G	%B	%G	%B	%G	%B	%G	%B
30	79.86	15.11	83.29	11.03	87.23	9.38	71.24	24.75	65.09	26.89	58.15	41.84
60	77.11	18.80	82.67	14.60	86.16	10.40	70.34	26.64	62.59	29.39	55.81	46.17
90	74.03	20.60	81.16	15.00	85.21	12.90	69.65	27.34	61.09	36.89	53.43	43.56
120	71.36	21.01	80.18	16.20	84.59	13.53	67.64	28.35	59.10	38.83	52.83	45.19

Time in minute	50 ppm MH		100 ppm MH		200 ppm MH		400 ppm MH		600 ppm MH	
	%G	%B	%G	%B	%G	%B	%G	%B	%G	%B
30	59.48	38.13	55.16	44.83	54.95	44.04	52.64	47.35	51.84	46.15
60	54.25	40.73	52.38	46.60	53.27	47.71	47.82	48.17	47.70	42.28
90	52.49	43.50	51.50	48.49	52.83	49.56	45.44	49.54	45.50	44.49
120	50.10	46.89	49.48	49.50	51.61	49.21	44.44	51.16	43.88	45.10

In *Zea mays* 5ppm GA<sub>3</sub> shows maximum percentage of pollen germination while in *Sorghum bicolor* L. Moench, 3ppm GA<sub>3</sub> shows maximum pollen germination (Jha, 2002). In *Datura metel* L. maximum percentage of pollen germination is reported in the medium having 1ppm GA<sub>3</sub> (Patel, 2002). GA<sub>3</sub> acts as strong stimulator in pollen germination and pollen tube elongation in *Abelmoschus esculentus* (Dabgar, 2002). Except ABA all other growth regulators (IAA, GA<sub>3</sub>, Kn and Ethylene) increased both germination and pollen tube growth at all the concentration used in *Cicer arietinum* (Setia *et al.*, 1985).

McLeod (1975) also reported that *Lycopersicon* pollen was almost completely insensitive to plant growth substances (including auxins, gibberellins and abscisic acid).

#### CONCLUSION:

Addition of exogenous GA<sub>3</sub>, IAA and kinetin vigorous pollen tube growth in *Datura metel* L. Salicylic acid and Maleic hydrazide proved to be lethal than other hormones.

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