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STUDIES ON POLLEN TUBE GROWTH AMD *IN VITRO* STORAGE OF POLLEN GRAINS IN *CICER ARIETINUM* L. AND *C. SOONGARICUM* J & S

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Bengal gram *(Cicer arietinum* L.*)* is one of the most important pulse crops of India. No work has been done on this genus with regard to the behaviour of its pollen grains *in vitro.* Attempts to cross *C. arietinum L.* with its wild relative *C. soongaricum* was not successful and the reason might be attributed to the failure of pollen tube growth on the stigma during cross fertilization (Mercy and Kakar, 1975). Therefore studies were taken up to analyse the *in vitro* behaviour of pollen grains and viability of the grains during storage.

Materials and Methods

The material was grown in the experimental research area of Haryana Agricultural University, Hissar, during the *rabi* seasons of 1969-'70 and 1970-'71. A nutrient medium of 0.5 M sucrose and 100 ppm boric acid in distilled water was standardized for studying *in vitro* germination and tube growth of pollen grains.

Each slide for percentage germination count was prepared with pollen from five random flowers from one plant and five random plants were selected from each species. From each slide 5 random microscopic fields were taken for determining the germination percentage. Tube length was determined by taking the average of the 10 longest tubes ie., 2 from each of the 5 random microscopic fields from each slide.

With a view to making pollen available for crossing with the late flowering wild species *C. pinnatifidum,* attempts were made to preserve the pollen «f *C. arietinum* and *C. soongaricum.* Fresh flowers were cut between 8 and 9 a. m., placed in a petridish and immediately transferred into a desicator and stored in deep freeze at −4°C. Pollen germination counts and tube growth measurements were made at intervals of two days for 42 days.

Results and Discussions

The data on the extent of germination and tube growth of *C. arietinum* and *C. soongaricum* pollen are given in Table 1. Sucrose solution of 0.5 M concentration was the most favourable for the germination of the pollen grains of both the species. This concentration was selected and used for testing the concentration of boric acid that would further increase the percentage of germination. The results of the experiments are given in Table 2. The 0.5 M

sucrose with 100 ppm boric acid medium gave the best results. Subsequently 0.5 M sucrose solution containing 100 ppm boric acid was further tested in combination with three concentrations of calcium nitrate, which normally accelerates tube growth. However, the effect of calcium nitrate was not significant (Table 3). Hence the sucrose-boric acid medium was adopted as the standard medium in later experiments.

C. arietinum is an early flowering species while *C. soongaricum* flowers about 15 days later. *C. pinnatifidum* is very late flowering. With a *view to attempting* hybridization of C. *arietinum* and C. *soongaricum* with *C. pinnatifidum* later, storage of pollen grains of *C. arietinum* and *C. soongaricum* was tried.

Table 1 Percentage germination of pollen in sucrose medium and average maximum tube length

Medium sucrose concentration molar used	Percentage germination						Average maximum tube length (μ)					
	1 hr		2 hrs		3 hrs		1 hrs		2 hrs		3 hrs	
	C.a	C.s	C.a	C.s	C.a	C.s	C.a	C.s	C.a	C.s	C.a	C.s
1.0 M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.5 M	54.6	44.6	56.3	45.2	61.4	55.0	4.5	3.2	6.0	4.9	6.6	5.8
0.25 M	43.7	41.7	46.3	43.8	48.1	45.1	3.7	2.6	3.5	2.9	4.1	3.0
0.125 M	39.3	30.0	41.9	35.8	45.6	49.1	2.7	2.6	3.1	2.7	3.3	2.8

C.a = C. *arietinum* C.S = *C. soongaricum*

Table 2 Percentage germination of pollen in sucrose—boric acid medium and average maximum tube length

Composition of medium	Percentage germination						Average maximum tube length (μ)					
	1 hr		2 hrs		3 hrs		1 hr		2 hrs		3 hrs	
	C.a	C.s	C.a	C.s	C.a	C.s	C.a	C.s	C.a	C.s	C.a	C.s
0.5 M sucrose + 50 ppm boric acid	71.0	56.8	81.5	65.9	84.5	73.1	5.5	4.0	8.5	7.7	9.7	9.6
0.5 M sucrose + 100 ppm boric acid	80.9	61.4	87.6	73.7	91.9	80.1	7.5	6.9	11.0	10.9	12.5	11.8
0.5 M sucrose + 200 ppm boric acid	78.0	58.6	83.4	69.7	85.5	74.3	6.0	3.1	8.4	8.1	9.7	9.0

C.a — C. *arietinum* C.s = C. *soongaricum*

Table 3 Percentage germination of pollen in sucrose—boric acid—calcium nitrate medium and average maximum tube length

Composition of medium	Percentage germination						Average maximum tube length (μ)					
	1 hr		2 hrs		3 3rs		1 hrs		2 hrs		3 hrs	
	C.a	C.s	C.a	C.s	C.a	C.s	C.a	C.s	C.a	C.s	C.a	C.s
0.5 M sucrose + 100 ppm boric acid + 50 ppm calcium nitrate.	37.2	30.2	31.2	64.4	70.7	66.0	5.5	3.6	7.8	6.9	8.9	8.6
0.5 M sucrose + 100 ppm boric acid + 100 ppm calcium nitrate.	76.2	66.9	81.5	73.9	89.7	81.1	8.0	8.7	9.2	12.1	10.4	12.9
0.5 M sucrose + 100 ppm boric acid + 200 ppm calcium nitrate.	43.7	33.5	52.9	42.7	56.5	56.8	4.9	4.4	5.8	5.7	6.7	6.9

C.a = *C-arietinum* C.s = *C, soongaricum*

Pollen stored at —4°C were tested after every two days for viability. Percentage germination of pollen in sucrose boric acid medium and the average length of longest pollen tubes of the fresh pollen and pollen stored upto 42 days were determined. The data are presented in Table 4.

A perusal of the data shows that even after storage for 42 days the pollen viability of *C. arietinum* was 43% while that of *C. soongaricum* was 46.3%. The pollen tube growth was also good. This indicates that pollen can be stored at —4°C without much loss of viability and ability to germinate and can be made use of in crossing experiments.

During the past 50 years, longevity of pollen has been attempted by controlling temperature and relative humidity (R. H.). Sub freezing temperatures (_5 to —10°C) and low R. H. have generally proved optimum for storing pollen in viable conditions. According to Bhojwani and Bhatnagar (1976 a), pollen of mango, remain viable for 8 days ordinarily but at a temperature of 4.5 to 9°C and 10, 25 or 50% R. H., they maintain a viability for about 5 months; at —23°C and 0% R. H. they remain viable upto 10 months. In jack fruit pollen stored at 0°C at 0%, 25% and 50% R. H. survived for eight, seven and seven months respectively (Sinha, 1973). Guava pollen, stored at 0°C at 25% or 100% relative humidity, remained viable upto 100 days (Mandloi, 1973).

Table 4 Percentage germination and average length of longest tubes of stored pollen grains

No. of days in storage	Percentage germination		Average maximum tube length (μ)	
	C. arietinum	*C. soongaricum*	*C. arietinum*	*C. soongaricum*
0	86.5	71.5	11.0	10.7
3	85.1	71.0	10.8	9.7
6	83.3	70.7	10.6	9.2
9	81.2	67.5	10.0	8.7
12	80.9	66.1	9.9	8.2
15	77.6	59.7	9.2	9.0
18	72.4	59.0	9.9	7.8
21	65.5	57.1	9.1	7.8
24	63.2	56.3	9.0	7.7
27	61.7	54.6	8.8	7.6
30	57.0	53.3	9.0	7.4
33	56.6	51.5	8.4	7.6
36	50.9	51.1	7.3	7.5
39	49.3	50.2	7.1	7.6
42	43.0	46.3	7.8	7.3

In the present study pollen grains of *Cicer arietinum* and *C. soongaricum* retained their viability even after storage for 42 days in a desicator at −4°C. This is in accordance with reports about pollen grains of other crops.

Sucrose, boric acid and calcium nitrate are the three major constituents used by various investigators to facilitate pollen germination *in vitro.* Among inorganic substances, boron in the form of boric acid or borate has most dramatic effect on pollen germination and pollen tube growth (Bhojwani and Bhatnagar, 1976 b). Pollen of most species are deficient in boron content and when such pollen grains are grown *in vitro,* high amounts of boron (10—200 ppm) is supplied exogenously. Boron reduces bursting of pollen tubes as well as enhances percentage germination and pollen tube growth.

The results of the different experiments conducted with these chemicals to germinate the pollen grains of *C. arietinum* and *C. soongaricum* have shown that when only sucrose was used for pollen germination, the most favourable results were obtained with 0.5 M sucrose solution. A significant difference in the percentage germination was noted between the two species

Indicating that *C. arietinum* pollen can more effectively utilize sucrose and show a greater response than C. *soongaricum* pollen. After one hour of germination in the sucrose solution, *C. arietinum* showed 54.6% germination while C. *soongaricum* had a germination percentage of 44.6. After three hours of germination, this difference was decreased considerably so that *C. arietinum* had a germination percentage of 61.4 while C. *soongaricum* had 55.0 (Table 1). The inefficiency of *C. soongaricum* pollen to utilize sucrose at the beginning was made up considerably with increased germination time. At 0.25 M and 0.125 M concentrations of sucrose, pollen of both species showed relatively lesser percentage germination. No germination took place at 1.0 M sucrose concentration.

The nature of pollen tube elongation was also similar in both species. C. *arietinum* pollen tube grew faster than those of C. *soongaricum* and the maximum tube elongation was obtained at 0.5 M concentration of sucrose.

When a combination of sucrose with boric acid was tried, 0.5 M sucrose with 100 ppm boric acid showed the best results for both *C. arietinum* and *C. soongaricum.* The addition of boric acid further increased the percentage of germination. This is in accordance with the observations of Sivaram *et al.* (1974). According to them when castor pollen grains were germinated in different concentrations of sucrose solutions with or without the addition of boric acid, the highest germination percentage was shown in 17% pure sucrose solutions with 50 ppm boric acid. While diploid rye pollen germinated best in a medium consisting of 1% agar, 40% sucrose, and 2 drops of 1% boric acid (Poddubnaya-Arnoldi and Ivanova, 1971), lucerne pollen germinated in a 30% sugar solution (Islamkulov 1971). In the present study, in sucrose alone the percentage germination after three hours in the medium was 61.4 for *C. arietinum* and 55.0 for *C. soongaricum,* while with the addition of boric acid, it increased 91.9 and 80.1 for C. *arietinum* and C. *soongaricum,* respectively. This indicates that certain pollen grains in the same species require only sucrose as an ingredient to facilitate germination. But there is a good percentage in the same lot which requires boric acid, in addition, as an essential ingredient for germination. This suggests physiological dimorphism in the pollen of *Cicer.* The tube elongation was also accelerated by boric acid in both the species. The pollen of C. *arietinum* showed better response to boric acid than the *C. soongaricum* pollen. The addition of calcium nitrate to the nutrient medium did not improve the germination of pollen in both species. The studies point to some kind of a physiological dimorphism in the pollen of the two species.

Summary

Pollen storage experiments showed that pollen of *C. arietinum* and *C. soongaricum* could be successfully stored below 0° for upto 40 or more

days if necessary. *In vitro* pollen germination and tube growth studies showed best germination in 0.5 M sucrose 100 ppm boric and medium. Boric acid increased the germination percentage significantly in both species as compared to the germination percentage in sucrose alone, thus indicating the presence pollen dimorphism is *C. arietinum* and *C. soongaricum*.

സംഗ്രഹം

പരാഗ സംഭരണ പരീക്ഷണങ്ങളിൽ നിന്നും സൈസർ അരീട്ടിനം, സൈസർ സൂംഗാരിക്കം എന്നിവയുടെ പരാഗം 0°C ൽ താഴെയുള്ള താപനിലയിൽ 40 ദിവസങ്ങളിലേറെ കേട്കൂടാതെ സൂക്ഷിക്കാവുന്നതാണെന്നു കണ്ടു. പരാഗ അങ്കുരണവും കുഴൽ വളർച്ചയും സംബന്ധിച്ച ഇൻവിട്രോ പഠനങ്ങളിൽ ഏറ്റവും കൂടുതൽ അങ്കുരണം നടന്നതു് 0.5M സൂക്രോസും 100 PPM ബോറിക്കു് rarajgcijo കലർത്തിയ മാധ്യമം ഉപയോഗിച്ചപ്പോഴാണെന്നു കണ്ടു. പഠന വിധേയമാക്കിയ rast^ സ്പീഷീസുകളിലും ബോറിക്കു് അമ്ലത്തിന്റെ സാന്നിദ്ധ്യം അങ്കുരണ ശതമാനം പ്രകടമാംവിധം വർധിപ്പിച്ചതായിക്കണ്ടു. സൈ. അരീട്ടിനത്തിലും സൈ. സൂംഗാരിക്കത്തിലും പരാഗ ദ്വിരൂപത ഉണ്ടെന്നാണു് ഇതു് സൂചിപ്പിക്കുന്നതു്.

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