Middle-East Journal of Scientific Research 12 (4): 560-562, 2012
ISSN 1990-9233

DOI: 10.5829/idosi.mejsr.2012.12.4.1709

Evaluation of FDA Staining Technique in Stored Maize Pollen

S.A. Kalkar and K. Neha

Department of Botany, Institute of Science, Nagpur, India

Abstract: Pollen viability of *Zea mays* L. varieties was examined by staining technique like flurochromatic diacetate. Viability under storage was determined by storing pollen in different conditions viz., room temperature, refrigeration, -20°C and -80°C. Pollen stored at cryopreservation (-20° and -80°C) showed good viability percentage as compared to pollen stored at room temperature and at +4°C. The freeze dried pollen (-80°C) showed the highest percentage of viability retained up to 20 days. Results also explained that esterase enzyme is quite active at cryopreservation than at room temperature and +4°C.

Key words: Pollen viability · *Zea mays* L. · FDA

INTRODUCTION

Pollen storage is the most efficient method to overcome barriers to hybridization between plants flowering at different time and or growing in different regions, as the pollen with this technique will be available whenever the female flower. Since the beginning of this century extensive studies have been carried out on pollen storage [1]. Various methods have been tried for successful storage of pollen of different taxa [2]. Pollen stored at low temperature presented germination capacity better than high temperature [3].

Maize forms part of the staple diet in many developing countries. It accounts for over 50% of calories provided by starchy cereals [4-6]. Maize is an anemophilous (wind pollinated) monoecious species [7-8]. Pollen viability is enhanced by cool temperature and high relative humidity (RH) levels [8-10].

In a study conducted by it was found that pollen longevity, under environmental conditions of 28 to 30°C and RH of >53% was between one to two hrs. However, the study was not able to conclusively determine the effect of temperature and relative humidity on pollen longevity [11]. The preservation of viable pollen is the subject of various investigators [12-14]. There are several records on germination tube growth and viability controlled by different chemicals [15]. For determining viability of stored pollen several tests have been applied, such as fruit and seed set, pollen tube growth in pistil, busting of pollen grains, germination on artificial nutrient media, staininability by tetrazolium salt, estimation of ATP content and peroxidase reaction etc. [16]. Enzyme stain-ability of the stored pollen by some specific redox dyes may also give some clues to the viability index. Pollen viability was tested in wheat stored pollen by Benzidine hydrogen peroxide test which proved to be a reliable test of viability and good predictor of seed set [17-18]. Viability of liquid air stored pollen by tetrazolium bromide was also measured [19].

In this study, an attempt was made to find out the percentage of viability of stored pollen of different varieties of maize with the help of flurochromatic diacetate (FDA).

MATERIALS AND METHODS

The three entries of maize *viz.,* MA-701, MA-702 and MA-703 were selected. A polliniferous material was collected from cultivated field in large quantity during the peak of flowering period of species. Fresh pollen was systematically subjected to preliminary viability test [20]. The FDA test assesses two properties of a pollen grain viz., the integrity of the plasma membrane of the pollen and the activity of esterase capable of hydrolyzing the fluorescein ester.

The fresh pollen grains were collected and viability was tested. The fresh pollen grains were also immediately kept in plastic vials, sealed with parafilm and were kept in Zip lock bag and then transferred at different temperatures, i.e. room temperature, 4°C, -20°C and -80°C. The viability of stored pollen grains was tested by fluorescein diacetate solution *in vitro*.

Corresponding Author: S.A. Kalkar, Department of Botany, Institute of Science, Nagpur, India.

Fluorescein Diacetate Solution: Stock solution of FDA is prepared in acetone (2 mg/ml). It can be stored in the refrigerator for months. Sucrose solution of 60% concentration is used which prevent bursting of pollen grains.

To 2-5 ml of sucrose solution in a small glass tube drops of stock solution of FDA were added until the resulting mixture shows persistent turbidity, this mixture was used within 30 minutes from preparation; otherwise most of the FDA would have been precipitated.

RESULTS AND DISCUSSION

The viability capacity of stored pollen of three entries of maize has been examined for 20 days in different storage conditions such as room temperature, 4°C, -20°C and -80°C. In contrast, pollen stored at room temperature for 2 days have shown viability decreases upto 1.22% and pollen stored at 4°C lost most of its viability after 4 days from 68.24 to 2.11%.

The -20°C temperature retain viability for 12 days which ranged from 89.41 to 3.75%. Freezer drying seems to be the best method for pollen retaining viability for 20 days (Table 1). These results explained that esterase enzyme is quite active at cryopreservation temperature and the integrity of plasmamembrane of pollen grains was more than pollen stored at room temperature and +4°C.

Temperature and other factors like humidity; organic solvents are the major factors influencing on the maintenance of pollen viability[21]. Pollen grains stored under cryopreservation (-20°C) had prolonged viability in *Terminalia paniculata* [22]. Pollen viability tests were also examined for *Eucalyptus marginata*, using fluorescein diacetate[21] whereas Alexander's staining method for testing viability of stored pollen of *Terminalia paniculata*[22].

Studies of pollen viability, fertility and storage are important for breeding programs. Thus the present work was the effort to evaluate FDA staining technique in order to check the percentage viability. It was found that FDA technique would prove more effective for testing viability of stored pollen. It seems that this method is less time consuming as compared to other viability tests.

Table 1: Percentage viability in Flurochromatic diacetate of storage pollen of maize

		Different temperature conditions			
Name of varieties	Date	Room temperature	-4°C	-20°C	-80°C
MA-1	15/12/07	57.37	68.24	87.36	89.41
MA-2		44.52	53.62	87.92	88.05
MA-3		40.10	40.30	70.33	71.34
MA-1	17/12/07	4.12	54.94	73.78	86.53
MA-2		2.77	42.84	71.11	82.77
MA-3		1.22	32.15	57.43	66.07
MA-1	19/12/07	-	5.34	66.70	81.79
MA-2		-	4.67	65.67	77.98
MA-3		-	2.11	51.23	62.78
MA-1	21/12/07	-	-	59.19	74.12
MA-2		-	-	52.33	71.88
MA-3		-	-	35.20	58.23
MA-1	25/12/07	-	-	28.89	65.11
MA-2		-	-	24.54	64.74
MA-3		-	-	15.44	52.33
MA-1	28/1/08	-	-	6.11	60.44
MA-2		-	-	5.87	53.15
MA-3		-	-	2.89	36.79
MA-1	1/1/08	-	-	-	30.54
MA-2		-	-	-	25.98
MA-3		-	-	-	17.34
MA-1	3/1/08	-	-	-	6.88
MA-2		-	-	-	6.47
MA-3		-	-	-	3.75

REFERENCES

1. Khan, S.A. and A. Perveen, 2006. Germination capacity of storde pollen of *Abelmoschus esculentus* L (Malvaceae) and their maintenance. Pak. J. Bot., 38(2): 233-236.
2. Vasil, K., 1960. Studies on pollen gemination of certain Cucurvitaceae. Amer. J. Bot., 47: 239-247.
3. Stanley, R.G. and H.F. Linskens, 1974. Pollen Biology, Biochemistry and Management. Springerverleg. New York.
4. Marediavet, M.K., D. Byerlee and P. Pee, 2000. Impact of food crop improvement research: evidence from sub-saharan Africa. Food Policy, 25: 531-559.
5. Pingaliv, P.L., 2001. CIMMYT 1999-2000. World maize facts and trends meeting world maize needs: Technological opportunities and priorities for the public sector. Mexico, D.F.: CIMMYT.
6. Rohrbach, D.D., I.J. Minde and J. Howard, 2003. Looking beyond national boundaries: regional harmonization of seed policies, laws and regulations. Food Policy, 28: 317-333.
7. Emberlin, J., B. Adams-Groom and J. Tidmarsh, 1999. A report on the dispersal of maize pollen. National Pollen Research Unit, University College, Worcester.
8. Glover, J., 2002. Gene flow study: Implications for the release of genetically modified crops in Australia. Bureao of Rural Sciences, Canberra, pp: 1-81.
9. Schoper, J.B., R.J. Lambert and B.L. Vasilas, 1987. Pollen viability, pollen shedding and combining ability for tassel heat tolerance in maize. Crop Sci., 27: 27-31.
10. Eastham and J. Sweet, 2002. Genetically modified organisms (GMO'S): The significance of gene flow through pollen transfer. Environmental Issue Report, pp: 38-42.
11. Luna, S.Y., J.M. Figueroa, B.M. Baltazar, R.L. Gomez, R. Townsend and J.B. Schoper, 2001. Maize pollen longevity and distance isolation requirements for effective pollen control. Crop Sci., 41: 1551-1557.
12. 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 Hort., 1: 286.
13. 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. (Solanaceae).
14. Thomas, C.J.R., 2000. Studies on pollen germination of 40 plants species on sucrose gelatin and on onion epidermis. Quekett J. Microscope, 38: 463-472.
15. Amma, M.S.P. and A.R. Kulkarni, 1979. Pollen storage in organic solvents J. Palynol., 15: 100-104.
16. Shivana, K.R. and B.M. Johri, 1985. The Angiosperm Pollen: Structure and Function. Wiley Eastern Ltd., New York.
17. King, J.R., 1961. The freeze-drying of pollen. Econ. Bot., 15: 91-98.
18. Khan, M.N., E.G. Hayne and J.A. Goss, 1971. Effect of relative humidity on viability and longevity of wheat pollen. Crop Sci., 11: 125-126.
19. Collins, F.C., V. Lertmongkol and J.P. Jones, 1973. Pollen storage certain agronomic species in liquid nitrogen Crop Sci., 13: 493-494.
20. Heslop-Harrison, J. and Y. Heslop-Harrison, 1970. Evaluation of pollen viability by enzymatically induced fluorescence; intracellular hydrolysis of fluorescein diacetate. Srain Technol., 45: 115-120.
21. Visser, T., 1955. Germination and storage of pollen. Meded. Landb. Hoogesch, 55: 1-68.
22. Thangaraja, A. and V. Ganesan, 2008. Studies on the pollen biology of *Terminalia paniculata* Roth. (Combretaceae). Afr. J. Plant Sci., 2(12): 140-146.