Research Paper

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IN VITRO POLLEN GERMINATION OF DATURA METEL L. [4] EFFECT OF POTASSIUM NITRATE (KNO3)

Botany

KEYWORDS : Potassium nitrate, Pollen germination, Pollen tube growth, Datura metel

Ms. Mital Laxmanbhai Katara — M.B.Patel Science college, Anand,Gujarat.

Dr. Archana Mankad — Department of Botany, School of Science, Gujarat University, Ahmedabad, Gujarat.

ABSTRACT

In vitro pollen 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, 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 7% Sucrose + 0.06% Boric acid + 0.04% Calcium chloride + 0.05% Potassium nitrate.

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. Pollen grains are known to be packed with different biochemical's like sugar,starch, lipids, phytic acid [Bertin,1988; Wetzel and Jensen,1992; Stephenson *et al*.,1994] and m-RNA [Stephenson,1994].These storage products get metabolized upon germination and elongation of pollen tube, thus, play an important role in germination and in initial stage of pollen tube growth [Vasil,1974; Wetzel and Jensen,1992; Stephenson *et al*.,1994]. There is sufficient evidence to suggest that Ca^{+2} plays an important role in pollen germination and direction of pollen tube growth (Taylor and Helper, 1997). It is known that pollen germination and tube growth are significantly regulated by the transport of inorganic ions, such as Ca^{+2} and K^{+}, across the plasma membranes of pollen and/or pollen tubes (Feijo *et al*.,1995; Taylor and Helper, 1997). It is also known that K^{+} is requiring for both pollen germination and pollen tube growth (Brewbaker and Kwack, 1963; Weisenseel and Jaffe, 1976; Liu-Min fan *et al*., 2001).

It is known that K^{+} is required for both pollen germination and pollen tube growth (Brewbaker and Kwack, 1963; Weisenseel and Juffe, 1976; Feijo *et al*., 1995). By the application of patch-clamp technique, the three types of K^{+} channels from *Lilium* pollen protoplasts were identified and the possible involvement of inward K^{+} channel-mediated K^{+} influx during pollen tube growth was suggested (Obermeyer and Kolb,1993). It was also shown that the inward K^{+} channels in *Brassica* pollen protoplast are significantly regulated by external Ca^{+2} , which is an important regulatory factor for pollen germination and pollen tube growth. These previous studies strongly suggest that the inward K^{+} channels may be essential components involved in the process of pollen germination and pollen tube growth, and that the regulation of K^{+} channels may play a regulatory role in pollen germination and pollen tube growth (Liu-Min Fan *et al*., 2001).

Malik *et al*., (1976) showed that nitrate concentrations up to 5mg promoted germination and pollen tube growth. Nitrate is an important source of nitrogen for the biosynthetic processes during germination and pollen tube growth. Most of the enzymes including nitrate reductases use nitrate as substance in addition to nitrates in the basic medium. KNO_3 may also regulate the osmotic potential for the swelling of pollen grains in poaceous plants (Matsui *et al*., 2000).

MATERIAL AND METHODS:

Collection of pollen in a viable condition is a primary requirement for any experimental study on pollen. Generally, the flowers of *Datura metel* L. were collected from the Botanical garden of our department at anthesis in the morning and the pollen grains were collected from the dehisced anther. The pollen grain were germinated in medium containing 7% Sucrose + 0.06% Boric acid +0.04% calcium chloride and different Potassium nitrate concentrations from 0.01% to 0.06%. The concentration of Potassium nitrate tried for *Datura metel* L. were 0.01%, 0.02%, 0.03%, 0.04%, 0.05% and 0.06%. The pollen were placed in the drop of medium on a clean slide and studied through the sitting drop method. The slides were carefully placed in large petridishes lined with moist filter paper to prevent evaporation. The percent germination and percent bursting of pollen was noted at regular intervals by keeping pollens in the medium up to 2 hour at room temperature. The concentration of Potassium nitrate giving maximum percent germination and pollen tube length was evaluated. The experimental design involved five replicates and readings were noted from ten different areas of the slide.

RESULTS AND DISCUSSION:

The various concentration of Potassium nitrate tried were 0.01%, 0.02%, 0.03%, 0.04%, 0.05% and 0.06% for *Datura metel* L. Potassium proved to be effective for pollen grains of the plant. 78.43% germination was obtained in *Datura metel.* 0.5% Potassium nitrate was best for *Datura metel* pollen grains.(Table-1)

0.01% of potassium nitrate was also incorporated in the standard media of *Gossypium hirsutum* (Shivastava, 1962) and was also good for *Plumeria alba* (Johri and Shivanna, 1985).

TABLE - 1 SHOWING PERCENT POLLEN GERMINATION (% G) & PERCENT POLLEN

BURSTING (%B) UNDER DIFFERENT CONCENTRATION OF POTASSIUM NITRATE (KNO_3) + 7% SUCROSE + 0.06% BORIC ACID + 0.04% $CaCl_2$ $6H_2O$

Time in minute	0.01%		0.02%		0.03%		0.04%		0.05%		0.06%	
	%G	%B	%G	%B	%G	%B	%G	%B	%G	%B	%G	%B
15	34.12	63.81	42.38	55.71	54.32	44.83	68.11	24.48	85.39	13.42	70.34	29.31
30	34.97	64.00	42.55	56.02	54.96	44.97	68.86	30.29	86.43	13.51	68.51	30.43
45	34.03	64.39	43.81	56.09	53.82	45.15	67.43	32.12	86.02	13.63	68.43	31.52
60	32.65	65.19	43.00	56.80	53.01	45.99	67.10	31.86	85.34	13.97	67.97	32.03
75	31.42	66.89	41.54	57.12	52.59	46.89	66.68	32.69	84.23	15.30	66.03	33.27
90	29.51	68.71	39.42	58.47	51.87	47.11	65.76	33.87	82.19	16.86	64.30	35.52
105	28.32	69.64	38.19	59.13	51.00	48.62	64.31	34.69	80.58	19.12	63.86	36.09
120	26.73	72.12	37.64	60.89	50.42	49.53	62.19	37.76	78.43	20.13	62.38	37.53

CONCLUSION:

Best germination ad least bursting were obtained in Datura metel L. Pollen grains. Optimum pollen germination and pollen tube growth of pollen grains of Datura metel L. was obtained at 7% Sucrose + 0.06% Boric acid + 0.04% $CaCl_2$ + 0.05% KNO_3.

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