pots. Twenty days old papaya seedlings were taken in four groups, each containing 25 seedlings. The seedlings of the first group were inoculated with the neutral phosphate buffer to serve as control. The second and the third groups of seedlings were inoculated with PMMV and PLDV respectively, while those of the 4th group were subjected to viruliferous (PLCV) white flies. The method of preparation of inoculum and the inoculation were followed according to Rao *et al*[1]. Carborundum powder (600 mesh) was used as abrassive. Leaf samples were collected separately from healthy and virus-infected plants on the 60th day after inoculation, when the symptoms of all the viruses were quite prominent.

For the measurement of ATPase activity, the fresh leaves of papaya from healthy and virus-infected plants were first crushed with activated charcoal which adsorbed the chloroplast of green leaves. This was filtered and the filtrate centrifuged. The clean supernatant was then taken for enzyme activity measurement. The assay procedure involved the incubation of ATP with leaf cytosol followed by the determination of the released phosphate using the method of Boyer *et al*[2] as modified by Woolfolk *et al*[3]. Phosphate was estimated by the method of Farnden and Robertson[4]. Enzyme activity was measured in terms of $MgPO_4\ g^{-1}$ fresh weight of the leaf. Each estimation was made in triplicate and the average value is presented. All the virus experiments were performed under glass house conditions. The results are presented in table 1.

ADP/ATP ratio is a pace maker reaction and controls the respiration rate, the latter depending upon ADP availability. ATPase enzyme is well-known to be involved in the production of energy by breaking ATP into ADP and the inorganic phosphate[5]. The increased ATP concentration in virus-infected tissues[6] implies more ATP generation from ADP during oxidative phosphorylation. The rapid ATP turnover results in the enhanced respiration of the infected tissues[7–9]. This might be the main reason for the increase in ATPase activity in the virus-infected leaves of papaya during the present investigation.

Table 1 *Effect of three different papaya viruses on the ATPase activity of papaya leaves*

Virus strains	ATPase activity ($MgPO_4\ g^{-1}$ fr. wt)
Control	5.196
PMMV	6.200
PLCV	12.450
PLDV	13.200

Thanks are due to CSIR, New Delhi, for financial assistance.

13 November 1987; Revised 13 June 1988

1. Rao, G. P., Baghel, A. K. S., Singh, R. K. and Chatterji, K. S., *Experientia*, 1984, **40**, 1257.
2. Boyer, P. D., Mills, R. C. and Fromm, H. J., *Arch. Biochem. Biophys.*, 1959, **81**, 249.
3. Woolfolk, C. A., Shapiro, B. and Stodtman, E. R., *Arch. Biochem. Biophys.*, 1966, **116**, 177.
4. Farnden, K. J. F. and Robertson, J. G., In: *Methods for evaluation of biological nitrogen fixation*, (ed.) F. J. Bergersen, John Wiley & Sons, New York, 1980, p. 265.
5. Beevers, H., *Respiratory metabolism in plants*, Harper and Row, New York, 1961.
6. Merrett, M. J. and Bayley, J., *Bot. Rev.*, 1969, **35**, 372.
7. John, V. T., *Bull. Nat. Inst. Sci., India*, 1963, **24**, 103.
8. Singh, R. and Mall, T. P., *Indian J. Exp. Biol.*, 1976, **14**, 376.
9. Singh, R. B. and Srivastava, A. K., *Rev. Biol.*, 1979, **72**, 3.

PRE-SOWING HYDRATION OF MAIZE SEEDS FOR STIMULATION OF LOW-TEMPERATURE GERMINATION AND ITS EFFECTS ON PHOSPHOLIPID CHANGES IN THE EMBRYOS

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THE research interest in pre-sowing treatments for improving seed performance has yielded information of both intrinsic and applied value[1–3]. Pre-sowing hydration treatments of seeds can be effective in accelerating the rate and reducing the spread of germination[2]. Such treatments allow the seeds to imbibe partially and undergo considerable metabolic activity but without radicle emergence.

The maize crop needs a high optimal temperature for germination and growth and belongs to the

category of thermophillic plant species. In India, maize has traditionally been grown as a *kharif* crop, however, its cultivation as a *rabi* crop is gaining importance in various states including the Punjab[4]. Partial seed hydration prior to sowing has been suggested to be a method for overcoming low soil temperature injury during germination of maize[5,6] but the underlying mechanisms remain elusive. The involvement of membranes during seed hydration has been implicated[7,8] without measuring changes in their constituents. The present study is an attempt in this direction.

Maize seeds (*Zea mays* L. cv. Partap-1) were obtained from the Department of Plant Breeding, Punjab Agricultural University, Ludhiana. The first seed pre-treatment comprised one, three and five imbibition-drying cycles. Imbibition for 16 h at 20°C followed by drying-back constituted one cycle. For imbibition, 25 seeds per petri dish (9 cm) were placed on two layers of filter paper moistened with 5 ml of distilled water. Subsequently, the seeds were either surface-dried or dried-back in a stream of air for 48 h at 20°C. In the second pre-treatment, the rate at which seeds imbibed water was controlled by varying the number of filter paper layers in a petri dish (9 cm) to a constant quantity of water (5 ml). Seeds were hydrated with 3 and 4 layers of filter paper for 2 and 3 days respectively and then either surface-dried or dried-back at 20°C as before.

Treated seeds, together with an untreated control, were germinated in the dark in 9 cm petri dishes lined with two layers of filter paper and moistened with 5 ml of distilled water at 10 ± 1°C. Four replicates of 25 seeds were used. A seed was credited with germination when its radicle protruded more than 2 mm. The mean germination time was calculated as $\Sigma D{\cdot}N/\Sigma N$ where D is the number of days counted from the beginning of germination test, and N is the number of seeds which germinate on day D. At the end of 2 days pre-sowing hydration over 3 layers of filter paper, the embryos were scooped out of the seeds and the amounts of total phospholipids, total sterols and individual classes of phospholipids were determined as detailed elsewhere[9].

Table 1 *Mean germination time of maize at 10°C when tested either untreated or following imbibition-drying cycles*

	Untreated control	Imbibition-drying cycles			LSD ($P = 0.05$)
		1	3	5	
Mean germination time (days)	11.1	10.9	10.5	5.7	1.8

Seeds subjected to 1 and 3 imbibition-drying cycles did not affect germination but the 5-cycle pre-treatment was highly effective in accelerating the rate of germination (table 1). The mean germination time was reduced to 5.7 days from 11.1 days of the untreated seeds. However, when the initial imbibition (i.e. pre-drying) time of seeds is taken into account, the total time for which the seeds are hydrated before completion of germination, is about the same as control (once-hydrated) seeds. Hence, the events occurring prior to drying are implicitly stable during drying and do not have to be repeated for the completion of germination. In fact, seeds are able to tolerate several cycles of hydration and dehydration without much injury, providing the hydration threshold is not exceeded[10,11].

Seeds hydrated on 3 or 4 layers of paper for 2 days were most effective in accelerating the low-temperature germination of seeds; the mean germination time was reduced by 6.2 days in surface-

Table 2 *Mean germination time of maize at 10°C when tested either untreated or following controlled hydration*

		Seed hydration on 3 filter paper layers				Seed hydration on 4 filter paper layers				
		Surface-drying		Drying-back		Surface-drying		Drying-back		
	Untreated control	1 day	2 days	1 day	2 days	1 day	2 days	1 day	2 days	LSD ($P = 0.05$)
Mean germination time (days)	10.8	7.6	3.6	10.8	5.8	9.1	3.6	10.3	6.8	1.0

Table 3 *Changes in phospholipids and sterols of maize embryos following controlled seed hydration over 3 filter paper layers at 20°C*

	Total phospholipids	Total sterols	Phospholipid classes				
			Phosphatidic acid	Lysophosphatidylcholine	Phosphatidylcholine	Phosphatidylethanolamine	Diphosphatidylglycerol
Untreated seeds	905 6	136 5	107.9	27 0	296 7	202.3	269.7
Hydrated seeds	1946	190 6	162 6	139 4	627 2	238.1	778 2

Amounts given are as μg 25 embryos, the values are significantly different according to t-test comparison at $P = 0.05$.

dried seeds (table 2). Drying-back the seeds slightly delayed germination because of the time taken to reimbibe water. The improvement in germination may be attributed to the slow rate of imbibition[12] which possibly helps in the restitution or metabolic repair of membranes[7].

Pre-sowing hydration for 2 days over 3 filter paper layers caused a significant increase in the embryo phospholipids and sterols which are membrane constituents (table 3). Individual amounts of phospholipids in the embryos of treated seeds also increased over dry seeds. The chemical constituents of a membrane influence its functional properties including fluidity, permeability, protein activity, responsiveness to hormones and the structural configuration that lipids assume under given thermal and ionic conditions[13,14]. Increased amounts of phosphatidylcholine and phosphatidylethanolamine in the treated embryos may contribute to a higher membrane fluidity upon reimbibition at the chilling temperature, as has been demonstrated during low-temperature hardening of plants[15]. It is interesting to point out that the amount of diphosphatidylglycerol (DPG) increased as compared with other phospholipids (table 3). There are no reports of DPG in higher plant plasma-membranes and its presence is probably restricted to inner mitochondrial membrane[16]. Maximum amounts of DPG in the primed embryos are suggestive of the well-formed internal organization of their mitochondrial membranes, which are also the sites for oxidative phosphorylation. The concomitant accumulation of ATP may hence be a mechanism for improved germination performance.

16 January 1988; Revised 7 May 1988

1. Heydecker, W. and Coolbear, P., *Seed Sci. Technol.*, 1977, **5**, 353.
2. Thomas, T. H., *Sci. Hortic.*, 1981, **32**, 47.
3. Bradford, K. J., *HortScience*, 1986, **21**, 1105.
4. Khehra, A. S. and Dhillon, B. S., *Agalmnus*, 1982, **16**, 6.
5. Cal, J. P. and Obendorf, R. L., *Crop Sci.*, 1972, **12**, 369.
6. Bennett, M. A. and Waters, L. Jr., *J. Am. Soc. Hortic. Sci.*, 1984, **109**, 623.
7. Burgess, R. W. and Powell, A. A., *Ann. Bot.*, 1984, **53**, 753.
8. Tilden, R. L. and West, S. H., *Plant Physiol.*, 1985, **77**, 584.
9. Basra, A. S., Seema Bedi and Malik, C. P., *Ann. Bot.*, 1988, **61**, 357.
10. Hegarty, T. W., *Plant Cell Env.*, 1978, **1**, 109.
11. Senaratna, T., McKersie, B. D. and Stinson, R. H., *Plant Physiol.*, 1984, **76**, 759.
12. Tully, R. E., Musgrave, M. E. and Leopold, A. C., *Crop Sci.*, 1981, **21**, 312.
13. Quinn, P. J. and Chapman, D., *CRC Crit. Rev. Biochem.*, 1980, **8**, 1.
14. Jusaitis, M., Paleg, L. G. and Aspinall, D., *Plant Physiol.*, 1982, **70**, 1486.
15. Kuiper, P. J. C., *Physiol. Plant.*, 1985, **64**, 118.
16. Bligny, R. and Douce, A., *Biochim. Biophys. Acta*, 1980, **617**, 254.

A NEW FLORAL CHARACTER INFLUENCING ALLOGAMY IN RICE

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A limitation in the development of hybrid rice is the floral structure which is basically not suited for cross-pollination. To facilitate out-crossing in male