Indian J. Pl. Genet. Resources 11(2): 229-236, 1998

MIDSTORAGE TREATMENTS FOR IMPROVING VIABILITY OF MUSTARD SEEDS

Kalyani Srinivasan, Sanjeev Saxena and B. B. Singh

Germplasm Conservation Division,
National Bureau of Plant Genetic Resources,
Pusa Campus, New Delhi 110 012

The effects of Osmo and hydropriming on partially aged seeds of mustard variety *Varuna* were studied. Seeds were osmoconditioned in PEG solution of strength-0.75 MPa and hydroprimed by (a) immersing in water for 3 hours; (b) allowing slow absorption of water from moist muslin for 36 hours (c) equilibrating the seeds in water saturated atmosphere for 48 hrs at 25°C. The treated seeds were then equilibrated at 20°C and 45% RH for 48 hours to 6% moisture and packed in aluminium foil packets. Primed seeds were assessed immediately as well as after 10 months of storage at ambient conditions for their germination, mean germination time, leachate parameters, protein content and activities of amylase, acid phosphatase, peroxidase and catalase. Osmoconditioning and slow hydration treatments resulted in significant improvement in germination and vigour parameters in contrast to direct soaking of seeds in water. These observations are supported by less leakiness of electrolytes and UV absorbing material and increased activity of free radical scavenging and reserve mobilizing enzymes.

Key words : Priming, viability, storability, mustard

Genebanks often encounter seeds with low viability at times due to improper storage conditions prior to their arrival in the genebank. It is important for genebanks to work out treatments so that the viability of these seeds is improved for storage and for the seeds regenerated or multiplied. Mid-storage treatments by priming the seeds has been used in some crops for seed lots that have lost vigour due to improper storage conditions (Lush *et al.* 1981, Pan and Basu 1985). In these treatments the seeds are hydrated to activate pre-germination metabolism and then dried again. The treated seeds thus reach the same state of readiness and there is shortening of time taken for radicle protrusion by the slow or fast seeds in a population, leading to synchronised germination. However, there are few contradictory reports wherein a reduction in storability has been observed (Argerich *et al.* 1989; Nath *et al.* 1991).

The present experiment was conducted to study the ameliorating effect of various priming treatments on seeds of mustard variety Varuna.

MATERIAL AND METHODS

One year old seeds of mustard variety Varuna were procured from the National Seeds Corporation, Pusa Campus, New Delhi. The seeds were subjected to the following priming treatments :

a) OP : Seeds were osmoprimed by spreading in a Petri dish lined with Whatman No. 1 filter paper soaked in PEG 6000 solution of -0.75 MPa osmotic potential and incubated at 20°C for 72 hours (Michel and Kaufmann 1973). After conditioning, the seeds were thoroughly rinsed in distilled water to remove any trace of osmoticum and wiped free of water.

b) HP : Seeds were hydroprimed by soaking in water at 25°C for 3 hours.

c) MM : Seeds were placed between two moist muslins and wrapped within a thin polythene at 20°C for 36 hours.

d) WS : Seeds were kept in a water saturated desiccator at 25°C for 48 hours.

The seeds from all the four treatments were then equilibrated at 20°C and 45% RH for 48 hours to 6% moisture content. One set of treated seeds from each treatment was tested immediately (PIE). Another set was sealed in tri-layered moisture proof aluminium foil pouches and stored at ambient conditions for 10 months and then tested to study the effect of priming on natural ageing (PNA). A set of untreated seeds served as control (C). Germination test was performed using 50 seeds incubated at 20°C. Germination count was taken every day considering the protrusion of the radicle as indication and is reported as the germination percentage (ISTA, 1985). Mean germination time (MGT) was calculated in days using the formula of Ellis and Roberts 1981. Root and shoot vigour were evaluated on the 7th day of planting the seeds. Twenty five seeds were weighed and soaked in 25 ml of deionised water at 20°C for 24 hours and electrical conductance (dS m^{-1}) was determined with a digital conductivity meter (Control Dynamics model APX 185E). The leachate was also used to determine the UV absorbance at 264 nm with spectrophotometer (DU 7400 Beckman). The hydrated seeds were used for estimation of soluble protein and enzyme analysis. Soluble proteins in the seed were extracted in 0.05M phosphate buffer pH 7 and estimated by Lowry's method (Lowry *et. al.* 1951). Seed extract prepared in Tris - HC1 buffer (0.05M, pH 7.6) was used for the assay of amylase (Bernfeld 1955) and peroxidase (Shannon *et. al.* 1966). Extract in 0.05M phosphate bufer pH 7 was used for the assay of acid phosphatase (Leigh and Walker 1980) and catalase (Maehly and Chance 1967).

Data were analysed as a factorial design with a minimum of three replicates in all cases. All data were subjected to an analysis of variance and tested for significance.

RESULTS AND DISCUSSION

Effect of priming on germination, vigour and leachate parameters are presented in Table 1. The germination percentage was increased by MM and OP when seeds were tested immediately after priming and by all the treatments after natural ageing. There was a decrease in the mean germination time immediately after treatment. Maximum decrease was by OP and MM followed by WS and HP. There was no significant decrease by treatment HP when the seeds were tested after natural ageing. The root vigour was increased immediately after priming by OP, WS and HP. When seeds were tested after natural ageing all the treatments increased the root vigour, maximum by OP followed by WS, HP and MM. The shoot vigour was also increased by all the priming treatments in both the cases. The electrical conductance of the leachate was significantly decreased by treatments OP and MM immediately after treatment. After natural ageing treatment WS also significantly decreased the conductance. The UV absorbance of the leachate followed the same trend as the conductance. The efflux of potassium ions into the leachate was significantly decreased by all the treatments after ageing. The natural ageing of seeds resulted in the decrease of soluble protein content. Primed seeds had higher protein content immediately after priming and also after natural ageing. Amylase activity decreased during natural ageing. Treatments OP and MM increased the amylase activity immediately and also treatment WS after natural ageing (Fig. 1a). Similar trend was observed for phosphatase activity with exception of HP which also increased the activity after natural ageing (Fig. 1b).

Peroxidase activity also decreased due to natural ageing, treatment OP increased the activity immediately after priming (Fig. 1c). Seeds subjected to natural ageing retained higher peroxidase activity in all the treatments. Primed seeds showed higher activity of catalase before and after ageing. Catalase activity decreased due to natural ageing (Fig. 1d).

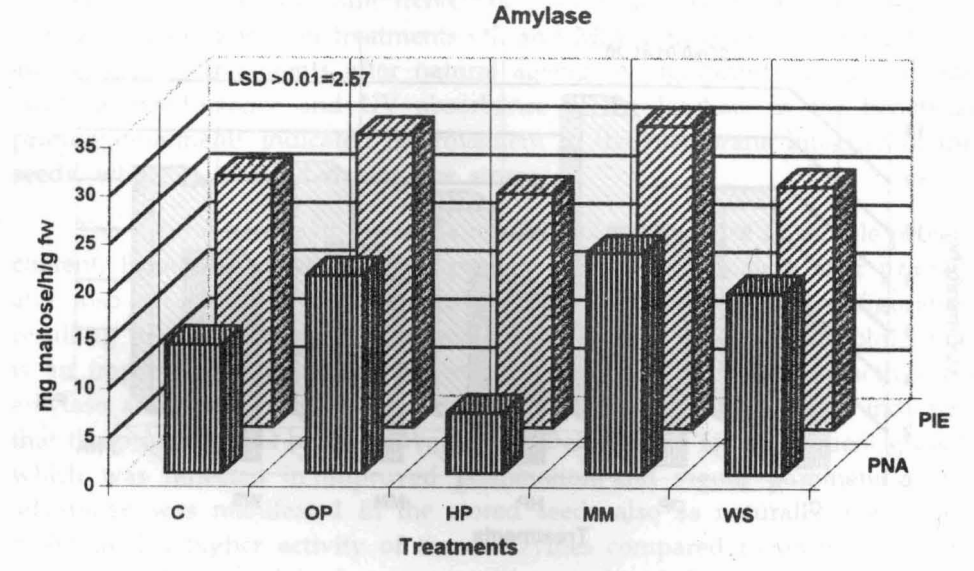
The seed quality had evidently deteriorated in control and treated seeds during the 10-month storage period as indicated by the decline in the germination percentage, and an increase in the MGT of aged seeds. The seeds primed by OP, MM and to some extent WS showed an improved quality immediately after treatment thus indicating the improvement in the storage life of the primed seeds. These priming treatments also increased the root and shoot vigour. Only the HP treatment was detrimental to seed quality probably due to the imbibitional injury occurring as a result of rapid uptake of water by the seeds (Ellis *et. al.* 1985) as the seeds in this treatment were directly soaked in water for hydration.

The electrical conductance of the leachate was significantly lower in the treated seeds after natural ageing. The maximum decrease was observed due

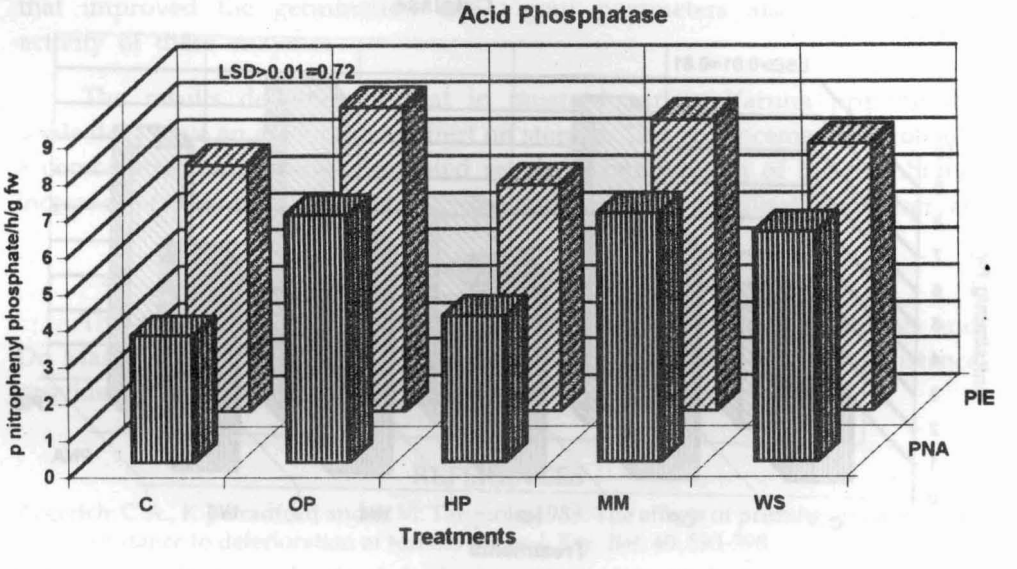
Table 1. Effect of priming on germination, vigour and leachate parameters immediately after priming (PIE) and after natural ageing (PNA) in mustard variety Varuna

Treatments	Germination		Vigour		Leachate			Soluble protein (mg 250 mg fw^{-1})
	Percentage	Mean Time (days)	Root (cm)	Shoot (cm)	UV absorbance (at 264 nm)	Conductance (dS m^{-1})	Potassium	
PIE								
C	79	2.40	3.24	3.14	0.062	52.3	11.0	4.26
OP	91	1.11	6.24	4.42	0.053	42.0	10.3	5.15
HP	80	1.50	3.93	4.28	0.066	55.2	12.3	4.28
MM	92	1.17	3.52	6.91	0.056	45.4	11.3	5.31
WS	84	1.54	4.33	5.69	0.060	48.6	10.6	4.56
PNA								
C	60	2.52	2.41	1.92	0.126	114.6	20.6	2.74
OP	85	1.23	4.69	2.98	0.076	63.6	14.3	3.75
HP	72	2.53	2.96	3.71	0.127	118.0	17.0	3.13
MM	86	1.32	2.84	6.05	0.074	66.0	15.3	3.74
WS	71	2.19	3.93	4.71	0.083	87.4	14.0	3.14
LSD>0.05%	6.3	0.29	0.42	0.84	0.004	4.30	2.56	0.28

(C-untreated control; OP-Osmo priming at -0.75 Mpa; HP- Hydropriming by soaking in water 3 hours; MM-Equilibration in moist muslin for 36 hours; WS-Equilibration in water saturated desicator for 48 hours)

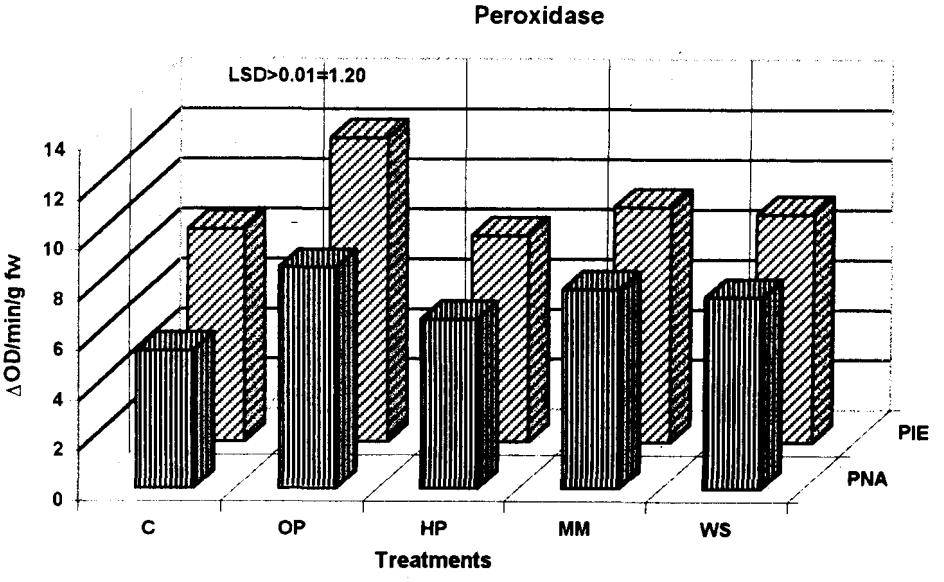


(a)

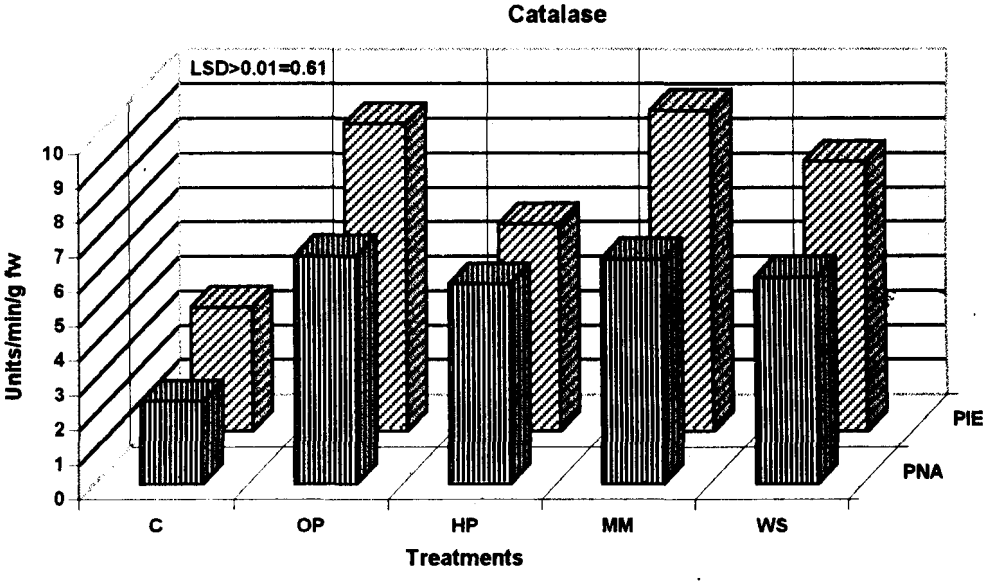


(b)

Fig. 1a&b. The effect of priming treatments on the activity of amylase, phosphatase, peroxidase and catalase in mustard variety Varuna. (C - untreated control; OP - Osmo priming at -0.75Mpa; HP- Hydropriming by soaking in water for 3 hours; MM - Equilibrationin moist muslin for 36 hours; WS - Equilibration in water saturated desiccator for 48 hours). The seeds were tested immediately after priming (PIE) and after natural ageing (PNA)



(c)



(d)

Fig. 1a&b. The effect of priming treatments on the activity of amylase, phosphatase, peroxidase and catalase in mustard variety Varuna. (C - untreated control; OP - Osmo priming at -0.75Mpa; HP- Hydropriming by soaking in water for 3 hours; MM - Equilibrationin moist muslin for 36 hours; WS - Equilibration in water saturated desiccator for 48 hours). The seeds were tested immediately after priming (PIE) and after natural ageing (PNA)

to treatments OP and MM followed by WS and HP. The UV absorbency of the leachate followed the same trend. The potassium ion content in the leachate significantly decreased in treatments OP and MM immediately after treatment and in all the treatments after natural ageing. A significant decrease in the electrical conductance and UV absorbance of the leachate in the beneficial priming treatments indicated improvement in the membrane integrity of the seeds, which is retained during the storage.

The natural ageing of the seeds resulted in the decrease of soluble protein content. Primed seeds had higher protein content immediately after priming and also after natural ageing, probably due to membrane reconfiguration resulting in re- synthesis of membrane bound enzymes. Reserve mobilisation is an important prerequisite for seed germination. The increased activity of amylase and acid phosphatase enzymes in the beneficial treatments indicated that the primed seeds had an advantage of being ready for subsequent growth which was reflected in improved germination and vigour parameters. This advantage was manifested in the stored seeds also as naturally aged seeds maintained a higher activity of these enzymes compared to untreated seeds. The free radicals and hydrogen peroxides produced from various metabolic reactions could be destroyed by the activity of peroxide savenging enzymes like peroxidase and catalase. The present study confirms that the treatments that improved the germination and vigour parameters also increased the activity of these enzymes.

The results demonstrate that in mustard variety Varuna priming the seeds does have an ameliorating effect on storage. This enhancement is probably a combination of priming stimulated repair/reconfiguration of the membrane, increased activity of scavenging enzymes and improved mobilisation of reserves.

ACKNOWLEDGEMENTS

The authors are grateful to the Director, for providing the facilities and Dr Manju Uprety, Technical Officer, NBPGR, New Delhi for the assistance provided in conducting the experiments.

REFERENCES

Argerich, C.A., K.J. Bradford and A.M. Tarquois. 1989. The effects of priming and ageing on resistance to deterioration of tomato seeds. *J. Exp. Bot.* **40**: 593-598

Bernfeld, P. 1955. Enzymes of carbohydrate metabolism. *In*: Methods in enzymology, Ed. Collowick and Kaplan, **1** 149-154

Ellis, R.H., and E.H. Roberts. 1981. The quantification of ageing and survival in orthodox seeds. *Seed Sci. and Technol.* **9** : 373- 409

Ellis, R.H., T.D. Hong and E.H. Roberts. 1985. Handbook of seed technology for genebanks. Vol. 1, Principle and methodology, 210 p. International Board for Plant Genetic Resources, Rome

ISTA. 1985. International rules for seed testing. Rules 1985. *Seed Sci. and Technol.* **13**, 57-64.

Leigh, R.A. and R.R. Walker. 1980. ATPase and acid phosphatase activities associated with vacuoles isolated from storage roots. *Planta* **150** : 222-229

Lowry, O.H., N.J. Rosenbrough, A.L. Farr and R.J. Randall. 1951. Protein measurement with the folin - Phenol reagent. *J. Biol. Chem.* **193** : 265-275

Lush, W.M., R.H. Goves and P.E. Kaye 1981. Presowing hydration- dehydration treatments and early seedling growth of wheat and ryegrass. *Aust. J. Plant Physiol.* **8**: 409-425.

Maehly, A.C. and B. Chance. 1967. Methods of biochemical analysis Vol 1 (Ed. D. Glick) Interscience Publ. Inc. NY., p 357-424

Michel, B.E. and M. K. Kaufmann. 1973. The osmotic potential of polyethylene glycol 6000. *Plant Physiol.* **51**: 914-916

Nath, S., P. Coolbear and J.C. Hampton. 1991. Hydration dehydration treatments to protect or repair stored "Karamu" wheat seeds. *Crop Sci.* **31**: 822-826

Pan, D and R.N.Basu. 1985. Mid storage and presowing seed treatment for lettuce and carrot. *Scientia Horticulturae* **25**: 11-19

Shannon, L.M., E. Kay and J.Y. Low. 1966. Peroxidase isoenzymes from horse radish roots. I. Isolation and physiological properties. *J. Biol. Chem.* **241**: 2166-2172