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Storability of Primed Seeds of Tomato (*Lycopersicon esculentum*), Egg Plant (*Solanum melongena*) and Chilli (*Capsicum annum*)

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Storage studies were conducted to compare four different methods of priming *viz*., hydropriming, halopriming, sand matricpriming and osmopriming accomplished for two durations. The storage studies were conducted for six months after imposing the priming treatment. The results revealed that viability of primed seeds were dependent on the method as well as duration of priming however, irrespective of method and duration all the priming methods were superior to control seeds through out the storage period. Among the protocols studied, hydropriming (48 h) for tomato and sand matricpriming (80% water holding capacity, 3 days) for egg plant and chilli are established as best methods of priming treatment capable of improving seed vigour as well as viability.

Key Words: Tomato, egg plant, chilli, seed priming, priming methods, storability

Literature on seed priming revealed that primed seeds may be vigourous but with reduced storage life. Bradford, (1986) stated that the results obtained so far are few, limited and contradictory because of varied response to treatments by cultivars and even seed lots. Mc Donald (1999) expressed that seed priming is capable of reversing the causes of seed deterioration. Seed priming entails the hydration of seeds using various protocols followed by reducing the moisture content to permit routine handling. The benefits include increased germination rate, uniformity in emergence, and germination under a broader range of environments and improved seedling vigour and growth. Although priming is acclaimed as a useful technique to invigourate the seed, yet it is also widely reported to cause detrimental effects on storage life of the subsequently dried seed (Argerich and Bradford, 1989; Tarquis and Bradford, 1992; Bruggink *et al.,* 1999). Against the popular opinion that priming reduces seed longevity, conflicting reports have been made about the viability of primed seeds during storage. In many species (eg., *Allium cepa* L. *Capsicum annum* L., *Pisum sativum*, *Daucus carota*) improvement in seed storability after osmotic priming has been reported by Savino *et al.*(1979), Dearman *et al.,* (1986) and Georghiou *et al.,* (1987). Gurusinghe and Bradford (2001) elaborated that desiccation tolerance *per se* is not lost in primed seeds, as they are capable of rehydrating and germinating rapidly following drying.

In the biochemical front also contradictory arguments have been made on impact of priming on seed storability. Mc Donald (1999) reported better storability of primed seeds owing to reversal of seed deterioration due to priming. Osborne (1983) proved that repair of damaged DNA due to seed priming will increased longevity. However, Saracco *et al* (1995) proposed increased sensitivity of primed seeds to controlled deterioration as a consequence of advanced germinative events, increasing the susceptibility of seeds to deteriorative factors imposed during storage. However, the results obtained so far are few, limited and contradictory because of the varied response to treatments of cultivars and even seed lots (Bradford, 1986) which require a careful choice of the compounds to be used as osmoticum and standardization of the treatment conditions. In deciding the adaptability of a seed invigouration treatment by seed companies, the effect on seed storability plays a crucial role. This criteria assumes significance because, for a seed company to adopt a specified seed treatment, two points have to be clarified i) the efficacy period of the treatment ii) viability period of primed seeds. This is important because the time taken for a treated seed lot to reach the farmers may be anywhere from three to six months. Moreover, the unsold seeds will have to be stored until the next season. These being the pre-requisites, imparting any seed treatment that will not "stay long in seed" or "not allow seeds to live longer" will not be practicable.

Against this background, in order to assess the storability as well as the efficacy period of the seed priming treatments, storage experiments were conducted on primed seeds of tomato, egg plant

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and chilli by investigating the physiological and biochemical indicators of seed vigour during storage.

Materials and Methods

Commercially available seeds of tomato cv. PKM 1, egg plant cv. CO 2 and chilli cv. K2 with 8% moisture content were obtained for the priming experiments. Seeds of each crop were submitted to seed priming protocols standardized by Venkatasubramanium and Umarani (2007) *viz.,* i) hydropriming (ii) halopriming iii) osmotpriming under room temperature (26+2°C) and iv) sand matricpriming at 25 ± 2°C , 100 % RH . The moisure content of primed seeds at the end of the treatment was about 35%. After the soaking period, seeds were air-dried to original moisture content under shade for three days at room temperature (26± 2°C). The priming methodologies followed are detailed here under:

Hydropriming

Each crop seed (10 g) were soaked in double the volume of water for 36 and 48 h followed by shade drying.

Sand matricpriming

The seeds of each crop were weighed upto 10 g with four replications. Seven kg of sand were kept in six trays (25 x 15 x 10 cm each) and water was added to attain 80 percent water holding capacity. The seeds were mixed with sand of same water holding capacity (80 per cent), placed in perforated plastic covers and buried deep in the tray (25 x 15 x 10 cm each) filled with sand of same water holding capacity. This was done to ensure uniform seed-substrate contact. The set up was kept in an incubator maintained at 25 ± 2°C and 95 ± 5 per cent RH, and samples were retrieved after 3 and 4 days and were shade dried.

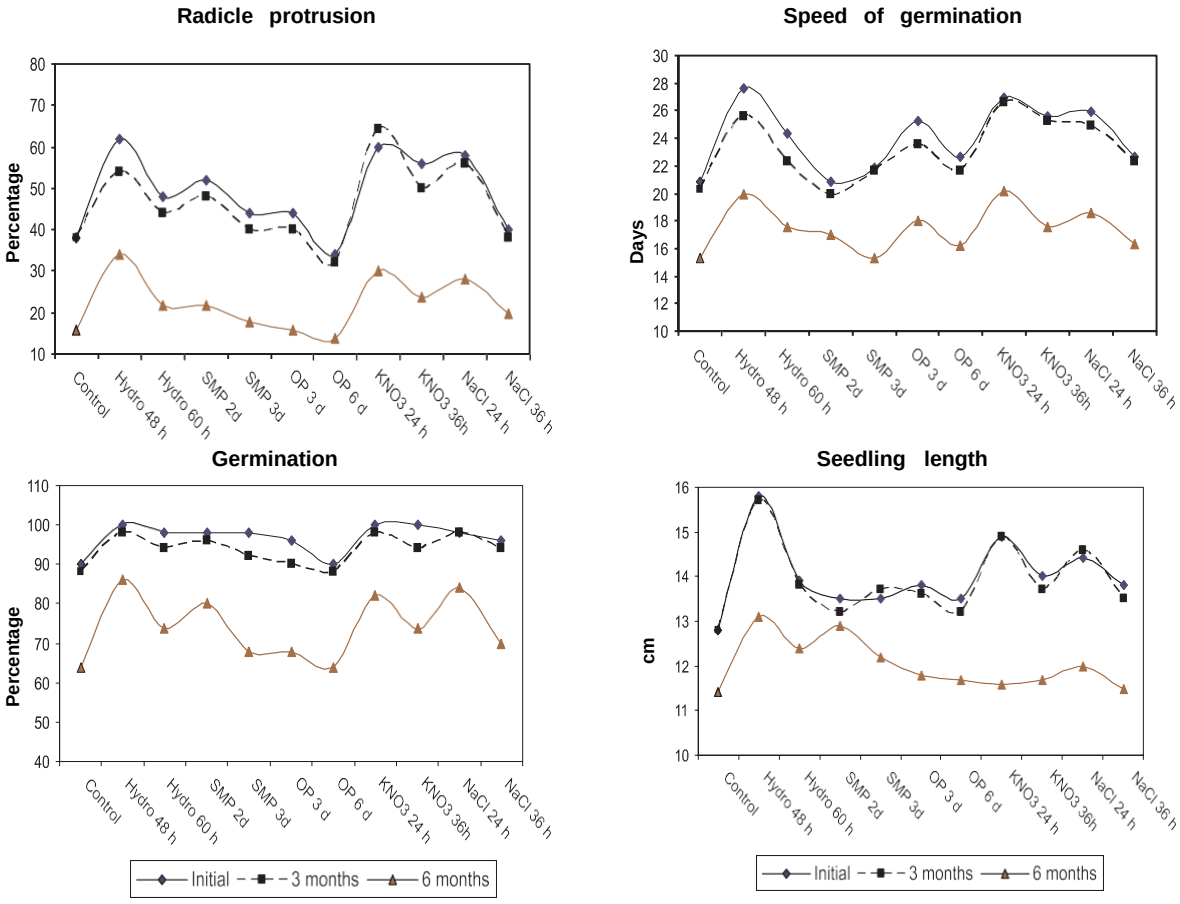

Figure 1. Effect of priming methods on radicle protrusion (%), speed of germination (days) , germination (%) and seedling length (cm) of tomato seeds (cv PKM 1).

Halopriming

Halopriming treatment was conducted with two salts at 5 % concentration *viz.,* KNO_3 and NaCl. Seeds were soaked for periods of 24 and 36 h. After priming, the seeds were removed from the solutions, rinsed in running tap water and shade dried.

Osmopriming

Osmopriming was performed using polyethylene glycol 6000 (PEG 6000) solution. Solutions with osmotic potential of -1.0 MPa were prepared by dissolving 273 g of PEG 6000 in one litre of water (Nienow and Bujaski, 1991). Seeds were soaked in

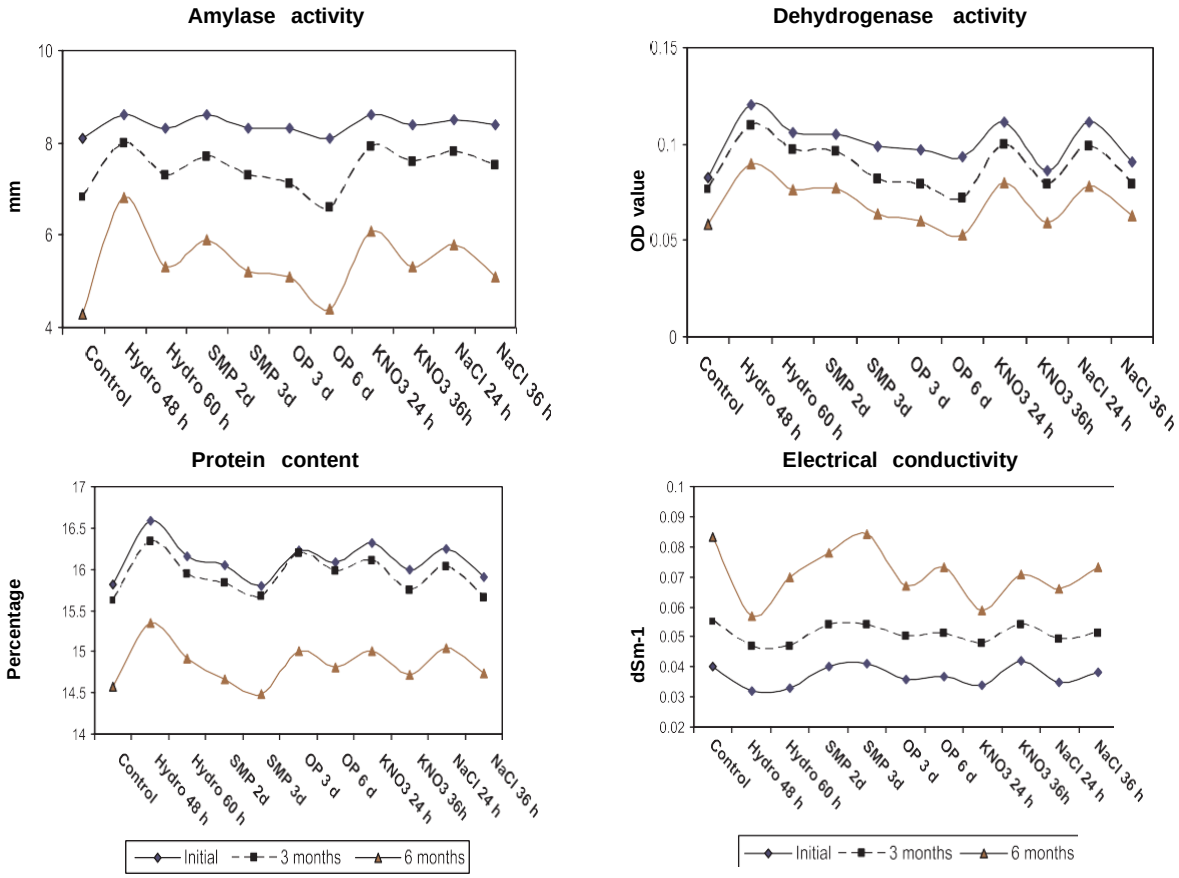

Figure 2. Effect of priming methods on amylase activity, dehydrogenase activity, protein content and electrical conductivity of tomato seeds (cv PKM 1).

the osmoticum of different osmotic potential for 3 and 6 days. At the end of the soaking period, the seeds were removed, rinsed in distilled water and shade dried.

Storage studies

The seeds were dried to 8 % moisture content and packaged aluminium foil bag. The containers were kept under ambient conditions (33°C and 57% RH) for six months. Seed samples drawn initially and subsequently at fortnightly intervals were subjected to germination test with four replicates of 100 seeds in between paper towels. The test conditions were 25 + 2°C and 95 ± 5 per cent RH, illuminated with fluorescent light. The seeds were checked daily upto 14 days for protrusion of radicle. The seeds showing less than 3.0 mm radicle protrusion were alone counted. The speed of germination was calculated according to Maguire (1962). The number of normal seedlings were counted after 14 days and expressed as germination percentage. The length of the seedlings were measured and expressed in cm. The seeds were also analysed for electrical conductivity (Presley, 1958), α- amylase activity (Simpson and Naylor, 1962), dehydrogenase activity (Kittock and Law, 1968) and protein content (Ali Khan and Youngs, 1973).

Data were analyzed using an analysis of variance (ANOVA) as a factorial combination of treatments. Means were separated on the basis of least significant difference (LSD) only if F test of ANOVA for treatments was significant at the 0.05 or 0.01 probability level. Values in percent data were arcsine transformed before analysis.

Results and Discussion

The results revealed that irrespective of the crops *viz.,* tomato, egg plant and chilli significant variations were observed in all the parameters observed both among the priming treatments as well as between the duration of the treatments. Tomato seeds, hydroprimed for 48 h recorded the highest values of both physiological and biochemical parameters observed (Figure 1,2). This was followed by KNO_3 (5%, 24 h), NaCl (5%, 24 h), sand matric priming (80%, 3 days) and osmopriming (-1.25 MPa, 3 days), respectively. The percentage improvement recorded by hydropriming (48 h) was observed throughout the storage period. At the end six months , the hydroprimed (48 h) seeds recorded highest percentage of radicle protrusion over rest of the treatments .The percentage improvement recorded was 0.30 (KNO_3 ,5%, 24 h); 21 (NaCl, 5%, 24 h); and 112 (osmopriming,-1.25 MPa,3 days). Hydropriming (48 h) also recorded higher speed of

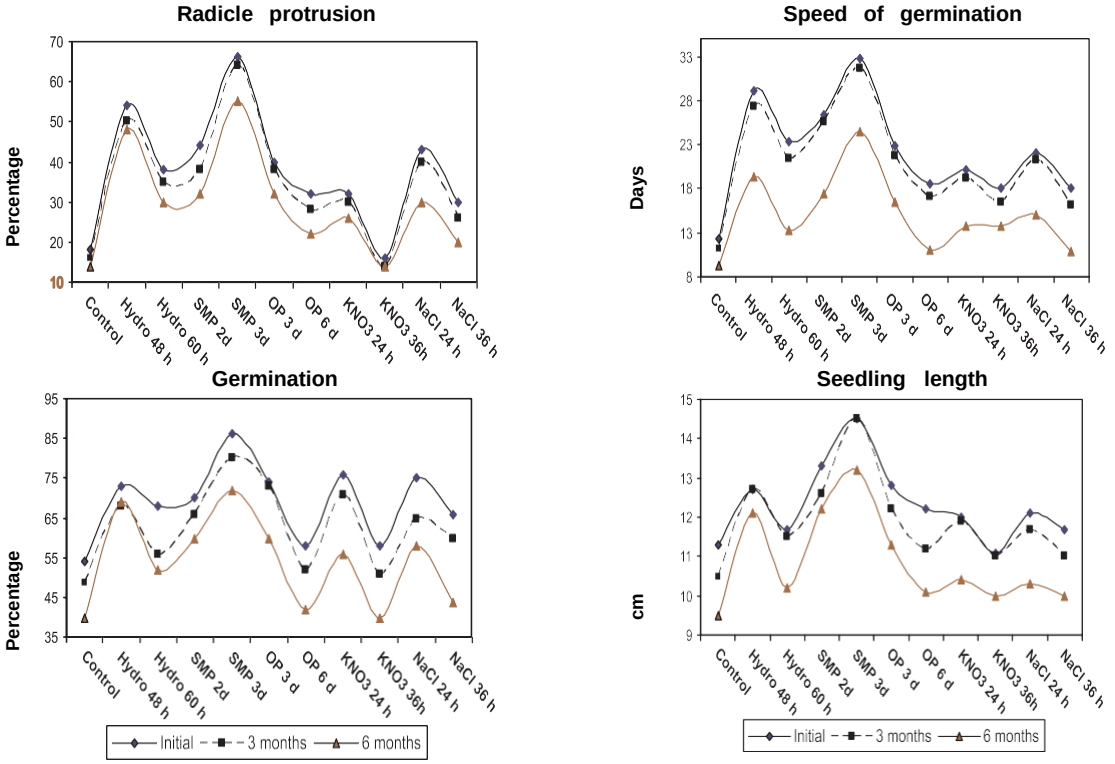

Figure 3. Effect of priming methods on radicle protrusion (%), speed of germination (days) , germination (%) and seedling length (cm) of egg (cv Co 2) plant seeds

germination. The improvement recorded was to the tune of 23, 13, 23 and 16 percent ;similarly the germination percentage recorded an increase of 4.8, 26, 7.5 and 26 percent, respectively. The advantage obtained by speed germination was also reflected in seedling growth (cm). The biochemical parameters made it clear that the priming treatments increased the amylase activity (cm), dehydrogenase

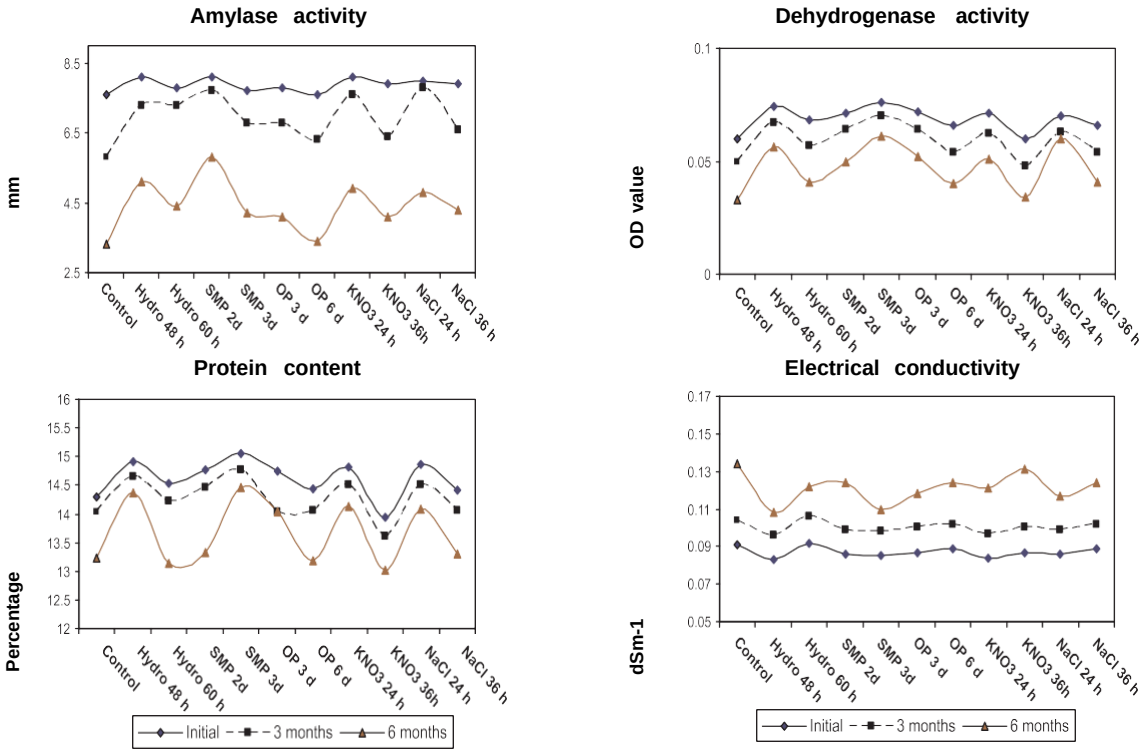

Figure 4. Effect of priming methods on amylase activity, dehydrogenase activity, protein content and electrical conductivity of egg plant (cv Co 2) .

activity (OD value) and protein content (%) positively and reduced the electrical conductivity (dSm^{-1}) considerably. The highest values of α- amylase (6.8 cm), dehydrogenase (OD value) (0.090) and protein content (15.35) was observed in hydropriming (48 h). The corresponding values recorded by control was 4.3, 0.058 and 14.58 , respectively. The electrical conductivity (dSm^{-1}) recorded by hydroprimed (48 h) seeds were lower than control by 45 percent.

Seed priming has been proved to influence the physiological and biochemical status of the seeds. In sweet corn, priming resulted in decreased conductivity, free sugars and DNA content while RNA content increased (Sung and Chang, 1993). Lower electrical conductivity readings following priming also were reported for egg plant and radish (Rudrapal and Nakamura, 1988) and onion (Choudhuri and Basu, 1988). Smith and Cobb (1991) reported increased protein, also. In sweet corn seeds, priming increased α- amylase and ß-amylase activity (Sung and Chang, 1993) and led to increased protein and DNA synthesis (Dell Aquilla and Trotto, 1990) in wheat. Saha et al (1990) showed that priming caused increased amylase and dehydrogenase activity in aged soybean seeds compared to unprimed seeds while lowering lipid peroxidation.

In the present study, unlike tomato, egg plant and chilli seeds, showed maximum improvement in seed quality parameters due to sand matric priming. In egg plant, the sand matric priming (80% WHC, 3 days) recorded the highest radicle protrusion (5.5), speed of germination (24.5), germination percentage (68) and seedling length

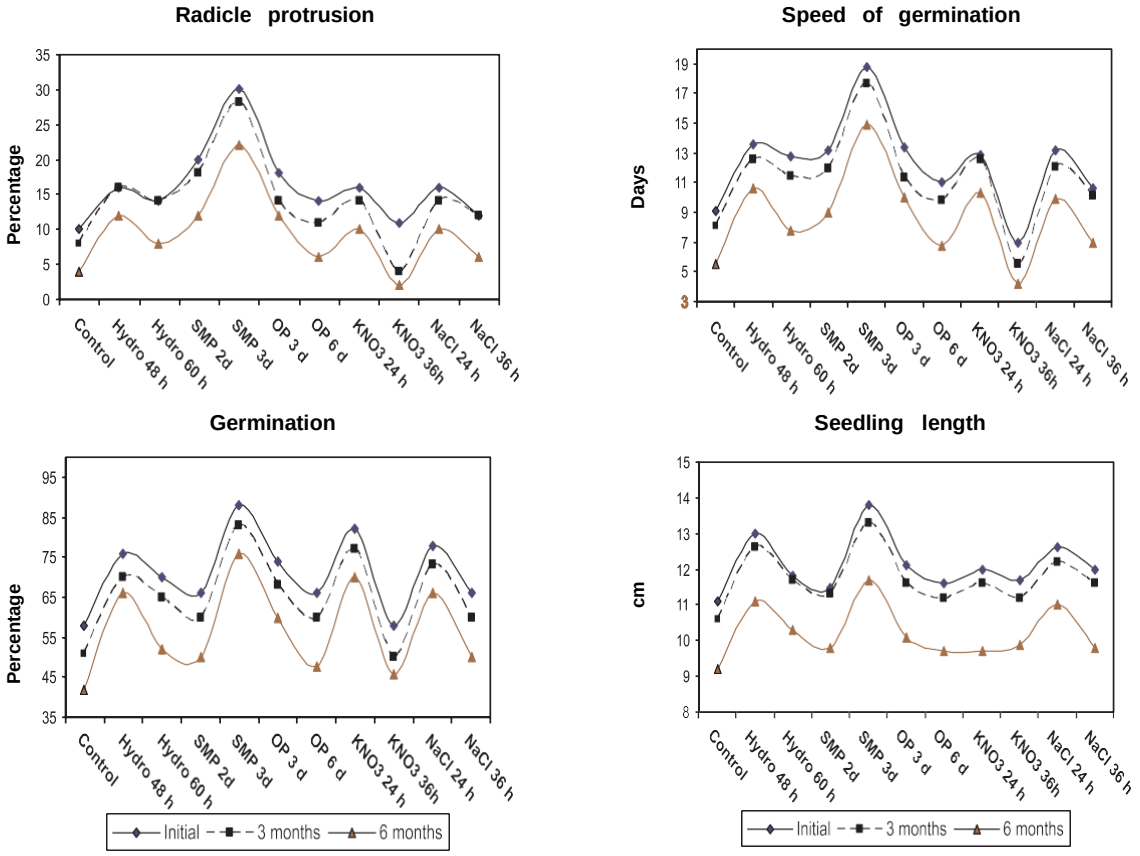

Figure 5. Effect of priming methods on radicle protrusion (%), speed of germination (days) , germination (%) and seedling length (cm) of chilli (cv K 2) seeds .

(cm) (13.2) whereas, control recorded 14, 97, 36 and 9.5, respectively (Figure 3,4). However, the other methods of priming were also found to be better than the control. The enzyme activities *viz.,* α-amylase (cm) (5.8) and dehydrogenase (OD value) (0.061) were found to be the highest when the seeds were treated with sand matricpriming (80 WHC% 3 days); the protein content (%) was also highest (14.46). The corresponding values recorded by control seeds were, 3.3, 0.033 and 13.22, respectively. The measure of membrane integrity ie., electrical conductivity (dSm^{-1}) was also lowest (0.110), compared to control (0.134).

The results for chilli seeds were similar to the egg plant seeds. Sand matric priming (80% WHC, 3 days) recorded the highest radicle protrusion, speed of germination and germination percent *viz.,* 22, 14.9 and 76 while the control recorded 4, 5,5 and 42, respectively. The seedling growth (cm) was

also maximum (13.3). It brought about an improvement in α- amylase (cm) (5.0) and dehydrogenase activity (OD value) (0.42) and protein content (%) (14.3) recorded was the maximum while the electrical conductivity (dSm^{-1}) (0.131) recorded was the lowest after six months period (Figure 5,6).

The review of available literature strongly endorsed that primed seeds may be vigourous but posses reduced storage life. Argerich *et al.* (1989) found that priming of tomato seed enhanced germination rate but lowered seed resistance to deterioration. Similar findings were reported for lettuce Tarquis and Bradford (1992) leek and carrot (Dearman *et al.,* 1987), Salvia splendens (Carpenter, 1989), celery (Singh, *et al.,* 1985), Trifolium spp (Jansen and Ison, 1994), pepper (Lanteri *et al.,* 1997) and leek (Maude *et al.,* 1994). Many studies were also made to investigate the biochemical basis of reduced storability of otherwise vigourous primed seeds. Yougging *et al.* (1996) reported that after 5 months of storage, most of the seed lots showed reduction in germination compared to control. They opined that, when the germination process has progressed to a point where endoduplication of nuclear DNA in the radicle tip started, the seed cannot stay at this stage without a reduction in storability (Osborne ,1983). Saracco *et al.* (1995) observed that longer duration of priming treatments may lead to replication of DNA making them more sensitive to controlled deterioration while seeds primed for optimum duration did not allow nuclei to enter the synthetic phase. It was clearly shown that seeds containing nuclei in G2 (after DNA synthesis) were comparatively more sensitive to ageing. Bruggink et al. (1990) experimented longevity fo seeds with *Impatiens walleriana* after subjecting them to hydropriming in aerated water for 3 days. They reported that fast drying of the seeds in 32 o Control was important to ensure seed longevity, which could 80% germination after 23 months of storage. They insisted that desiccation tolerance and longevity are related characteristics.

Gurusinghe and Bradford (2001) hypothesized that loss of RFOs (raffinose oligosaccharide) during priming and failure to accumulate them again during drying , could compromise glassy state formation in the seeds, allowing faster deteriorative reactions. However, they observed that restoration of tolerance to controlled deterioration by post- priming treatment was not associated with concomitant changes in tissue sucrose or oligosaccharide content. Further, they confessed that the data did not support the hypothesis that oligosacharide loss is responsible for the reduced longevity of primed seeds. Probert

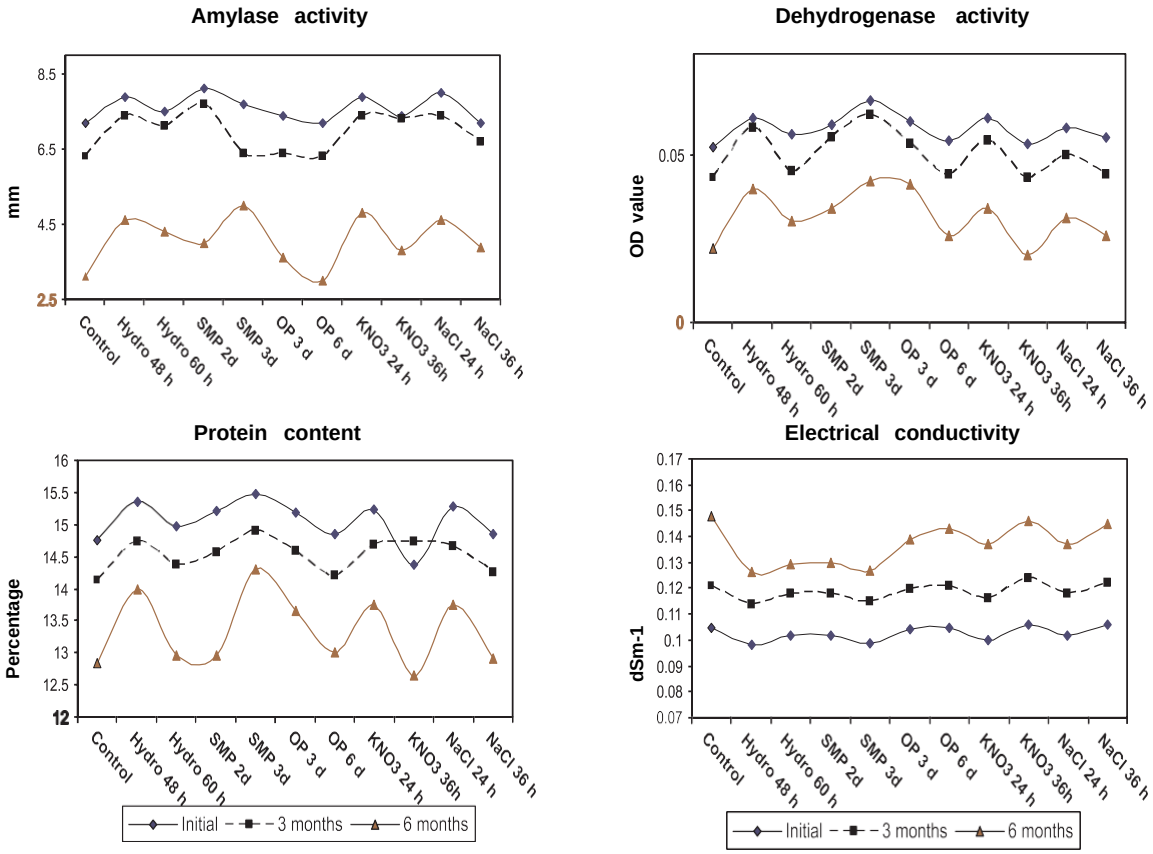

Figure 6. Effect of priming methods on amylase activity, dehydrogenase activity, protein content and electrical conductivity of chilli (cv K 2) seeds.

et al (1991) primed *Ranunculus sceleratus* seeds in PEG (-1.5 MPa) for 7 days followed by drying and observed 4-5 fold increase in seed longevity when stored at 35°C and 9.2 % moisture content . Priming for one day resulted in a smaller but significant increase in longevity independently of whether the seeds were primed in PEG distilled water or a saturated atmosphere (100 % RH). They suggested that besides repairing the accumulated damage, the priming treatment also obviated subsequent damage. They unequivocally demonstrate that desiccation followed by carefully controlled ageing conditions can result in a significant increase in seed longevity.

Many studies have established that seed priming reverses seed deterioration and these beneficial effects generally occur in the meristamatic axis or the radicle tip. Sivritepe and Dourado (1994) found that humidification of aged pea seeds decreased chromosomal aberrations. During the natural process of ageing, damage in membranes and DNA leads to a loss of seed quality, this damage can be repaired during a hydration (Villiers and Edgecumbe, 1975). Sur and Basu (1990) found that hydration - dehydration treatment of wheat seeds enhanced germination of stored seed and reduced the production of volatile aldehydes. In onion seed, Choudhuri and Basu (1988) demonstrated that hydration - dehydration treatments effectively slowed physiological deterioration under natural (5 months) and accelerated ageing conditions with the effect dependent on the level of seed vigour.3

The present study also established that, seeds primed by adopting crop specific protocol for optimum duration and drying back to original moisture content ensured the efficacy of the treatment is retained in the seed during storage, besides maintaining viability of primed seeds. These results corroborates that priming benefits seeds through rectification of DNA damage, decreased chromosomal aberration, reduced volatile aldehyde production etc., The problem of reduced storability of primed seeds arises only if duration of seed priming extends to an advanced stage wherein seeds enter into the Synthetic phase leading increase in DNA content. Based on the results of the present study, it is recommended that tomato seed can be hydroprimed for 48 h in water (double the volume of seeds) while, egg plant and chilli seeds can be subjected to sand matricpriming (80% water holding capacity) for 3 days. The seeds dried back to original moisture content can be stored in aluminium foil pouches for at least six months without losing the efficacy of the priming treatment.

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