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GERMINATION CAPACITY OF STORED POLLEN OF *ABELMOSCHUS ESCULENTUS* L. (MALVACEAE) AND THEIR MAINTENANCE

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Abstract

Pollen germination and viability of *Abelmoschus esculentus* L., (Malvaceae) upto 48 weeks was examined by "Hanging drop Technique" in different concentration of sucrose and boric acid solutions (10-100%). Viability under storage was determined by storing pollen in different conditions, like refrigerator, freezer, in vacuum oven silica gel and in organic solvents (Acetone, Benzene and chloroform). Pollen stored at low temperature (-30°C – 20°C) showed better germination as compared to pollen stored at +4°C and in the fresh. Freeze dried pollen (-60°C) showed the best of germination. Benzene showed more germination than acetone and chloroform.

Introduction

Pollen physiology especially germination and viability has received considerable attention for its application in plant breeding, conservation, adaptation and under standing of physiological behavior of different taxa (Kapoor, 1976; Zeng-Yu Wang *et al*., 2004) with varied aims and objective. Pollen grains of Angiosperm can be classified into two groups, Binucleate and Trinucleate. The later 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 affects the maintenance of pollen germination capacity eg., relative humidity (RH), and temperature surrounding pollen (King, 1961; Gill *et al*., 1992; Malik & Thind, 1992; Shivanna & Rangaswamy, 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.

Pollen storage is the most efficient method to overcome barriers to hybridization between plants flowering at different times and or growing in different regions, as the pollen with this technique will be available whenever the female flowers. Since the beginning of this century extensive studies have been carried out on pollen storage. Various methods have been tried for successful storage of the pollen of different taxa (Vasil, 1964). An attempt has been made in the present paper to determine the optimum condition for pollen storage *Abelmoschus esculentus* by controlling the storage temperature and humidity levels.

The preservation of viable pollen is the subject of various investigators (Piney & Polito, 1990; Poulton *et al*., 2001; Thomas, 2000). There are several records on germination tube growth and viability controlled by different chemicals (Amma & Kulkarni, 1979). The present study is the first systematic attempt to analyze storage condition and viability test method of *Abelmoschus esculenteus* L. No reports are available on maintenance and germination capacity of stored pollen of this economically important plant.

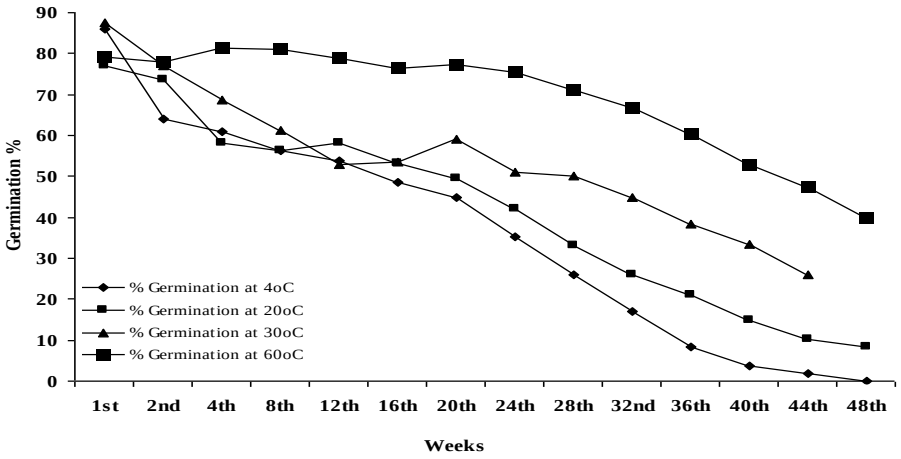

Fig. 1. Germination capacity of stored pollen of *Abelmosclus esculentus* L., (Malvaceae) at different temperature and humidity conditions in sucrose and boric acid solutions.

Material and Methods

Methodology: A polliniferous material was collected from cultivated fields and plants growing in green house in large quantity during the peak of flowering period of species. Fresh pollen was systematically subjected to preliminary viability tests (Alexander, 1996). Pollen culture media was prepared using Brewbaker & Kwack, (1963) techniques. Pollen grains equal to at least twice the diameter of pollen grain, counted as germinated, burst pollen grains were not counted as germinated. The viability of stored pollen was assessed in terms of percentage germination. The stored pollen was germinated in 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 also prepared for light (LM) and scanning (SEM) microscopy using (Erdtman, 1952) procedure. For light microscopy pollen grains were mounted in unstained glycerin jelly and observation were made with a Nikon type-2 microscope. The measurements are based on 15 readings.

Results and Discussion

Germination capacity of stored pollen of *Abelmoschus esculenteus* L., (Malvaceae) has been examined for 48 weeks in different storage conditions such as refrigerator, freezer, vacuum and in organic solvents (Fig. 1). Pollen stored at +4ºC showed reasonable germination in 10% solution upto 20 weeks but later on the germination decreased as solution percent increased (30%). At -20ºC the germination scored 30% solution but after 28 and 32 weeks 40% and 20% solutions showed more germination. Pollen stored at -30ºC showed more germination upto 30 weeks in higher solutions (30%, 60%). Freezer drying seems to be the best method for pollen germination and upto 48 weeks the germination was 40%. In organic solvents pollen grains were treated from 1-24 hours and then stored at +4ºC. Benzene showed best germination compared to acetone and chloroform in which pollen lost viability very rapidly.

Pollen was also treated over silica jel for 1-24 hrs., and then germinated. This condition showed germination in early hours but later on no germination was observed. The controlled temperature and humidity condition were found to be effective in prolonging pollen viability in *Abelmoschus esculentus* L., although the extent of prolongation was highly variable between different storage conditions. Temperature and humidity are the major influencing factors in the pollen behavior of different conditions.

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References

Alexander, M.P. 1996. Different staining of aborted and non aborted pollen. *Stain Technology*, 44: 117-122.

Amma, M.S.P. and A.R. Kulkarni. 1979. Pollen storage in organic solvents. *J. Palynol*., 15: 100-104.

Brewbaker, J.L. and B.H. Kwack. 1963. The essential role of calcium ion in pollen tube growth. *Amer. J. Bot*., 50: 859-865.

Erdtman, G. 1952. Pollen morphology and plant taxonomy of Angiosperms. In: *Introduction to Palynology*, vol. 1. Almquist and Wiksell, Stockholm.

Gill, M.S.N. and C.P. Malik. 1992. Pollen biotechnology storage and viability. In: *Pollen Physiology and Biotechnology*. (Ed.): C.P. Malik. Today and tomorrow's Printer and Publisher, New Delhi, India.

Kapoor, S.K. 1976. Pollen germination in some Cucurbits. *J. Palyn*., 12(1&2): 87-93.

King, J.R. 1961. The freeze drying of pollen. *Economic Botany*, 15: 91-98.

Malik, C.P. and S.K. Thind. 1992. Pollen biotechnology and fertilization engineering in crop improvement. In: *Pollen physiology and biotechnology*. (Ed.): C.P. Malik. New Delhi, India.

Pinney, K. and V.S. Polito. 1990. Olive pollen storage and *in vitro* germination. In: *International Symposium on Olive growing*. (Eds.): L. Rallo, J.M. Caballero and R.S. *Rscaba. ISHS Acta Horticulture*, 286. Vol. 1.

Poulton, J.L., T.K. Roger and G.S. Andrew. 2001. Effects of mycorrhizal infections and phosphorus availability on *In vitro* and *In vivo* pollen performance in *Lycopersicon esculentum* L. (Solamaceae).

Shivanna, K.R. and N.S. Rangaswamy. 1992. Pollen biology. A laboratory manual. New Delhi, India.

Stanley, R.G. and H.F. Linskens. 1974. Pollen biology, biochemistry and management. Springer, Verlag Berlin, Heidelberg, New York.

Thomas, C.J.R. 2000. Studies on pollen germination of 40 plants species on sucrose gelatin and on onion epidermis, *Quekett Journal of Microscope*, 38: 463-472.

Vasil, I.K. 1964. Effect of boron on pollen germination and pollen tube growth. In: *Pollen physiology and fertilization*. (Ed.): H.F. Linskens. pp. 107-119. (North Holland Amsterdam).

Zeng-Yu Wang, Yaxin Ge, Megam Scott and German Spangenberg. 2004. Viability and longevity of pollen from transgenic and non-transgenic tall fescue (*Festuca arundinaceae*) (Poaceae) plants. *Am. J. Bot*., 91: 523-530.

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