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GERMINATION CAPACITY OF STORED POLLEN OF *MORUS ALBA* (MORACEAE) AND THEIR MAINTENANCE

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Abstract

Present investigations pertain to pollen germination and viability of *Morus alba* L. (Moraceae) for up to 48 weeks. Pollen germination was made by hanging drop technique in different concentration of sucrose and boric acid solutions (10%-100%). In a refrigerator, freezer, in vacuum over silica gel and in organic solvents (Acetone, Benzene, and chloroform). Pollen stored at low temperature (-30°C, -20°C) showed better germination percentage compared to pollen stored at 4°C and fresh. Freeze dried pollen (-60°C) showed the best of germination. The study indicates that 30% and 40% solutions favoured pollen germination. Benzene showed more germination than acetone and chloroform.

Introduction

Recently pollen physiology especially germination and viability, has received considerable attention for its application in plants breeding, conservation, adaptation and understanding of physiological behavior of fertilizing pollen grains. There are several reports on pollen germination and viability from different taxa (Nair & Singh, 1972; Vijay *et al*., 1972; Kapoor, 1976; Zeng-Yu Wang *et al.*, 2004.

Pollen grains of angiosperm can be classified into two groups, binucleate and trinucleate. The latter one lost viability very rapidly and can hardly germinate on artificial media. Pollen has considerable potential to achieve genetic transformation. There are some critical external factors which affect the maintenance of pollen germination capacity eg., relative humidity (RH), and temperature surrounding pollen (King 1961, Gill *et al*., 1992, Malik & Thind. 1992, Shivanna & Ranaswamy, 1992). Pollen grains of different plants require varying range of growth media like water, sugar solution, inorganic salts and vitamins for successful germination. Pollen stored at low temperature presented germination capacity better than high temperature (Stanley & Linskens 1974).

It has been widely acknowledged that temperature and relative humidity of the storage environment are two important factors, which profoundly influence the viability of stored pollen. Pinney & Polito (1990) reported that the germination of Olive pollen improved markedly in storage condition. Thomas (2000) studied pollen germination of 40 plant species on sucrose gelatin on Onion epidermis. According to Aslantus & Pirlak (2002) the germination capacity of strawberry pollen increase at low temperature.

The present study is the first attempt to analyze storage condition and viability test method of *Morus alba* L. No reports are available on maintenance and germination capacity of stored pollen of this economically important plant.

Materials and Methods

Methodology: A polliniferous material was collected from cultivated fields of Khuzdar, Quetta and plants growing in Karachi University campus in large quantity during the peak of flowering period of species. Fresh pollen were systematically subjected to preliminary viability tests (Alexander, 1969). Pollen culture media were prepared using Brewbaker & Kwack (1963) techniques. Pollen grains equal to at least twice the diameter of pollen grains counted as germinated, burst pollen grains were not counted as germinated pollen. The viability of stored pollen was assessed in terms of percentage germination pollen. The stored pollen was germinated in a humidity chamber in different solutions. The germination was determined after 3-6 hrs of incubation. The hanging drop technique was used for culturing pollen grains in liquid media, culture was stored at room temperature.

The pollen grains slides were prepared for light microscope (LM) using Erdtman (1952) procedure. For light microscopy pollen grains were mounted in unstained glycerin jelly and observations were made with a Nikon type-2 microscope. The measurements are based on 15 readings.

Results and Discussions

Germination capacity of stored pollen of *Morus alba* L., has been examined for 48 weeks in different conditions as refrigerator, freezer, vacuum and in organic solvents. Pollen stored in freezer (-30°C, -20°C) showed better germination percentage as compared to pollen stored at 4°C and in organic solvents (Table 1). Fresh and 4°C showed more or less equal germination percentage. At 4°C the germination capacity decreased as compared to germination at freezer where the germination is reasonably high after 24 weeks (Table 1). The freeze-drying condition seems to be the best method where the germination goes on increasing and after 48 weeks it is up to 53.70% (Table 1). Different solutions are required in different conditions.

In organic solvents pollen grains were treated from 1-24 hrs and then stored at +4°C. Benzene showed the best of germination compared to acetone and chloroform in which pollen lost viability after 6-9 hrs. Benzene showed best of germination up to 21 hrs. In vacuum pollen was treated over silica jell for 1-24 hrs and then germinated. This condition showed reasonable germination up to 18 hrs and then lost the viability.

The controlled temperature and humidity conditions were found to be effective in prolonging pollen viability in *Morus alba* L., (Fig. 1) although the extent of prolongation was highly variable between the different storing conditions

The present investigations are the first systematic attempt to compare the efficacy of pollen of *Morus alba* in storage through conventional methods of controlled temperature (4°C, -20°C, -30°C, -60°C) and humidity, in organic solvents. The pollen grains were fairly uniform in their response to the organic solvents tested. Germination percentage was maximum in freeze-dried pollen (Table 1).

Conclusively temperature and humidity are the major influencing factors in the pollen behavior of different conditions.

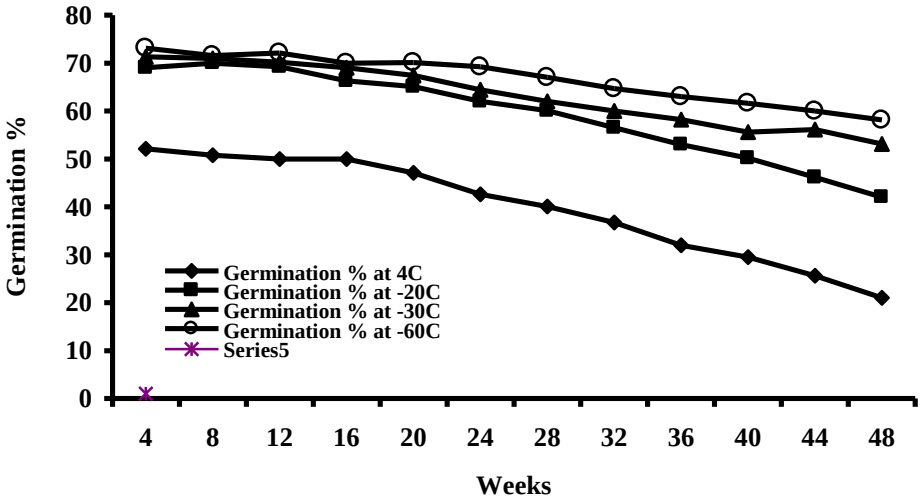

Fig. 1. Germination rate of fresh and stored *Morus alba* pollen over 48 weeks.

Table 1. Germination capacity of stored pollen of *Morus alba* L., (Moraceae) at different temperatures and humidity conditions in sucrose and boric acid solutions.

Weeks	Germination % at 4°C	% Solutions	Germination % at - 20°C	% Solutions	Germination % at -30°C	% Solutions	Germination % at -60°C	% Solutions
1	52.10	20	69.00	30	71.30	30	73.12	40
2	50.80	20	70.00	30	71.00	30	71.56	40
4	50.00	30	69.20	50	70.20	40	72.10	30
8	50.00	20	66.30	60	69.00	30	70.00	30
12	47.10	30	65.10	30	67.40	30	70.10	40
16	42.60	30	62.00	30	64.40	70	69.20	40
20	40.10	40	60.10	30	62.00	30	67.00	50
24	36.70	40	56.50	30	60.00	30	64.70	40
28	32.00	20	53.00	30	58.20	40	63.00	30
32	29.50	30	50.20	40	55.60	50	61.60	30
36	25.60	30	46.15	30	56.10	30	60.00	30
40	21.00	30	42.00	30	53.10	40	58.10	40
44	18.10	30	39.70	30	51.50	40	56.00	40
48	16.00	30	35.40	20	50.00	40	53.70	60

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