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Effect of Hormones and Antibiotics on Pollen Grain Germination

MUHAMMAD ALI KHAN, I.A. MAHMOOD AND M. ASLAM
Applied Microbiology Section, National Agricultural Research Centre, Islamabad, Pakistan

ABSTRACT

The effect of different hormone concentrations (0.0, 0.001 and 0.0001% W/V) and antibiotics as single as well as in various combinations were studied on the germination of pollen grains of four plants i.e. *Stellaria media, Euphorbia heliscopia, Brassica compestris* and *Phaseolus lablab.* The pollen grains of these plants were also treated with 2% colchicine and 2% combined solution of colchicine and tetracycline. Time taken for germination was recorded. It was 100 and 115 minutes in *Stellaria media* and 115 and 120 minutes in *Euphorbia heliscopia*, and 105 and 120 minutes in *Brassica compestris* and in *Phaseolus lablab* the germination time was 100 and 125 minutes. In 20% sugar and distilled water, pollen grains of *Stellaria media, Euphorbia heliscopia, Brassica compestris* and *Phaseolus lablab* germinated after 40, 65, 90, 45, 75, 40, 70, and 30 minutes, respectively. Similarly pollen germination was noted in hormones. In Naphthalene acetic acid (NAA), in concentration of 0.001% solution, the germination time was 25, 15, 20, 25 minutes and in solution of concentration of 0.0001% dilution, germination time recorded was 20, 20, 15, 20 minutes in Stellaria media, *Euphorbia heliscopia, Brassica compestris* and *Phaseolus lablab.* Indole butyric acid (IBA) in 0.001% solution consumed 20, 25, 20, and 15 minutes and in concentration of 0.0001% dilution, the pollen grains of afore-said serial wise plants germinated after 15, 20, 15 and 10 minutes. Indole propionic acid (IPA), in concentration of 0.001% solution, the pollen grains germinated after 20, 20, 25, 25 minutes and in solution of concentration in 0.0001% dilution, the pollen grains germinated after 15, 15, 20 and 20 minutes, in the serial wise above mentioned plants alone and similar results were recorded in different combinations with one an other. Indole butyric acid applied in 0.0001% concentration proved the best among all the chemicals for *in vitro* pollen germination.

Key Words: Plant growth substances; Antibiotics; Pollen germination

INTRODUCTION

The study of the pollen grain behaviour is important for plant breeders and geneticists for getting better germination of field crops. Better the pollen germination, better will be the crop establishment. Size of the pollen, which is important for fertilization, varies from specie to specie i.e. 5 microns to 200 microns represent the male gametophyte. Hence better pollen germination means good fruit setting, better and high yield of crops.

Apart from other conditions, humidity and temperature play an important role in determining the rate of growth of a pollen tube. *in vitro* studies of pollen germination are helpful for observing the pollen tube growth. For this purpose various media i.e. agar, sucrose, various hormones, antibiotics, colchicine, herbicides and insecticides had been used. Koteswari and Mary (1983) reported that the average fertility and germination of *Dactyloctenium agyptium* pollen was 85.0% and 57.5%, respectively. The germination and pollen tube growth was increased by Indole acetic acid (IAA) and Kinetin and were inhibited by Carbaryl and BHC. Similar studies were undertaken by Gupta and Murty (1985) for the pollen of *Vicia faba.* Pollen germination and tube growth were increased with increasing concentration (0.5–1.0 ppm) of Boric acid (BA), Gibberellic Acid (GA), IAA (in a basic sucrose) and agar medium, without injurious effect. The highest percentage of pollen germination was obtained with growth regulators, B or Mo at 0–35°C. Optimum concentrations of hormones for pollen germination after 5 h at 25°C were 10 ppm of 2–4 D, 20 ppm of NAA, 2 ppm of tricentennial, 20–30 ppm by Diamnozide, 70 ppm boric acid, 5 ppm ammonium molybdate. Germination time was lowest with 10 ppm, 2–4 D (about half of the time in control). In a trial for determining the effect of storage on germination it was found that pollens stored at 10°C had shortest germination time.

The aim of this study was to know the process of self and cross pollination, to understand self incompatibility and self sterility and to explore germination of pollen grains *in vitro* treated with different hormones as well as antibiotics. The ultimate objective was to find out chemical agents efficient in fertilization and seed production under a particular environmental.

MATERIALS AND METHODS

A laboratory study was conducted on freshly collected pollen grains of the following plant species to explore germination of pollen grains *in vitro* with different hormones as well as antibiotics.

Table I. Effect of sugar and hormones on time of pollen tube emergence (minutes) (ave. of 4 repeats)

Treatment	Dilution (%)	Brassica compestris	Euphorbia heliscopia	Phaseolus lablab	Stellaria media
Sucrose	10	50	30	40	25
	15	45	35	35	30
	20	40	45	30	40
NAA*	.001	25	15	20	25
	.0001	20	20	15	20
IBA**	.001	20	25	15	20
	.0001	15	20	10	15
IPA***	.001	25	20	25	20
	.0001	20	15	20	15
NAA+ IBA	.001	20	25	25	20
	.0001	25	30	20	25
IBA+ IPA	.001	15	20	25	25
	.0001	20	25	30	20
NAA+ IPA	.001	20	15	20	25
	.0001	25	20	25	30
Distilled Water	-	75	90	70	65
Tap Water	-	85	80	60	70

*NAA = Napthaline acetic acid, **IBA = Indole butyric acid ***IPA = Indole propionic acid

1. *Brassica compestris.*
2. *Euphorbia heliscopia.*
3. *Phaseolus lablab.*
4. *Stellaria media.*

All the pollens were viable. They germinated and produced pollen tubes in tap as well as distilled water (control treatments). All the glassware including bottles, watch glasses and slides were thoroughly washed before use. In case of individual treatments, freshly prepared solutions of the hormones, antibiotics, colchicine and sugar (shown in Tables I and II) were separately taken in beakers and from these a drop of each solution was transferred on microscopic slides by droppers. After this the pollens were transferred with needles. For each solution or chemical, separate dropper was used to check contamination. In each case initial and final time was recorded with care. Initial time was the time when pollen grains of the given species were placed in a drop of specific solution over the slides. Final time was the time at which the pollen tube appeared.

Environmental conditions. All the observations were performed under ordinary room temperature, pressure and humidity conditions, under the microscope. During the period of investigation, the temperature ranged 17.5°C to 20°C, whereas the humidity ranged 88.55 to 94.50%.

RESULTS AND DISCUSSION

In present studies sugar (sucrose), distilled water, tap water, hormones, colchicine and antibiotics were applied to study *in vitro* the emergence of pollen tube. Accordingly the species material in Table I and II, distilled water soaking took 75, 90, and 65 minutes respectively for the emergence of pollen tubes. These results agree with those given by Sharma (1984) and results do not agree with Dhar (1983) for *in vitro* germination and pollen tube growth of Belladonna species. However, the pollen grains of *Stellaria media, Euphorbia helisscopia, Brassica compestris* and *Phaseolus lablab* plants (soaked in tap water) germinated after 85, 80, 60 and 70 minutes which is near to the distilled water. Colchicine 1% took 95, 100, 105 and 90 minutes whereas 2% took 105, 115 110 and 100 minutes. The time taken by colchicine for pollen grain germination is more than distilled as well as tape water. The antibiotics took similar time as colchicine, but the combination of 2% colchicine and 1% tetracycline took 120, 120, 125 and 115 minutes for pollen grain germination, which is longer time than others.

The treatment with 20% sucrose solution took 40, 45, 30 and 40 minutes, respectively, and proved to be better than afore mentioned treatments indicating that early emergence of pollen tubes might be due to the action of enhancement for growth promoting hormones. These results are in line with those of Isaev and Domracheva (1973), Sing (1978), Asif *et al.* (1983), Dhar (1983) and Sharma (1984).

Growth hormones, NAA, IBA and IPA alone and in combinations in dilutions of 0.001% and 0.0001% took only 10–15 minutes for all the above mentioned chemicals and antibiotics. Many researchers are of the view that the most important mechanism of direct growth promotion may be the production of plant growth regulators by plant growth promoting Rhizobacteria (Hussain & Vancura, 1970; Brown,

Table II. Effect of colchicine and antibiotics on time of pollen tube emergence (minutes) (ave. of 4 repeats)

Treatment	Dilution	*Brassica compestris*	*Euphorbia heliscopia*	*Phaseolus lablab*	*Stellaria media*
Colchicine	1%	95	100	105	90
"	2%	105	115	110	100
Penicillin	1%	85	75	80	70
"	2%	95	85	90	80
Tetracycline	1%	80	85	75	65
"	2%	90	95	85	75
Streptomycin	1%	85	80	70	75
"	2%	90	95	85	80
1+2	1%	95	110	105	100
"	2%	105	110	115	110
1+3	1%	110	115	120	100
"	2%	120	120	125	115
1+4	1%	100	105	110	95
"	2%	110	115	120	105
2+3	1%	105	100	95	85
"	2%	120	115	110	100
2+4	1%	100	90	85	90
"	2%	115	105	100	105
3+4	1%	100	90	85	90
"	2%	115	105	100	105

1974; Arshad & Frankenberger, 1991; Sarwar, *et al.*, 1992; Zarnstroff, *et al.*, 1994). Several studies have frequently demonstrated the ability of various plant growth promoting Rhizobacteria to produce plant growth regulators *in vitro* (Mei, 1989).

Plant growth regulators released as secondary metabolites by applied hormones may contribute to the growth promoting effects that enhanced early emergence of pollen tubes. The beneficial effects of plant growth promoting hormones on plant growth could also be explained on the basis of various mechanisms such as production of siderophores, antibiotics, extracellular metabolites and induced systemic resistance. Besides this, many plant growth promoting Rhizobacteria synthesise other plant growth promoting Regulators (gibberellins, cytokinins, ethylene and abscisic acid) in addition to auxins (Sanchez-Calle, 1989; Kloepper, 1994; Frankenberger & Arshad, 1995). From this study, it thus could be concluded that growth hormones are the best chemicals for *in vitro* pollen germination and tube emergence.

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