Study of Pollen Viability in Various Protogynous Genotypes of Indian Mustard for Hybrid Seed Production

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Abstract—*Pollen viability is significant for multiplication of a genotype and hybrid seed production. It is an indicator of fecundity and fertilization efficiency of the male gametes. The present investigation was aimed at evaluating the pollen viability of F_4 and F_5 population of four cross combinations of protogynous (Pg) mustard lines at divisional research field of Seed Science and Technology, Indian Agricultural Research Institute, New Delhi during rabi 2011-12 and 2012-13. Staining pollen with Accetocarmine was done to study the pollen grain viability from day of stigma exertion to the 8th day after stigma exsertion. Observations were recorded under microscope with 40x magnification. Differences in pollen viability were observed during different days after stigma exsertion. Maximum viability was observed in ($PGxGP_{60}$) x Pusa Bold on 3rd day (89.29%) and minimum was observed in ($PGxGP_{60}$) x Pusa Mahak (0.42%) on 8th day after stigma exsertion during rabi 2011-12 and also maximum pollen load as well as pollen viability was observed in ($PGxGP_{60}$) x Pusa Bold and minimum was observed in ($PGxGP_{60}$) x NPJ_{105} during rabi 2012-13. It can be concluded that protogynous plants/ lines can be used as pollen source using its pollen during 3rd and 4th day after stigma exsertion for hybridization purpose.*

Keywords*: Pollen grain viability, Protogyny, Stigma exertion, Mustard.*

1. INTRODUCTION

Mustard (*Brassica* spp.) is a major oil seed crop of India. It belongs to the family Brassicaceae. It is third most important source of vegetable oil in the world after soybean and palm oil, out of six cultivated species of *brassica* more than 80% of total area is occupied by Indian mustard (*B. juncea*) (Anonymous,2011). Pollen grain physiology, especially viability has received considerable attention for its application in hybrid seed production. Understanding of physiological behavior of fertilizing pollen grains is a very important researchable area. There are several reports on pollen grain viability from different plants (Khan and Perveen, 2006). Test of pollen viability may be done with direct or indirect methods. The direct method includes germination induction *in vitro* and indirect method is based on the reaction of stains and fluro chromes (Kearns and Inouye, 1993). *In vitro* germination makes use of culture media to determine the capacity of pollen grains to develop pollen tubes (Stanely and Linskens, 1974). Different staining methods were used to evaluate the integrity of pollen grains with regard to their cellular components (Munhoz *et al.*, 2008). Stains such as Lugol are associated with starch detection (Ge *et al.*, 2011), where as Alexander's solution reflects the integrity of structures such as nucleus and plasma membrane (Alexander, 1980). Two important dyes orcein and Accetocarmine are used to test the pollen viability which results in to the cytoplasmic coloration in pollen grains, which are then classified as normal (viable) with colored cytoplasm and abnormal/ non-viable, when showing little or no cytoplasmic coloration (Vargas *et al*., 2009).

Information on pollen viability at different developmental stages in Pg mustard is scarce. In this context, the present study was conducted to evaluate the pollen viability of F_4 and F_5 population of protogynous mustard crosses.

2. MATERIAL AND METHODS

The present experiment was conducted at experimental field of Division of Seed Science and Technology, I.A.R.I., New Delhi during *rabi* 2011-12 and *Rabi* 2012-13. Seeds of protogynous (Pg) lines and genotypes of *Brassica* were sown and observations were recorded in 10 randomly selected plants from Pg population of F_4 and F_5 generation of 4 genotypes ($PGxGP_{60}$) x Pusa Mahak, ($PGxGP_{60}$) x Pusa Bold, ($PGxGP_{60}$) x Pusa Tarak and ($PGxGP_{60}$) x NPJ 105. Data were recorded from the buds of day 0 (day of stigma exsertion) to day 8 (8th day after stigma exsertion). These were collected and used for pollination and study of pollen viability. Pollens were obtained by smearing the anthers on a glass slide and stained with Accetocarmine solution(Dafni *et al.* 2005). Experiment was conducted in three replications and pollen viability was assessed under a compound microscope (40 x magnifications). Pollen viability test was performed on five random microscopic fields. Partially stained or unstained pollen grains were counted as non-viable and brightly stained as fertile statistical analysis was carried out by following usual method (Panse and Sukhatme, 1957)

3. RESULT AND DISCUSSION

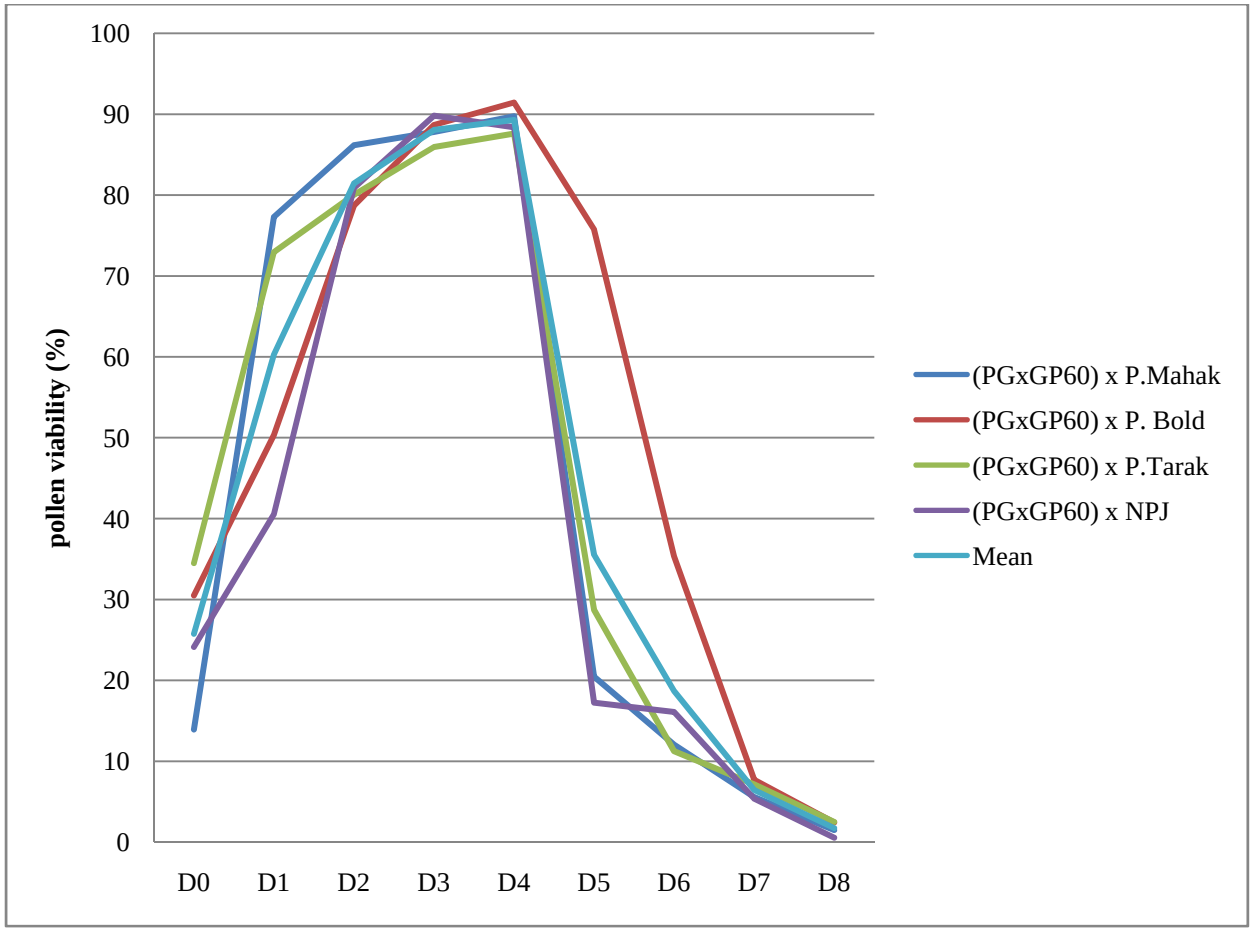
Pollen viability differed among flower/ bud from day of stigma exertion to 8 days after its exertion. Maximum pollen

viability was observed on 3rd day after stigma exsertion in (PGxGP60)x Pusa Bold (89.29%) and minimum in (PGxGP60)x Pusa Tarak (87.01%). Mean pollen viability (87.98%) of all genotypes was maximum on day 3 after stigma exsertion and minimum (1.91%) on day 8 after stigma exertion during *rabi* 2011-12 (Table 1).

Table 1: Pollen viability (%) in different genotypes after stigma exsertion during *rabi* 2011-12

Days	(PGxGP 60) x P.Mahak	(PGxGP 60) x P. Bold	(PGxGP 60) x P.Tarak	(PGxGP 60) x NPJ 105	Mean
D0	11.96	25.61	30.84	38.60	26.75
D1	74.84	46.59	43.11	34.98	49.88
D2	45.33	34.43	35.37	35.47	37.65
D3	87.49	89.29	87.01	88.14	87.98
D4	85.85	88.53	88.21	86.01	87.15
D5	17.55	69.93	34.25	27.29	37.25
D6	26.63	35.87	18.41	11.88	23.20
D7	3.45	9.19	8.60	9.73	7.74
D8	0.42	3.42	2.69	1.13	1.91
CD(0.05)	Days	9.33			
	Genotypes	6.22			
	Days vs. genotypes	16.16			

D0, D1, D2……D8: 0, 1, 2,…..8 Days after stigma exsertion



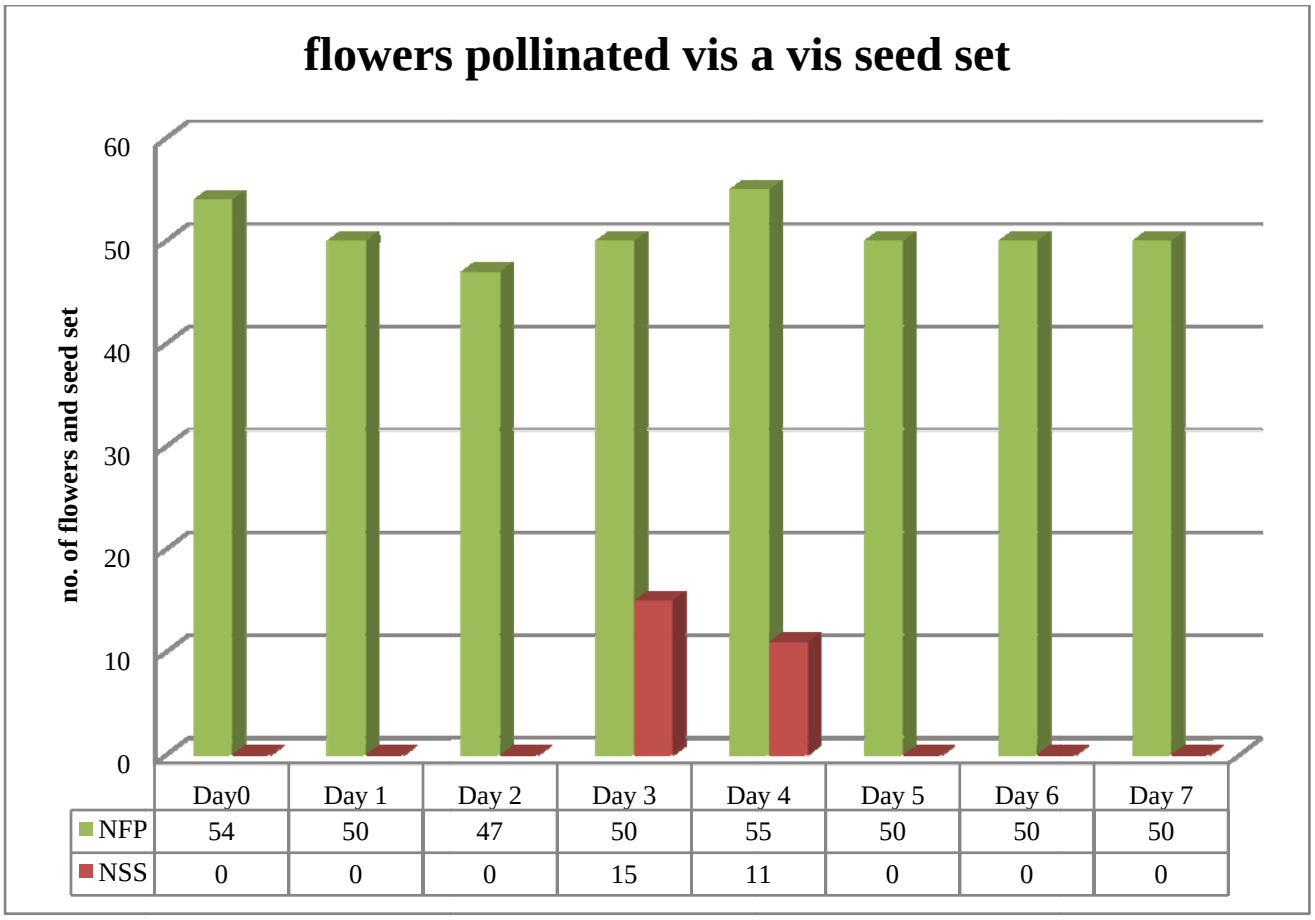
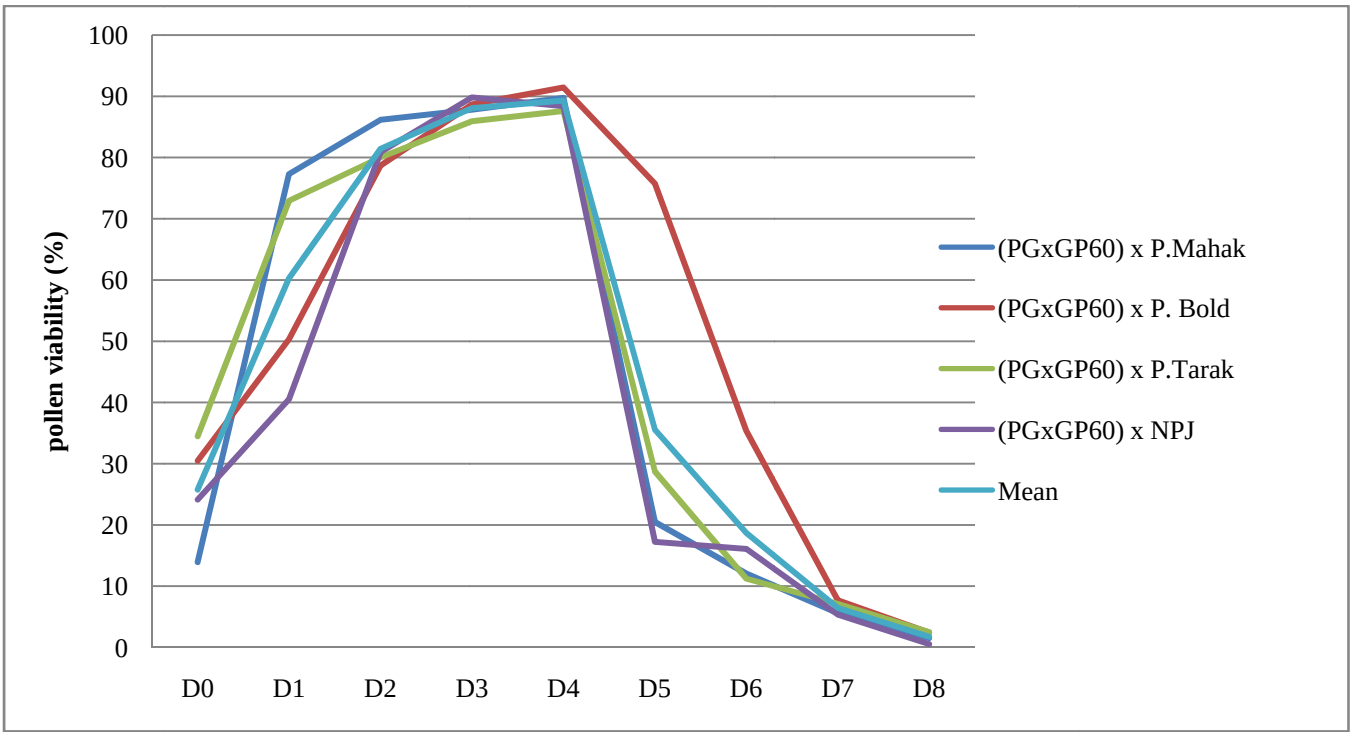


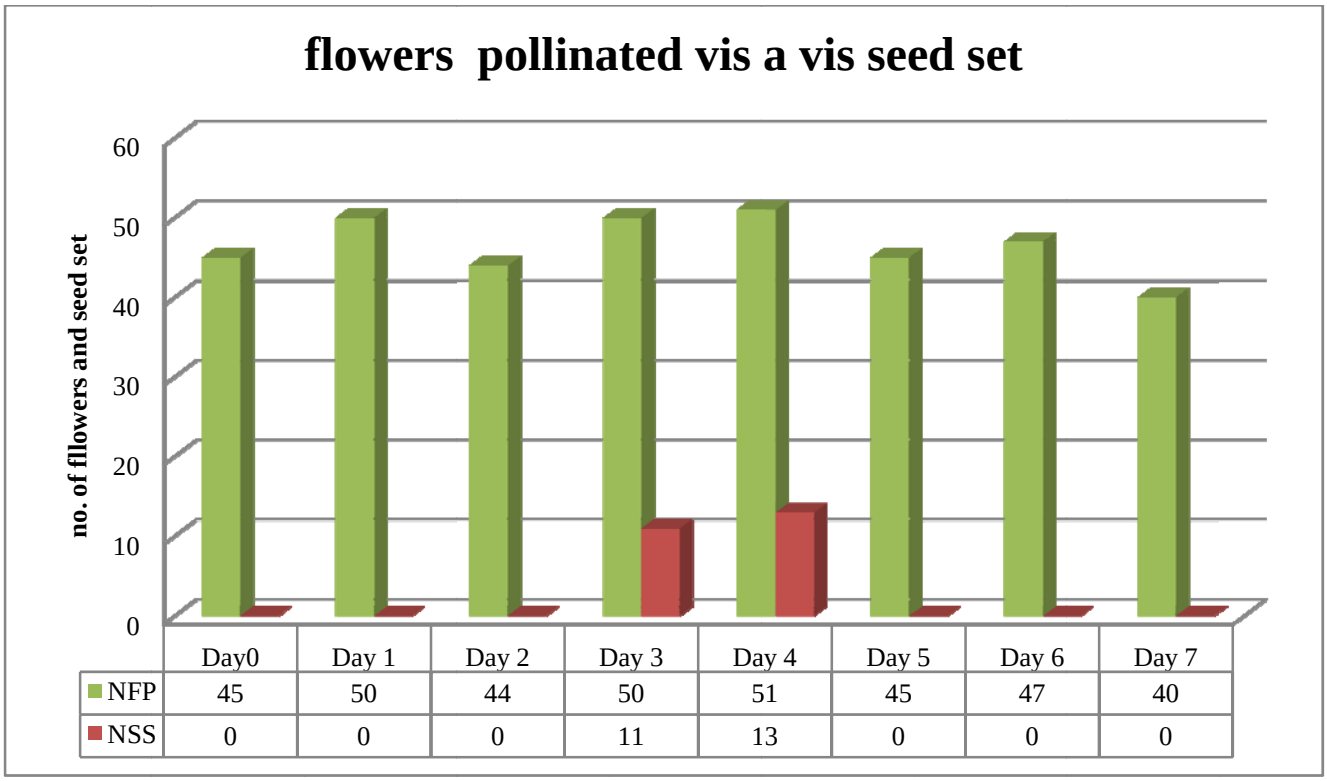
Fig. 1: Comparison of pollen viability in different genotypes of Indian mustard at different stages of development (day 0 to day 8 after stigma exsertion) during *rabi* 2011-12

Genotypes showing viability over 70%, are considered high according to the Souza *et al.* (2002). On day 3 and 4 most of the genotypes showed maximum viability ranging from 86.01 ($PGxGP_{60}$) x NPJ 105 to 89.29% ($PGxGP_{60}$) x Pusa Bold. Pollen viability from day 0 to day 8 after stigma exsertion ranged from 0.42% {day 8 in ($PGxGP_{60}$) x Pusa Mahak} to 89.29% {day 3 in (PGx GP_{60}) x Pusa Bold}. Seed setting takes place on those inflorescences where pollens of day 3 and day 4 buds after sigma exsertion were used for pollination.

Table 2: Pollen viability (%) in different genotypes after stigma exertion during rabi 2012-13

Days	**(PGxGP60) x P.Mahak**	**(PGxGP60) x P. Bold**	**(PGxGP60) x P.Tarak**	**(PGxGP60) x NPJ**	**Mean**
D0	13.92	30.49	34.49	24.13	25.76
D1	77.3	50.35	72.96	40.54	60.29
D2	86.15	78.7	80.03	80.88	81.44
D3	87.81	88.69	85.95	89.82	88.07
D4	89.74	91.46	87.61	88.37	89.30
D5	20.46	75.76	28.71	17.23	35.54
D6	12.06	35.37	11.24	16.08	18.69
D7	5.57	7.71	7.13	5.36	6.44
D8	1.5	2.42	2.5	0.54	1.74
CD(0.05)	Days	1.55			
	Genotypes	2.22			
	Days vs. genotypes	3.80			





	Day0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
NFP	45	50	44	50	51	45	47	40
NSS	0	0	0	11	13	0	0	0

NFP=No. of flowers pollinated, NSS= No. of seed set

Fig. 2 comparison of pollen viability in different genotypes of Indian mustard at different stages of development (day 0 to day 8 after stigma exsertion) during *Rabi* 2012-13

Based on the data on pollen viability in tables and figures, it can be recommended that best results can be obtained by using the buds for pollination of day 3 and 4 after stigma exsertion and it can give the good seed setting in pollinated flowers.

REFERENCES

[1] Alexander M.P. 1980. A Versatile Stain for Pollen, Fungi, Yeast and Bacteria. *Stain technology*. **53**:13-18.

[2] Chakrabarty S.K., Chandrashekar U.S., Prasad M., Yadav J.B. (2011). Protogyny and self incompatibility in Indian mustard (*Brassica juncea* (L.) Czern and Coss): A new tool for hybrid development. Indian J. of genet. and plant breed. 71(2): 170-173.

[3] Coser, S.M., Fontes, M.M.P and Ferreria, M.F.S. 2012. Assessment of Pollen viability in Guava genotypes. *Acta horticulture* No. 959.

[4] Dafni A., Kevan PG, Husband B.C. (2005). Practical pollination biology. Enviroquest, Canada, p. 590

[5] Deniz Karakaya 2011. Effects of Inflorescence on Pollen viability and Morphology of Strawberry (*Fragaria vesca* L.). *Journal of Science and Technology* **1:** 43-47

[6] Nepi, M. and Pacini, E. 1993. Pollination, pollen viability and pistil receptivity in Cucurbita pepo. Ann. Bot. **72**: 527-536

[7] Rodriguez- Riano, T. and Dafni, A. 2000. A new procedure to asses pollen viability. Sex. Plant reprod. **12**: 241-244.

[8] Souza, M.M., Pereria, T.N.S. & Martins, E.P. 2002. Microsporogenese e micro gametogenese associadas ao tamanho do botao floral e da anterae viabilidade polinica em maracuja zeiro- amarelo (passiflora edulis sims f. flavicarpa degener). Ciencia agrotecnica **26**: 1209-1217.

[9] Stainley R.G., Linskens, H.F. 1974. Pollen biology biochemistry management. Springer verlag, Berlin p. 307