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Storage potential of primed seeds of okra (*Abelmoschus esculentus*) and beet root (*Beta vulgaris*)

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Abstract

Seed priming increases the germination rate, uniformity and emergence under a broader range of environmental conditions besides improving seedling vigor and growth. However, reports on subsequent seed storage potential of primed seeds are more variable. Most of the studies reported have not compared the effects of different methods of priming on seed longevity. In the present study, the seeds of okra and beet root were subjected to four methods of priming , by including two durations *viz.*, hydro priming (12, 24 h), sand matricpriming (60 % WHC; 3, 6 h), halo priming (3% NaCl; 12,24 h) and osmopriming (PEG,24 h) two osmotic levels (-1 and -1.5 Mpa). In okra, among the priming methods, hydro priming (both 12 and 24 h) was detrimental to seed quality both initially and over six months of storage. Besides, longer durations of sand matricpriming (60 % WHC; 6 h) and halo priming (3% NaCl; 24 h) were found to be inferior to shorter duration counterparts, as they showed 10 and 15 per cent reduction in initial germination, respectively. However, in beet root, all the seed priming treatments were found to be superior to control, both initially and after six months of storage. Among the treatments, highest improvement in seed quality parameters was recorded by hydro priming (12 h). It recorded 30.9 per cent increase in germination over control even after six months of storage. Among the protocols studied, sand matricpriming (3 h in 60% WHC of sand) was found to be the best for okra, while for beet root; hydro priming (for 12 h in water at double the volume of seed) was most suitable.

Keywords: Okra, beet root, seed priming, priming methods, storage and seed vigor.
Abbreviations: WHC_Water Holding Capacity.

Introduction

Seed quality enhancement is possible through various seed priming techniques including hydropriming, halopriming, osmopriming, thermopriming, solid matrix priming, and biopriming (Ashraf and Foolad 2005; Venkatasubramanian and Umarani, 2007). Seed priming entails hydration of seeds using various protocols to permit routine handling resulting in increased germination rate, uniformity in emergence, and germination under a broader range of environments. Seed priming is known to favor initiation of basic pre-germination biochemical changes which include breaking of dormancy, imbibition, hydrolysis or metabolism of inhibitors and enzyme activation (Ajouri et al., 2004). To sum it up, Asgedom and Becker (2001) stated that seed priming triggers few or all pre-germination processes, which persist in the seed even after re-drying leading to quick re-imbibition and rapid revival of germination metabolism. Ruan et al. (2002) established that priming also allowed some repair of membrane damage that occurs due to seed deterioration. According to Basra et al. (2003) in normal and low vigour seeds, germination improvement may happen as a result of reserve mobilization, activation and re-synthesis of some enzymes as well as initiation of RNA and DNA synthesis. Although priming is acclaimed as a useful technique to invigorate the seed initially, yet it is also widely reported to cause detrimental effects on storage life of the subsequently dried seed (Schwember and Bradford, 2005; Hill et al., 2007). Against this popular opinion, reports have also been made in support of improved viability in primed and stored seed (Basra, 2003; Dearman et al., 2008; Venkatasubramanian and Umarani, 2010). Contradictory statements have been made on biochemical impact of seed priming on stored seeds. Mc Donald (1999) reported better storability of primed seeds owing to reversal of seed deterioration. Gurusinghe et al. (2002) reported that when primed seeds are slowly dried back, it induces synthesis of LEA (late embryogenesis abundant) proteins, while rapid drying at higher temperatures may induce heat shock proteins, promoting protective mechanisms which increased the seed storage life. However, Schwember and Bradford (2005) reported that irrespective of drying rates, longevity of lettuce seeds were lower than non-primed seeds. In response to these conflicting opinions, Tzortzakis (2009) stated that each of the priming methods had its advantages and disadvantages and may have varying effects depending upon plant species, stage of plant development, concentration/ dose of priming agent, and incubation period. Earlier, Nirmala and Umarani (2008) reported on efficacy of four methods of priming *viz.*, hydropriming, sand matricpriming, halopriming and osmopriming, in imparting initial improvement of seed germination and seedling vigor of okra and beet root. However, the study did not address the issue of variability in post-priming storage life of primed and non-primed seeds. Therefore, in the present experiment, okra and beet root seeds were subjected to different priming methods and subsequently, the post-priming storage potential were analyzed by studying the seed physiological and biochemical characteristics.

Results

In the present study, the seeds of okra and beet root were subjected to four methods of priming, by including two durations for hydropriming (12, 24 h), sand matricpriming (60 % WHC; 3, 6 h), halopriming (3% NaCl; 12,24 h) and with two osmotic levels (-1 and -1.5 Mpa) for osmopriming (Polyethylene glycol, 24 h).

Storage potential of primed okra seeds

In this present experiment, the initial evaluation of primed seeds also revealed that among the treatments, longer duration of priming *viz.*, hydropriming (24 h), halo priming (3% NaCl, 24 h) and sand matric priming (60 % WHC, 6 h) were inferior to the shorter duration counterparts *viz*., hydropriming (12 h), halopriming (3% NaCl, 12 h) and sand matric priming (60 % WHC, 3 h) (Fig.1). The percentage reduction recorded in germination was to the tune of 10, 15, and 10, respectively. The monthly evaluation of seed germination and vigor of primed seeds revealed that among the treatments, hydropriming (both 12 and 24 h) was the only priming treatment which proved to be detrimental to seeds, in terms of radical protrusion, seed germination, and speed of germination as well as seedling length (cm) (Fig 1) (Table 1). After one month of storage, hydroprimed seeds showed 2 and 1 per cent reduction in germination due to 12 h and 24 h of hydropriming, respectively. After six months, the percentage of reduction increased to the tune of 31 and 37 per cent, respectively. The results clearly suggested that hydropriming was not suitable for okra seeds. When the seeds were subjected to to controlled imbibition through halopriming and sand matricpriming also, longer duration was found to be detrimental to seed quality. Among the priming methods, sand matricpriming with 60 % WHC for 3 h recorded the highest improvement in terms of radical protrusion percentage, seed germination percentage, speed of germination and seedling length (cm) (Fig.1). The percentage of improvement over control recorded initially after treatment was 26, 27.5, 10 and 24, respectively. After six months of storage also sand matricpriming (60 %; 3 h) recorded superiority over control seeds by recording highest percentage of improvement amounting to 13, 20, 18 and 47.4 over control, respectively (Fig.1). It was obvious that the efficacy of the priming treatments obtained initially after treatment were retained in the okra seeds, even after a period of six months. The observations on biochemical parameters made in this study, revealed that halopriming, osmopriming, and sand matricpriming, recorded superiority over control seeds throughout the six months of storage. Among the methods, sand matricpriming (60 % WHC, 3 h) recorded the lowest electrical conductivity (dSm^{-1}), and highest protein content (%), dehydrogenase activity (OD value) and ∞-amylase activity (mm), throughout the storage period compared to control seeds as well as seeds primed by other methods (Fig.2) (Table 1). Even after six months of storage, sand matricpriming (60 % WHC, 3 h) recorded a lower electrical conductivity (0.580 dSm^{-1}) compared to control seeds (0.780 dSm^{-1}) demonstrating better membrane integrity in primed and stored okra seeds. With respect to protein content, dehydrogenase activity and amylase activity also, highest values were recorded by sand matricpriming (60 % WHC, 3 h), after six months of storage by recording 21.9, 107 and 60 per cent increase over control, respectively (Fig.2). The data stands proof for the enhanced metabolic potential of the primed and stored okra seeds.

Table1. Statistical significance of okra (*Abelmoschus esculentus*) seed germination and biochemical characters given in Fig 1 and Fig 2

	Priming Method (T)	Period (P)	TXP
Radicle protrusion (%)			
SEd	0.13	0.12	0.36
LSD (0.05)	0.27	0.23	0.70
Speed of germination			
SEd	0.18	0.16	NS
LSD (0.05)	0.36	0.32	NS
Germination (%)			
SEd	0.38	0.33	1.0
LSD (0.05)	0.74	0.65	1.96
Seedling length (cm)			
SEd	0.31	0.28	NS
LSD (0.05)	0.61	0.54	NS
Electrical conductivity (dSm^{-1})			
SEd	0.017	0.011	0.033
LSD (0.05)	0.033	0.022	0.067
Protein content (%)			
SEd	0.07	0.04	NS
LSD (0.05)	0.13	0.09	NS
Dehydrogenase (OD value)			
SEd	0.004	0.003	0.008
LSD (0.05)	0.008	0.006	0.017
α-amylase (mm)			
SEd	0.03	0.02	NS
LSD (0.05)	0.05	0.04	NS

Storage potential of primed beet root seeds

Unlike okra, in beet root, all the seed priming treatments were found to be superior to control irrespective of treatments and durations, both initially and after 6 months of storage. Among the treatments, highest improvement in seed quality parameters were recorded by hydropriming (12 h) followed by sand matricpriming (60 % WHC, 4 days). The initial values recorded by hydropriming (12 h) for radical protrusion percentage (79), germination percentage (100), speed of germination (8.0) and seedling length (19.5 cm) were 56, 7, 17.6 and 19.6 percentage higher than control. The same trend was observed after six months of storage also, by recording a percentage increase of 55, 30.9, 16 and 55, respectively, over control (Fig.3) (Table 2). The data recorded for electrical conductivity (0.972 dSm^{-1}), protein content (4.1 %), dehydrogenase enzyme (OD value), and amylase activity (70 mm), after six months of storage also revealed the superiority of hydropriming (12 h) (Fig.4) (Table 2) compared to control and other priming methods. The corresponding values recorded by control were 1.568 dSm^{-1}, 3.6 %, 0.141 OD value, and 15 mm, respectively. The data proved that primed seeds had better membrane integrity and higher synthetic rates, even after six months of storage. It reveals that seed priming is in fact, capable of improving the storage life of beet root seeds.

Discussion

Seed priming has been demonstrated to improve germination and emergence in many seeds particularly vegetables (Demir Kaya et al., 2006). The positive effect has been attributed to the initiation of biochemical changes which include breaking of dormancy, imbibition, hydrolysis or metabolism of inhibitors and enzyme activation in primed seeds (Ajouri et al., 2004). Asgedom and Becker (2001) summed up that seed

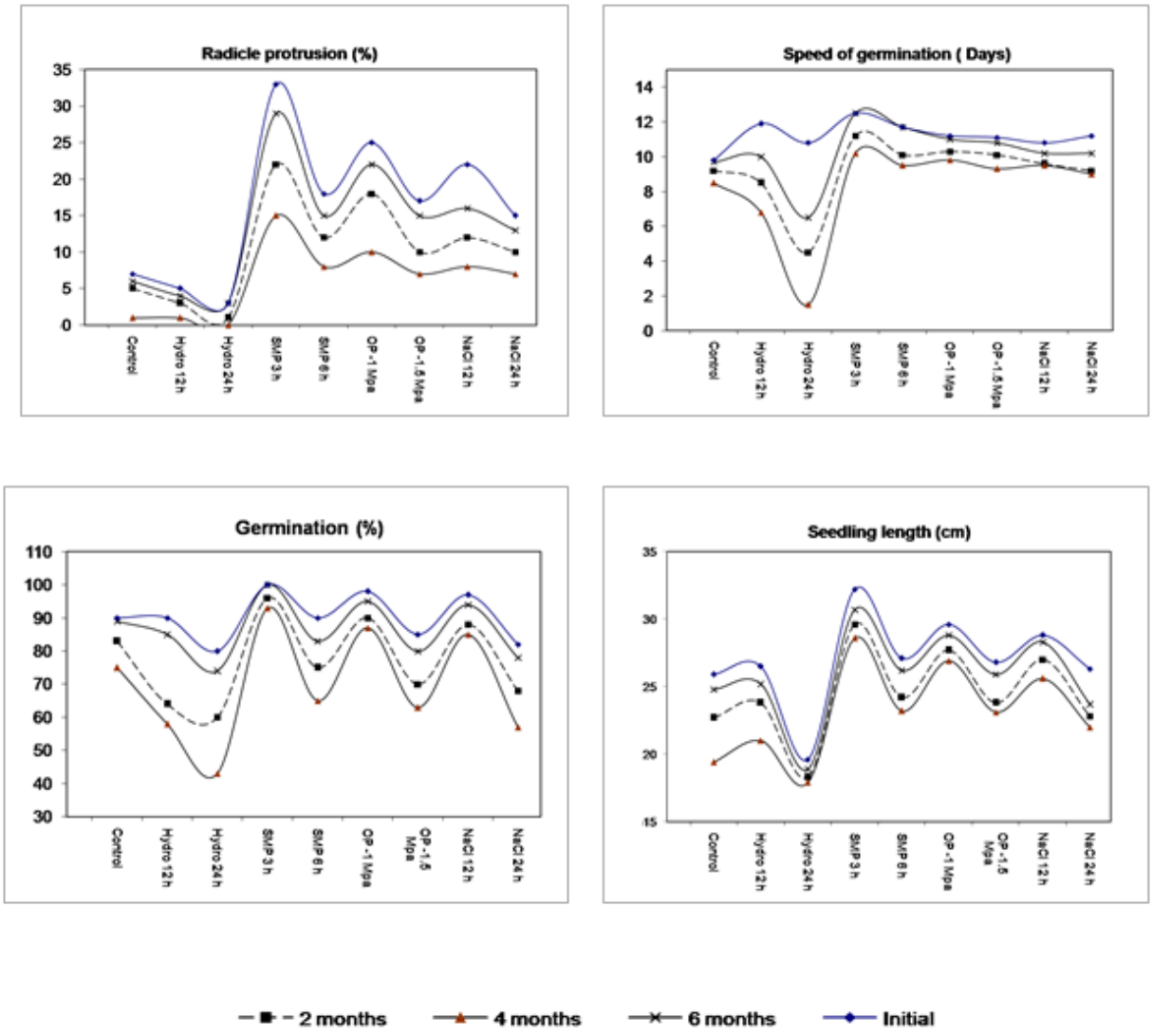

Fig1. Influence of different priming methods on radicle protrusion (%), speed of germination (days), germination (%) and seedling length (cm) of okra (*Abelmoschus esculentus*) seeds, as recorded over six months of storage, at bimonthly intervals.

priming triggers few or all pre-germination processes, which persist in the seed even after re-drying leading to quick re-imbibition and rapid revival of germination metabolism. Faster germination, uniform seedling emergence (Halmer, 2004) and increased shoot length and root length of seedlings observed in primed seed might be due to the induction of above metabolic activities in the seed embryo (Wahid et al., 2008). In close conformity with the above reports, the initial evaluation of primed seeds of okra and beet root revealed that priming methodologies invariably enhanced radical protrusion percentage, germination percentage, speed of germination and seedling length, except in the case of hydropriming, in okra. Negative impact of hydroypriming has already been reported in freshly harvested seeds of dry bean (*Phaseolus vulgaris* L.) particularly due to nutrient leakage (Abush Tesfaye and Modi, 2009). Since okra seeds also contain high protein content like dry bean seeds, which are highly hygroscopic, hydropriming could have negatively impacted seed quality as a result of excess nutrient leakage. It has also been noted that in okra, over six months of storage, longer duration of priming *viz.*, hydropriming (24 h), halo priming (3% NaCl, 24 h) and sand matric priming (60 % WHC, 6 h) were detrimental to seed quality (Fig.1). This negative impact could have been due to advancement of germination (during priming) to a state wherein seeds could have lost the desiccation tolerance (S' liwin´ska and Jendrzejczak, 2002). It is also contemplated that longer duration of priming could have resulted in dehydration damage and nutrient leakage as reported by Kalsa *et al.*, (2011) in hydro-primied seeds of in Common vetch (*Vicia sativa* L.). Among the treatments, sand matricpriming with 60 % WHC for 3 h recorded the highest improvement in terms of seed germination and seedling vigor as well as biochemical parameters, in okra *viz.*, lowest electrical conductivity (dSm^{-1}), and highest protein content (%), dehydrogenase activity (OD value) and ∞-amylase activity (mm), throughout the six months storage period (Fig 2). Solid matrix priming had also improved germination of hot pepper seed by 10–16 % depending on temperature (Pandita et al. 2007). Amylase, protease and lipase are important enzymes which have great role in initial growth and development of embryo; any increase in their activity leads to rapid initial growth of the embryo (Abnavi and Ghobadi, 2012). Unlike okra, in beet root, all the seed priming treatments were found to be superior to unprimed control seeds, irrespective of priming method and duration, both initially and after six months of storage (Fig 3). Optimum method of seed priming has improved seed membrane integrity as revealed by the

Table 2. Statistical significance of beetroot (*Beta vulgaris*) seed germination and biochemical characters given in Fig 3 and Fig 4.

	Priming Method (T)	Period (P)	TXP
Radicle protrusion (%)			
SEd	0.30	0.26	0.79
LSD (0.05)	0.59	0.52	1.56
Speed of germination			
SEd	0.07	0.06	0.18
LSD (0.05)	0.13	0.12	0.35
Germination (%)			
SEd	0.41	0.36	1.08
LSD (0.05)	0.80	0.71	2.12
Seedling length (cm)			
SEd	0.22	0.19	0.58
LSD (0.05)	0.43	0.38	1.14
Electrical conductivity (dSm^{-1})			
SEd	0.003	0.002	0.006
LSD (0.05)	0.006	0.004	0.011
Protein content (%)			
SEd	0.08	0.05	NS
LSD (0.05)	0.15	0.10	NS
Dehydrogenase (OD value)			
SEd	0.004	0.003	0.009
LSD (0.05)	0.009	0.006	0.017
α-amylase (mm)			
SEd	0.06	0.04	0.12
LSD (0.05)	0.11	008	02

decreased electrical conductivity besides improving the enzyme activity and protein synthesis thereby enhancing the metabolic potential of the seeds. All these positive biochemical activation in fact culminated in the better speed of germination, seed germination and seedling growth, in the primed and stored seeds, compared to control (unprimed) seeds (Fig 4). Kalsa, et al. (2011) has also reported that there was no significant reduction in germination percentage, speed of germination and emergence index of Common vetch (*Vicia sativa* L.) seeds stored upto two years as compared to freshly harvested seeds. Seed priming increased the membrane stability, presumably due to repair of cell membrane damaged during seed deterioration (Ruan et al., 2002; Arif et al., 2008). Bijanzadeh et al. (2010) observed that hydropriming and solid matrixpriming had a positive effect on membrane stability and minimized the seed electrical conductivity in rapeseed. Basra et al. (2003) and Dearman et al.(2008) reported that subjecting the seeds to priming before storage enhanced the seed longevity by activating antioxidant enzymes in seeds which can scavenge reactive oxygen species and lower lipid peroxidation (Farooq et al. 2009; Braccini et al.2000, Pukacka and Ratajczak, 2005). According to Braccini et al. (2000) hexanal accumulation in seed during storage may be controlled through priming. The increase in seed protein content subsequent to priming might have been due to synthesis of LEA (late embryogenesis abundant) proteins, if dried slowly, or heat shock proteins, if dried at high temperatures. Both the proteins offer protective mechanisms that help to improve the storage life of seeds (Gurusinghe et al., 2002). Water imbibitions during seed priming process leads to initiation of germination process, DNA cloning, RNA activity and consequently protein synthesis, which leads to improvement in seed germination and seedling growth (Bailly et al.2000; Ashraf and Foolad, 2005). Bedi et al. (2005) reported that hydropriming of brassica seeds increased the total protein content. Enzymes such as amylase, protease and lipase play an important role in initial growth and development of embryo. Singh et al. (1999) reported that priming of muskmelon seeds helped to increase the amylase and dehydrogenase activity thereby increasing the germination rate in saline conditions. As observed in the present study, detrimental effects of seed priming on storability of seeds are largely due to negative impact of extended durations of priming. Yongging 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 endo-duplication 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. Thus, the decrease in storability may be a feature of adverse effects of over priming or extending the pre-hydration period too long (Tarquis and Bradford, 1992).

Materials and Methods

Plant materials

Seed priming is a potential tool to reduce cold related seedling emergence problems. Beet root (*Beta vulgaris)* is grown in higher hill regions of India where in case of advanced seeding, low soil temperature often delays seedling emergence and increases the probability of soil crusting thereby disrupting seedling emergence. On the contrary okra (*Abelmoschus esculentus*) is a summer crop grown in plains with great intolerance to frost; okra seedlings do not emerge when temperature is below 20^{o}C. In the present study, seeds of okra (*Abelmoschus esculentus*) cv. Arka Anamica and beet root (*Beta vulgaris)* cv. Detroit Dark Red with 9 and 8 % moisture content, respectively were submitted to seed priming protocols standardized by Nirmala and Umarani (2008) *viz,,* (i) hydropriming (ii) halopriming iii) osmopriming under room temperature (33 ± 2°C) and iv) sand matricpriming at 25 ± 2 ^{o} C, 100 % RH. The moisture content of primed seeds at the end of the treatment was about 35%. The priming methodologies followed are detailed here under:

Seed priming treatments

Hydropriming

Okra seeds (10 g) were soaked in water at four times the volume of seed, while beet root seeds (10 g) were soaked at double the volume both for a period of 12, and 24 h followed by shade drying.

Sand matricpriming (SMP)

The seeds of each crop were weighed upto 10 g with four replications. Six trays (25 × 15 × 10 cm) were filled with 7 kg of sand to which water was added @ 180ml Kg^{-1} of sand to

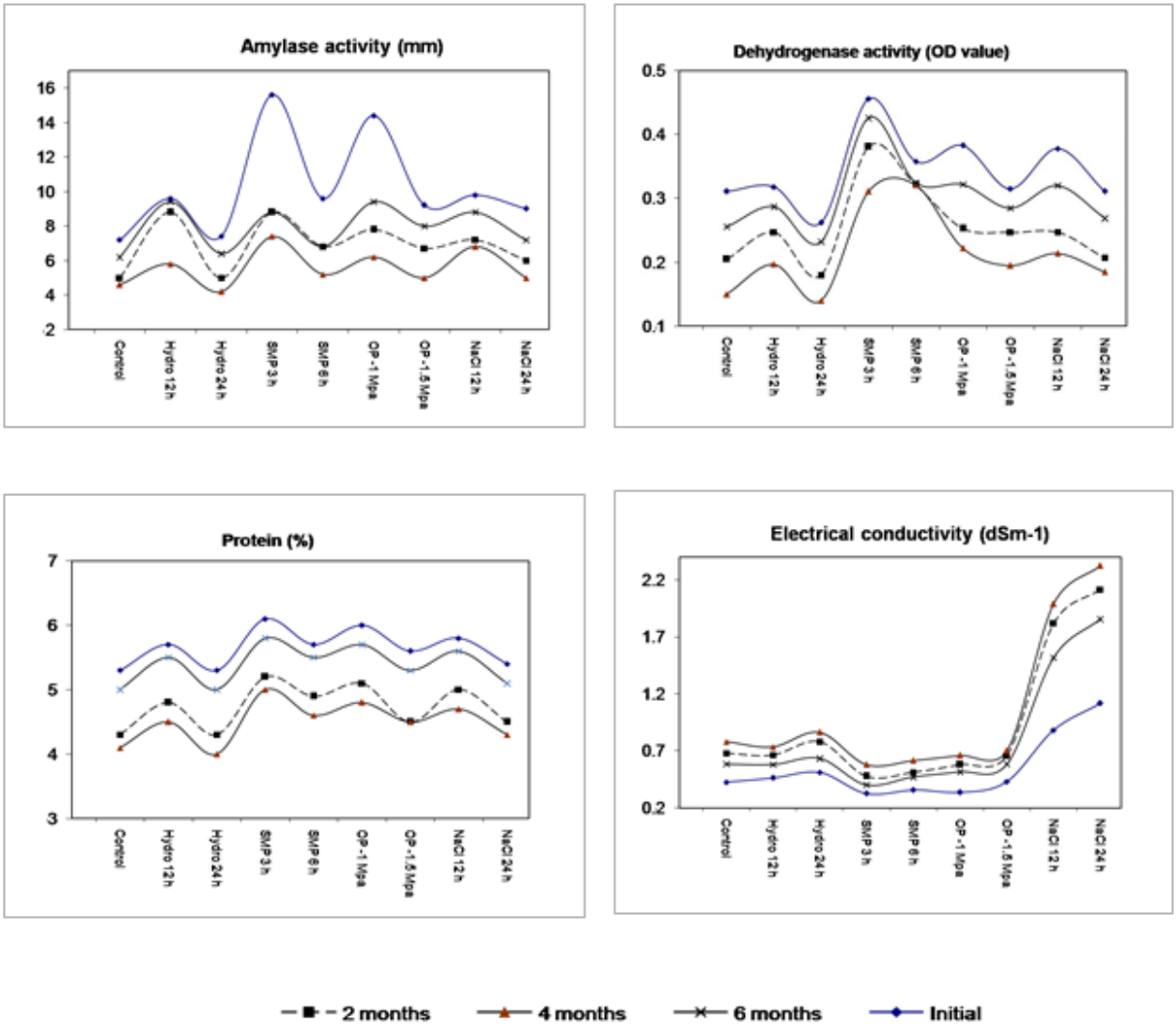

Fig 2. Influence of different priming methods on amylase activity (mm), dehydrogenase activity (OD value), protein content (%) and electrical conductivity (dSm^{-1}) of okra (*Abelmoschus esculentus*) seeds, as recorded over six months of storage, at bimonthly intervals.

create water holding capacity (WHC) of 60 per cent. The seeds mixed with such sand were placed in perforated plastic covers, and placed deep in the tray filled with sand of the same WHC. This ensured uniform seed-substrate contact. Seed samples were retrieved after 3 and 6 h for okra and 4 and 5 days for beet root and shade dried to original moisture content.

Halopriming

Halopriming treatment was conducted with NaCl for okra and KNO_3 for beet root. The salt solution at a concentration of 3 % was prepared and respective seeds were soaked for 12 and 24 h. After priming, the seeds were removed from the solutions, rinsed in running tap water and shade dried.

Osmopriming

Osmopriming was done using polyethylene glycol 6000 (PEG 6000) solution. Solutions with osmotic potential of -1.0 and -1.5 MPa were prepared by dissolving 273 and 342 g of PEG 6000 in one liter of water, respectively (Nienow and Bujaski, 1991). Seeds were soaked for 1day and subsequently rinsed thoroughly in in distilled water and shade dried. After the soaking period, seeds were air- dried to original moisture content under shade for three days at room temperature (33 ± 2°C). Later the seeds were subjected to initial assessment of improvement in germination potential and vigor by subjecting to germination test with four replicates of 100 okra seeds in sand and 100 beet root seed balls in paper roll towels. The untreated seeds of okra and beet root served as control to compare the performance of primed seeds. The test conditions were 25 ± 2°C and 95 ± 5% RH, illuminated with fluorescent light. The seeds were checked daily upto 14 days for radicle protrusion. The seeds showing less than 3.0 mm protrusion were expressed as percentage of protrusion of the radicle. The speed of germination was calculated according to Maguire (1962). After 14 days, germinating seeds which showed normal development of root and shoot alone were counted and expressed as germination percentage. The seedlings were measured from the tip of the root to tip of the growing meristem of the shoot and mean seedling length was expressed in cm.

Seed Storage studies

The seeds were dried to 8 % moisture content and packaged in aluminium foil bag. The containers were kept under ambient conditions (33 ± 2°C and 57% RH) for six months.

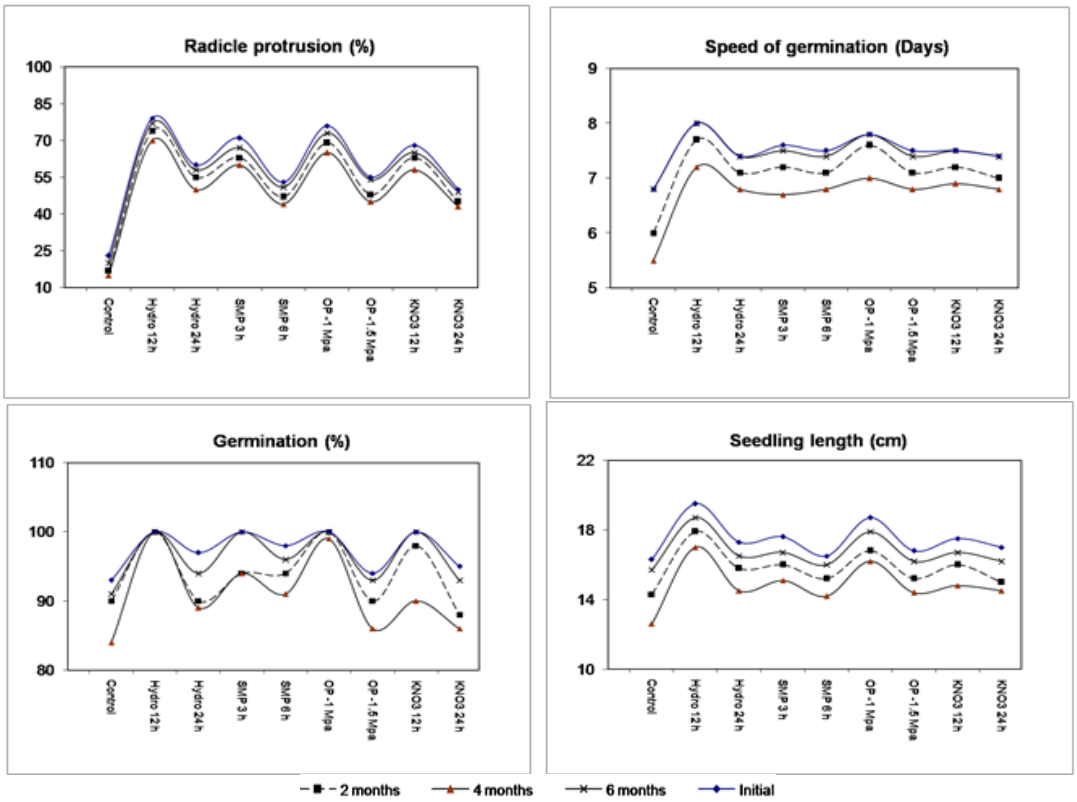

Fig 3. Influence of different priming methods on radicle protrusion (%), speed of germination (days), germination (%) and seedling length (cm) of beet root (*Beta vulgaris*) seeds, as recorded over six months of storage, at bimonthly intervals.

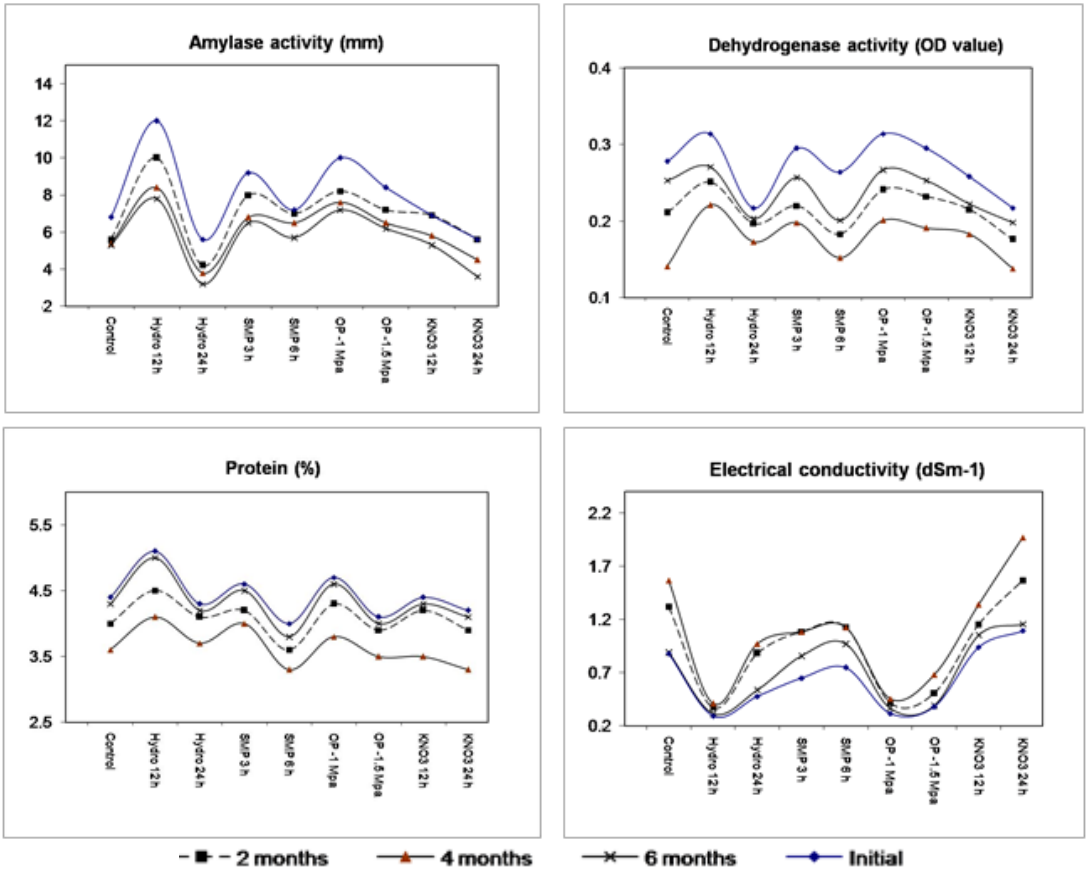

Fig 4. Influence of different priming methods on amylase activity (mm), dehydrogenase activity (OD value), protein content (%) and electrical conductivity (dSm^{-1}) of beet root (*Beta vulgaris*) seeds, as recorded over six months of storage, at bimonthly intervals.

Seed samples drawn initially and subsequently at bimonthly intervals were subjected to germination test with four replicates of 100 seeds in between paper towel as stated above. After the test period, observations were made on percentage of radical protrusion, speed of germination, germination percentage and seedling length (cm). The seeds were also analyzed 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).

Experimental design and statistical analysis

The applied experimental design was a two-factor factorial experiment arranged in completely randomized design; with three replications and 50 seeds per replication. The first factor was priming treatments while the second factor was storage periods. Result data (in percentage) were transformed to arcsine values before statistical analysis in order to unify the variance of the data (Ansari et al., 2012). The data were statistically analyzed using analysis of variance and treatment means were compared using LSD test at 0.05 level of probability, when the F-values were significant (Steel and Torrie, 1984).

Conclusions

Based on the results of the present study, it is recommended that better improvement in seed quality with good storability can be obtained in okra by subjecting the seeds sand matricpriming (60 % WHC) for 3 h while for beet root seeds, hydropriming for 12 h in water (double the volume of seeds) can be adopted. Primed seeds can safely be dried back to original moisture content and stored in sealed aluminium foil pouches to harness maximum efficacy of priming treatments and better storability. The results underscore the importanceof adopting optimum duration of priming, specific to each crop since lower durations may not be sufficient to achieve maximum effect of priming treatment, while longer duration of priming may permanently damage the seed quality altogether. This in conformity with Mubshar et al. (2006) who stated that improvement in priming is largely dependent on factors such as plant species, water potential of priming agent, priming duration, temperature, seed vigor and storage condition of primed seed.

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