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IN VITRO POLLEN GERMINATION CAPACITY OF *CITRULLUS LANATUS* L., (CUCURBITACEAE)

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Abstract

Pollen germination capacity of *Citrullus lanatus* L., (Cucurbitaceae) in "hanging drop" technique was evaluated up to 48 weeks. The collected pollen were stored at different temperatures (4ºC, -20ºC, -30C and -60ºC). The pollen were also treated in organic solvents (acetone, benzene & chloroform), in vacuum over silica gel and in freeze dryer (-60ºC) for 30 minutes. The study indicates that low temperature is far better than high temperature with respect to pollen germination capacity and viability. In organic solvents benzene showed better results as compared to vacuum dried pollen. Freeze dryer (-60ºC) seems to be the best method to store pollen grains for a long period of time.

Introduction

The artificial culture of pollen grains attracted the attention of botanists with the discovery of pollen tubes by Amici (1824) in *Portulaca oleraceae.* Mangin (1886) and some other workers discovered pollen tube longevity in 80 species in air dry conditions. Earlier work has been mainly centered on pollen storage in fruits. It has been widely acknowledged that temperature and relative humidity of the storage environment are the two major factors which predominantly influence the viability of pollen grains in storage conditions. A humidity of 50% is optimum for the storage of different species and varieties of *Prunus*, *Pyrus* and *Vitis* (King, 1965a). The optimum temperature favours the storage of pollen which is different for different species. The important aspects for pollen conservation are storage temperature and humidity of the material (Gill *et al.,* 1992; Malik & Thind, 1992; Shivanna & Rangaswamy, 1992). There are different reports on pollen storage of different taxa using low temperature and humidity. Using low temperature and humidity, King, (1965b) stored *Medicago sativa* pollen for about 11 years in freeze dryer. Angiosperm has two groups of pollen viz., binucleate and trinucleate among which the former one is easy to germinate on artificial media, while the latter one is hard to germinate (Stanley & Linskens, 1974). Pollen grains of different species required various growth media such as water, sugar solution, salts, hormones etc. The literature relating to the germination of pollen grains of angiosperm has been reviewed by several workers (Visser, 1955; Singh, 1961; Vijay, 1972; Vasilakakis & Porlingis, 1985; Pinney & Polito, 1990; Tyagi & Mccomb, 1992; Thomas, 2000). Subramanyam (1959) and Vasil (1960) observed that the pollen grains of apple, papaya, grape, guava and *Cucumis* germinate in boron. Different investigators also assessed pollen viability and germination in vacuum over silica gel and in organic solvents (Iwanami & Nakamura, 1972, Khan & Perveen, 2009). Iwanami & Nakamura (1972) for the first time soaked pollen grains in different solvents as acetone, benzene, ethanol etc. Iwanami (1975) reported reduction of dormancy in pollen during the period of storage in organic solvents. The present report gives an account of the pollen germination capacity of *Citrullus lanatus* of the family Cucurbitaceae.

Material and Methods

During peak flowering season of *Citrullus lanatus* L., polliniferous material was collected in large quantity from cultivated fields of Sakro and Gharo. Fresh pollen were systematically subjected to preliminary viability tests (Alexander, 1969). The standardized medium suggested by Brewbaker & Kwack (1963) was used for pollen germination. The germination was scored after 3-6 hours of incubation at room temperature using humid chambers. Pollen tubes equal to at least twice of the pollen diameter was counted as germinated, while burst grains were considered as ungerminated. The viability of stored pollen was assessed in terms of percent germination. The pollen grains slides were also prepared for light microscopy (LM) using the standard methods of Erdtman (1952). For light microscopy the pollen grains were mounted in unstained glycerine jelly and observations were made with a Nikon type-2 microscope.

Results and Discussion

Pollen germination capacity of *Citrullus lanatus* L., (Cucurbitaceae) was examined up to 48 weeks using different concentrations of sucrose and boric acid solution (10%-100%). Pollen were preserved at different conditions like refrigerator (4ºC) and freezer (-20ºC, -30ºC & -60ºC). The stored pollen samples were taken out after specific intervals and tested for viability. Fresh pollen grains showed only 53% of germination in 20% sucrose boric acid solution at room temperature, while in stored conditions the percentage of germination was high. The percentages of pollen germination were 56.10%, 72.00% and 79.10% at 4ºC, -20ºC and -30ºC, in 4 weeks stored pollen, the germination observed in 30% and 40% sucrose boric acid solutions (Fig. 1). The rate of pollen germination decreased slowly with the passage of time which was quite clear in refrigerator (4ºC). Whereas freeze pollen showed better viability for a long time and after 48 weeks the rate of pollen germination was 26.70% and 34.00% at -20ºC and -30ºC respectively as compared to 11.5% observed in refrigerator at 4ºC (Table 1). The pollen grains soaked in organic solvents also showed good percentage of germination. After 1 hour of soaking period of pollen in acetone, chloroform and benzene showed 41.70%, 51.30% and 39.7% germination in 20%, 30% and 10% solutions respectively while after 6 hours the germination rate decrease which was 30.50%, 41.60% and 21.30% in the same solutions. Among organic solvents benzene seems to have good potential because after 15 hours of soaking period acetone and chloroform showed no germination while in benzene 9.70% germination was observed. Pollen grains dried in desiccator over silica jell showed low germination. After 1 hr drying period pollen showed 26.35% germination and after 12 hours no germination was observed.

Among all the methods freeze drying method seems to be the best method for pollen storage and maintaining viability. After 4 weeks the pollen grains showed 61.00% of germination in 20% solution but after 8 weeks the percentage of germination increased to 80% in 20% solution which is the highest. This condition also showed best viability for a long time and after 48 weeks the percentage of germination was 51.50% in 20% sucrose boric acid solution. It is concluded that both low temperature and humidity, freezer (-20ºC, -30ºC) and freeze dryer (-60ºC) promote pollen germination capacity as compared to fresh, refrigerator, vacuum and organic solvents. This is the first scientific effort to find out the pollen germination capacity of *Citrullus lanatus* L. (Cucurbitaceae) in Pakistan.

Table 1. Germination capacity of *Citrullus lanatus* L. (Cucurbitaceae) at different temperatures in sucrose boric acid solutions.

Weeks	Germination % at 4ºC	% solution	Germination % at -20ºC	% solution	Germination % at -30ºC	% solution	Germination % at -60ºC	% solution
4	56.10	30	72.00	40	79.10	30	61.00	20
8	52.00	30	67.10	30	72.60	30	80.00	20
12	47.30	30	64.39	30	69.00	30	79.10	20
16	43.50	30	61.00	30	65.20	20	76.00	30
20	40.10	30	56.30	30	61.60	20	75.10	20
24	35.40	30	53.70	30	56.70	20	72.50	20
28	31.70	30	50.00	30	53.00	20	70.00	20
32	27.50	30	43.60	30	51.10	20	67.50	20
36	24.00	30	40.00	30	47.00	30	63.00	20
40	19.60	30	35.60	30	42.50	20	59.70	20
44	15.10	30	31.60	30	37.10	20	57.10	20
48	11.50	30	26.70	30	34.00	20	51.50	20

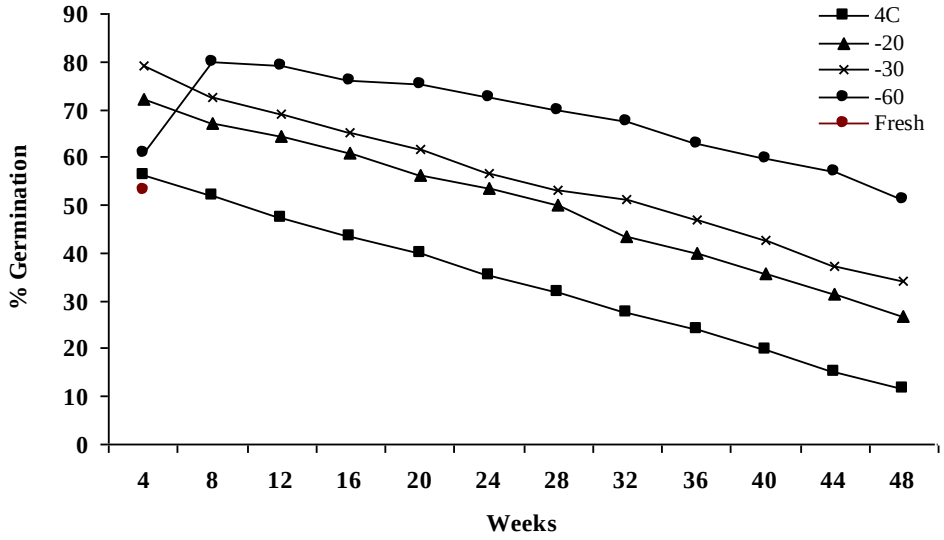

Fig. 1. Graph showing percentages of pollen germination of *Citrullus lanatus* L., (Cucurbitaceae) pollen at 4- 48 weeks.

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