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Optimization of Priming Benefits in Tomato (*Lycopersicon esculentum* M.) and Changes in Some Osmolytes During the Hydration Phase

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Abstract: This study was intended to detect optimum benefits of seed priming in tomato with respect to treatment solutions, after priming storage period and concomitant changes in certain osmolites. For this target, seeds of two varieties of tomato (*Lycopersicon esculentum* M..), namely Castle rock and Super strain B were osmoprimed for 7 days in PEG 6000 (20%), K_2HPO_4 (200 mM) and KNO_3 (250 mM) solutions, at 25°C in dark. Air-dried primed seeds were sown either directly (without storage), or after dry storage for 2 or 4 weeks. PEG was superior in enhancement of the germination percentage and rate, as well as growth vigor and uniformity of transplants (45-day-old) at the different storage periods. The increased growth rate was concomitant with evoked levels of photosynthetic pigments in leaves of transplants. Significant results were also obtained on osmoconditioning with K_2HPO_4, but to a lower extent than PEG, whereby KNO_3 exhibited least efficiency. Castle rock seeds showed higher positive responses to priming processes than those of Super strain variety. Maximum significance of the priming benefits was shown on direct sowing of seeds without storage period. This substantiated that the priming benefits of tomato seeds are gained during the hydration phase with no requirements to the following drying phase. Long storage periods of osmoprimed Castle rock seeds (in PEG or K_2HPO_4) up to six months indicated that priming benefits could be maintained till 2 months and then started fading out afterwards. The contents of soluble sugars, free amino acids, proline and glycine betaine were compared in the control seeds, hydroprimed and those osmoprimed in PEG or K_2HPO_4 after 8 h, 3 and 7 days. Interpretation of the results augmented variations between the control seeds channeled to germination and corresponding quiescent seeds in the osmotica from 8 h to 7 days.

Key words: Tomato seeds, hydropriming, osmopriming, Polyethylene Glycol (PEG), K_2HPO_4, KNO_3, dry storage, germination, transplant growth, photosynthetic pigments, soluble sugars, free amino acids, proline glycine betaine

INTRODUCTION

Rapid and uniform field emergences of seeds are two essential pre-requisites to increase yield, quality and ultimately profit in annual crops[1]. Slow germination ability of some seeds results in smaller seedlings and consequently plants[2]. This also makes such seedlings more vulnerable to soil-born diseases[3]. Uniform performance of seeds of cultivated plants is seldom achieved, where a seed represents an amalgam of individuals, each with different germination vigor[4]. For these reasons, seed priming has become a common treatment to increase the rate and uniformity of emergence in many vegetable and flower species, where it results in a more rapid and uniform germination when the seeds are re-imbibed[5]. Physiologically, seed priming allows the seeds to imbibe water and goes through the initial stages of germination, but does not permit radicle protrusion through the seed coat[6].

Seed osmopriming, using either Polyethylene Glycol (PEG), inorganic salt solutions, or both (each separately) improved germination potential of some herbaceous perennials[7], pigeonpea[8], brassica[9], carrot[10], sorghum and pearl millet[11], tomato, pepper and cucumber[12], burmodagrass[13], *Nigella damascena*[14], sugar beet[15] and lettuce[16].

The rate of germination (inverse of time to germination) was enhanced as a result of seed priming[17-19]). Other researchers also reached the same conclusion, where a reduction in T_{50} (time to 50% germination) was recorded in response to seed priming of many plants[15,20-22]. Enhanced growth and hence yield, as a result of seed priming, have been also recorded by a relatively large number of research researchers[15,20,23-25].

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One of the most critical aspects of seed priming is the longevity of dry storage period through which the benefits of priming are still maintained. In this connection, Alvarado and Bradford[26,27] revealed that primed tomato seeds were more susceptible to loss of viability and vigor than unprimed seeds. Argerich and Bradford[28] revealed that the viability and germination of primed tomato seeds were severely reduced after 6 months storage at 30°C. Gradual loss of priming benefits with increased duration of dry storage was also recorded in many plants[29-31]. On the other hand, Drew *et al.*[32] concluded that the germination percentage of primed leek and onion seeds was not changed by drying and storage, but seedling development was adversely affected and the number of abnormal seedlings increased with extension of storage duration.

Arguments are continuous on how the osmoprimed seeds are kept quiescent but active, under the stressful effects of osmotica and complete germination when left in water during hydropriming. It is generally known that osmopriming increases Osmotic Pressure (OP) in the cell sap[33]. One of the ways that plant cells protect themselves against loss of water is by accumulation of compatible solutes or molecules which increase OP (osmolytes) and thus lower the value of free water potential (ψ).[34].

Thus, the present study intended to improve germination potential of seeds and uniform growth vigor of transplant stands in two varieties of tomato (*Lycopersicon esculentum* M.), namely Castle rock and Superstrain B, in response to seed priming. It has been planned to optimize conditions with regard to the type of priming solution and its concentration, as well as the duration of priming (at 25°C). Detection of the critical dry storage duration, after which the priming benefits would start culmination, has also been targeted. It has been also aimed to investigate variations in osmolyte contents between the control seeds and those either hydro primed with water or primed in osmotica at narrower intervals during the priming course (after 8 h, 3 and 7 days). Tomato was selected as it ranks the first position among vegetables in Egypt due to its high nutritional value and various uses as fresh or processed form, for both local consumption and export (Economics and Statistics, Ministry of Agriculture, Egypt.)

MATERIALS AND METHODS

Pure lots of seeds of tomato (*Lycopersicon esculentum* M.) varieties Castle rock and Super strain B were obtained from the Seed Technology Department, Horticulture Research Institute, ARC, Ministry of Agriculture, Giza, Egypt, May/2003. The priming agents PEG 6000, K_2HPO_4 and KNO_3 were pure chemicals from Sigma-Aldrich Company.

Time course experiment: Seed priming of the two tomato varieties; Castle rock and Super strain B was carried out. On the basis of a preliminary experiment (results not shown), osmopriming duration lasted for 7 days in either Polyethylene Glycol (PEG) 6000 (20%), dipotassium hydrogen phosphate (K_2HPO_4; 200 mM), or potassium nitrate (KNO_3; 250 mM). To avoid fungal growth during priming, a fungicide topsin (1g L^{-1}) was added at a constant amount in the priming medium. Equal numbers of seeds were primed in petri dishes (12 cm diam.) on filter paper (Whatman No. 1); each containing a constant amount of the priming solution. The petri dishes were placed in a controlled-temperature cabinet (germinator) at 25°C in darkness for one week. After the priming period, the seeds were rinsed thoroughly in water, surface dried on filter paper, then spread on dry blotters and left to be air dried overnight on the lab bench. Seeds were stored in paper envelops at room temperature until planted after 0, 2, or 4 weeks. The primed seeds and the control (normal dry lot) were allowed to germinate in a glass green house using special trays, each with 82 pyramidal-shaped cells. The cells were filled with equal amounts of a commercial transplanting mixture [1 peat moss: 1 vermiculite (v/v)] amended with macro-and micro-nutrients and adjusted to pH 6.0. Fifty seeds, replicated four times from each lot and each treatment, were planted in the trays. Sowing was carried out so that each cell of the tray had one seed covered with 0.5 cm of the sowing mixture. The trays were watered regularly, with a constant amount of water, twice or three times per week, or as needed. The trays were checked daily and newly emerged shoots were recorded until this process was completed. Seedlings were considered as have emerged when the cotyledons became free above the soil surface. Daily temperatures were recorded (maximum 25± 2°C and minimum 15±2°C), during the experimental period. The germination percentage and rate were calculated as mentioned by Edmond and Drapala[35]

At least 10 randomly choice transplants (45-day-old) of each treatment were taken for measurements of growth criteria: shoot length (cm), root length (cm), number of leaves per plant and fresh and dry weights (g) per plant.

Statistical analysis was done, using the LSD. test to show significant ($p = 0.05$) and highly significant ($p = 0.01$) variations of the treated plants from their corresponding controls[36].

Table 1: Germination potential of seeds of two varieties of tomato (Castle rock and Super strain B) primed for 7 days, in 20% polythylen glycol (PEG) K_2HPO_4 (200 mM) and KNO_3 (250 mM), primed seeds were subjected to dry storage for 0, 2 and 4 weeks. Seeds of the control are normal (unprimed) dry lot. The rate of germination represents time (days) required for maximum germination. Each value is the mean of 4 replicates (each of 50 seeds)±SD

Castle rock

Stages →	Storage time (week) 0		2		4	
Priming Solutions	Germination percentage	Rate of germination	Germination percentage	Rate of germination	Germination percentage	Rate of germination
Control (0.0)	80±3.26	5.29±0.34	80±3.29	5.56±0.34	78.5±4.42	5.82±0.35
PEG (20%)	92±0.82	4.04±0.13	90±2.04	4.29±0.15	89±2.31	4.40±0.15
K_2HPO_4 (200 mM)	85±2.90	4.09±0.24	85±2.31	4.24±0.20	83±3.19	4.54±0.24
KNO_3 (250 mM)	82±2.04	4.26±0.21	81±2.31	4.98±0.29	80±2.61	5.00±0.29
LSD 5%	4.21	0.35	2.7	0.47	4.6	0.28
1%	6.05	0.51	3.89	0.67	6.7	0.4

Super strain B

Stages →	Storage time (week) 0		2		4	
Priming solutions	Germination percentage	Rate of germination	Germination percentage	Rate of germination	Germination percentage	Rate of germination
Control (0.0)	76±2.91	5.05±0.31	75±3.35	5.30 ±0.31	72±3.05	5.56±0.39
PEG (20%)	86±2.05	4.00±0.15	86±2.81	4.10±0.20	80±1.98	4.20±0.35
K_2HPO_4 (200 mM)	82±2.49	4.29±0.23	80±2.93	4.31±0.21	78±2.00	4.33±0.35
KNO_3 (250 mM)	80±2.83	4.76±0.23	78±2.99	4.96±0.26	75±2.51	5.16±0.39
LSD 5%	3.95	0.25	3.21	0.41	4.05	0.32
1%	5.81	0.43	4.90	0.59	6.10	0.49

Photosynthetic pigments were measured in the first fully expanded leaf from the top of transplants (45-day-old), following the method of Metzner *et al.*[37].

Determination of osmolytes during priming: Soluble sugars, free amino acids, free proline and glycine betaine were extracted from seeds of the control (dry seeds) and those hydroprimed for 8 h, germinated for 3 and 7 days and osmoprimed in PEG or K_2HPO_4 solution for 8 h, 3 and 7 days, following the same procedure mentioned above.

Soluble sugars: The method used for extraction and quantitative determination of soluble sugars were that described by Blakeney and Mutton[38]. Monosaccharides and sucrose (after alkali hydrolysis) were estimated colorimetrically at 620 nm, using anthron (0.1g anthron in 76% H_2SO_4).

Free amino acids: The method used was originally that of Müting and Kaiser[39] with slight modification as described in details by El-Araby[40] .

Free proline: Extraction and determination were done according to the method described by Bates *et al.*[41].

Glycine betaine: Extraction and estimation of glycine betaine content were carried out according to Grieve and Maas[42].

RESULTS AND DISCUSSION

Evaluation of the priming benefits with different priming solutions and short storage periods: An increase in the germination percentage was obtained in both tomato varieties (Castle rock and Super strain B) in response to priming treatments with PEG and K_2HPO_4 solutions, followed by different storage periods (0, 2, 4 weeks). In Castle rock seeds; this increase was highly significant in PEG solution. Osmopriming in K_2HPO_4 also showed a similar trend, but to a lower extent than PEG. On the other hand, a non significant increase from the control was shown on osmopriming in KNO_3 solution. Seeds of Super strain B showed also a similar trend, but with a lower magnitude of responsiveness, as compared to those of Castle rock variety (Table 1). These results agree with those of other researchers[12-14,16,25] in tomato as well as many other plants. The rate of germination (Table 1A and B), i.e. inverse of time to obvious germination (appearance of radicle) was also highly significantly enhanced in Castle rock seeds as a result of osmopriming with the differently used osmoconditioning solutions and with all storage periods. In Super strain B a similar trend was shown on using P EG and K_2HPO_4 solutions, whereby with KNO_3, a significant result was only obtained on direct sowing of the primed seeds (0 storage). Increased germination rate, was also found in tomato by Bradford and Haigh[17]. Enhanced germination rate

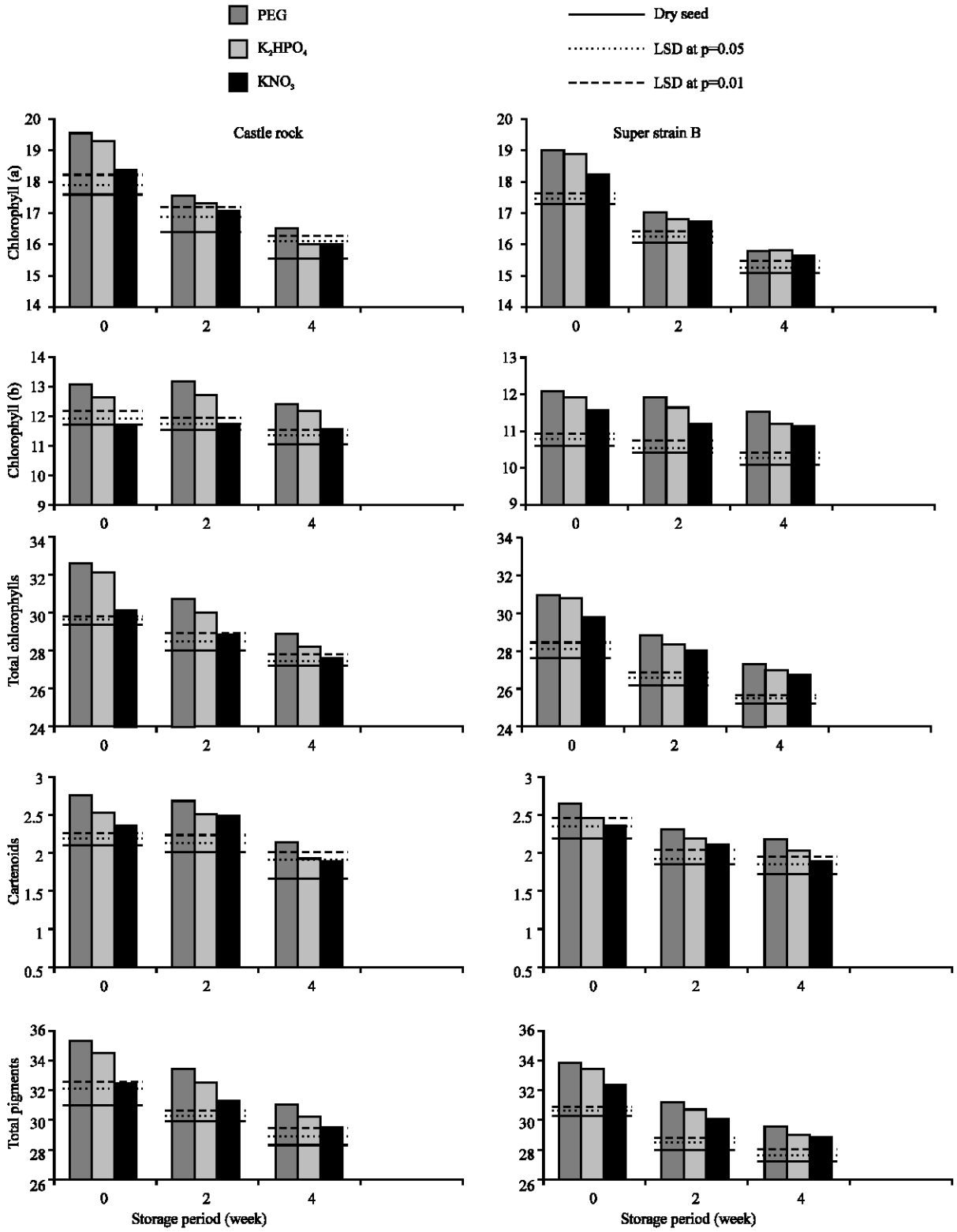

Fig. 1: Photosynthetic pigments ($mg^{-1}g$ f.wt.) of leaves of transplants (45-days old) from two varieties of tomato seeds (Castle rock and Super strain B) primed for 7 days in PEG(20%) K_2HPO_4 (200 mM), or KNO_3 (250 mM). Then dry stored for 0, 2 and 4 weeks, Each value is the mean of 3 replicates

Table 2: Growth criteria of transplants (45-day-old) from seeds of two varieties of tomato (Castle rock and Super strain B) primed for 7 days in 20% Polythylen glycol (PEG) K_2HPO_4 (200 mM) and KNO_3 (250 mM), primed seeds were subjected to dry storage for 0, 2 and 4 weeks. Seeds of the control are normal (unprimed) dry lots. Each value is the mean of 10 replicates±SD

Castle rock

Stages → / Priming solution		Length of shoot (cm)			Length of root (cm)			Number of leaves/plant		
		0	2	4	0	2	4	0	2	4
Control (0.0)		14.25±0. 85	13.51±1.05	13.32±0.58	6.5±0.28	6.15±0.37	5.74±0.64	6.20±0.47	5.88±0.85	5.75±0.70
PEG (20%)		17.28±0.43	16.03±0.43	15.07±0.09	8.25±0.12	7.06±0.07	6.78±0.29	7.67±0.18	7.75±0.50	7.50±0.41
K_2HPO_4 (200 mM)		16.63±0.43	15.37±0.50	14.25±0.46	7.83±0.16	6.57±0.17	6.32±0.23	7.40±0.30	7.25±0.50	6.70±0.87
KNO_3 (250 mM)		15.94±0.80	13.67±0.84	13.73±0.42	7.40±0.20	6.48±0.19	5.78±0.24	7.00±0.46	6.67±0.39	6.00±0.47
LSD	5%	1.12	0.95	0.55	0.48	0.33	0.38	0.61	0.99	1.04
	1%	1.16	1.37	0.79	0.70	0.47	0.55	0.88	N.S.	N.S.

Castle rock (Continue)

Stages → / Priming solutions		Fresh weight (g)/plant			Dry weight (g)/plant		
		0	2	4	0	2	4
Control (0.0)		1.49±0.39	1.25±0.18	1.20±0.23	0.15±0.03	0.13±0.02	0.12±0.03
PEG (20%)		2.28±0.12	2.05±0.05	1.72±0.03	0.22±0.00	0.20±0.00	0.16±0.03
K_2HPO_4 (200 mM)		2.21±0.14	1.65±0.12	1.46±0.12	0.19±0.00	0.17±0.00	0.15±0.02
KNO_3 (250 mM)		2.13±0.18	1.47±0.12	1.40±0.33	0.18±0.01	0.14±0.02	0.14±0.12
LSD	5%	0.40	0.21	0.26	0.026	0.019	0.014
	1%	0.58	0.30	0.37	0.037	0.028	0.020

Super strain B

Stages → / priming solutions		Length of shoot (cm)			Length of root (cm)			Number of leaves/plant		
		0	2	4	0	2	4	0	2	4
Control (0.0)		12.63±0.87	12.61±0.78	11.57±0.65	6.65±0.53	6.11±0.41	5.48±0.37	5.43±0.88	4.63±0.48	4.35±0.80
PEG (20%)		15.00±0.28	14.17±0.28	14.01±0.21	7.45±0.06	7.13±0.19	6.99±0.22	6.56±0.22	5.50±0.22	5.50±0.36
K_2HPO_4 (200 mM)		14.99±0.29	13.86±0.51	13.03±0.35	7.38±0.30	6.85±0.20	6.47±0.15	6.44±0.25	5.40±0.28	5.33±0.26
KNO_3 (250 mM)		13.97±0.41	13.13±0.70	12.31±0.50	7.27±0.38	6.57±0.35	5.84±0.12	5.96±0.55	5.13±0.39	4.99±0.75
LSD	5%	0.84	0.65	0.70	0.48	0.47	0.41	0.53	0.57	0.73
	1%	1.2	0.94	1.01	0.69	0.67	0.60	0.76	N.S.	N.S.

Super strain B (Continue)

Stages → / Priming solution		Fresh weight (g)/plant			Dry weight (g)/plant		
		0	2	4	0	2	4
Control(0.0)		1.03±0.21	0.99±0.17	0.97±0.19	0.093±0.11	0.093±0.08	0.089±0.09
PEG (20%)		1.56±0.08	1.54±0.05	1.48±0.08	0.15±0.03	0.14±0.01	0.13±0.00
K_2HPO_4 (200 mM)		1.49±0.11	1.44±0.07	1.35±0.15	0.13±0.06	0.129±0.02	0.11±0.02
KNO_3 (250 mM)		1.35±0.11	1.27±0.09	1.25±0.15	0.12±0.08	0.124±0.05	0.106±0.01
LSD	5%	0.20	0.17	0.25	0.021	0.021	0.014
	1%	0.28	0.25	0.36	0.031	0.031	0.020

Table 3: Germination potential of tomato seeds variety Castle rock primed for 7 days in 20% Polythylen Glycol (PEG) K_2HPO_4 (200 mM) and KNO_3 (250 mM), primed seeds were subjected to long dry storage for 0, 2, 4 and 6 months. Seeds of the control are normal (unprimed) dry lot. The rate of germination represents time (days) required for maximum germination. Each value is the mean of 4 replicates (each of 50 seeds)±SD

Storage time (months)	Priming solution	Germination (%)	Rate of germination
0	Control (0.0)	78.57±3.68	4.57±0.76
	PEG (20%)	91.48±1.13	2.84±0.11
	K_2HPO_4 (200 mM)	90.67±1.35	3.01±0.19
2	PEG(20%)	85.95±2.16	3.33±0.23
	K_2HPO_4 (200 mM)	83.95±2.84	3.40±0.20
4	PEG(20%)	78.36±3.0	4.59±0.50
	K_2HPO_4 (200 mM)	78.19±3.19	4.63±0.41
6	PEG (20%)	74.88±4.05	4.81±0.85
	K_2HPO_4 (200 mM)	74.00±3.96	4.90±0.92
LSD	5%	2.84	0.26
	1%	3.86	0.35

(expressed as time to 50% germination or T_{50}) was recorded in many plants, including tomato[15,20-22]. As mentioned above, the best performance of PEG in osmopriming tomato seeds agrees with the general views in the literature. In this instance McDonald[4] stated that PEG would be the preferred osmoticum because it is inert and its large molecular size precludes it from being taken up by the embryo. The positive effects of inorganic salt osmotica in seeds such as tomato is related to the presence of a selectively permeable tissue layer surrounding the embryo, which allows the uptake of water but prevents the diffusion of solutes into the seeds[4]. McDonald[4] added that seeds without such layer absorb the salts and become damaged. However, other research data are available where salts are in some cases superior

to priming in PEG with seeds of some plants including tomato[43,44]. Thus, it is herein recommended that different approaches to priming must be re-evaluated for each species and seed lot to determine best treatment.

Table 2A and B shows different growth criteria of the transplants (45-day-old) of the control and primed seeds. In Castle rock, a general trend was obvious concerning the extension growth of shoot and root, the number of leaves per plant, as well as the fresh and dry weights per plant. In this respect, the transplants of the seeds osmoprimed with PEG showed highly significant increases, as compared with those of corresponding control values after 0, 2 and 4 weeks storage. The results obtained on osmopriming Castle rock seeds in K_2HPO_4 solution were also significant but with a lower magnitude than PEG. On the other hand, priming in KNO_3 solution was mostly of non significant effect, except on sowing directly after water rinsing and surface drying of the primed seeds (0 storage). In seeds of Super strain B, a similar trend was also shown, but with a lower responsiveness to the priming solutions than in Castle rock seeds. Another interesting point was that PEG, particularly in Castle rock variety, resulted in maximum uniformity of the produced transplants with regard to the values of standard deviations of the different growth parameters (Table 2 A and B).

The results could be further confirmed by those obtained with photosynthetic pigment contents of transplants (Fig. 1). Thus, the values of chlorophylls (a) and (b) total cholorophylls, carotenoids and total photosynthetic pigments were generally significantly increased in response to osmopriming of seeds. The results obtained were in a clear alliance with those of the different growth criteria, where the magnitude of responsiveness of photosynthetic pigments was highest with PEG, followed by K_2HPO_4 and in Castle rock variety than Super strain B.

Enhancement of different growth parameters as a result of seed priming was also recorded by Al-Karaki[20] in wheat and barley, Lee *et al.*[23] in rice, Mauromicale *et al.*[45] in cucurbita pepo, Singh *et al.*[24] in sesame, Huang *et al.*[46] in spinach, Orzesko and Podlaski[15] in sugar beet and Groot *et al.*[25] in seeds of many plants for productivity under organic cultivation.

The results obtained in the present study showed maximum effect of priming on germination potential and transplant growth after zero dry storage. This agrees with the conclusion of Armstrong and McDonald[4] that osmopriming soybean seeds without further air drying treatment increased germination vigor as well as plumule and radicle length and seedling weight. But, when the seeds were air dried, the performance was decreased due to excessive cellular leakage of electrolytes from cracked cotyledons.

Effect of dry storage longevity, up to six months, on the priming benefits of castle rock seeds: In the previous experiment (experiment I), of tomato seeds variety Castle

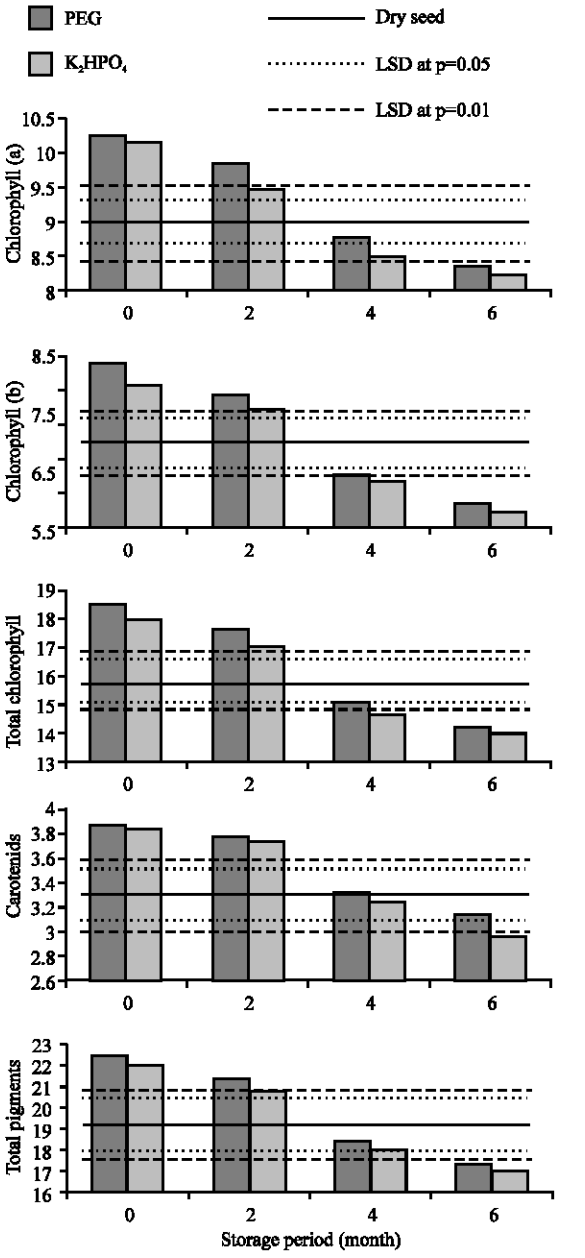

Fig. 2: Photosynthetic pigments ($mg^{-1}g$ f.wt.) of leaves of transplants (45-days old) from tomato seeds variety Castle rock primed for 7 days in PEG (20%) or K_2HPO_4 (200 mM), then dry stored for 0, 2, 4 and 6 months. Each value is the mean of 3 replicates

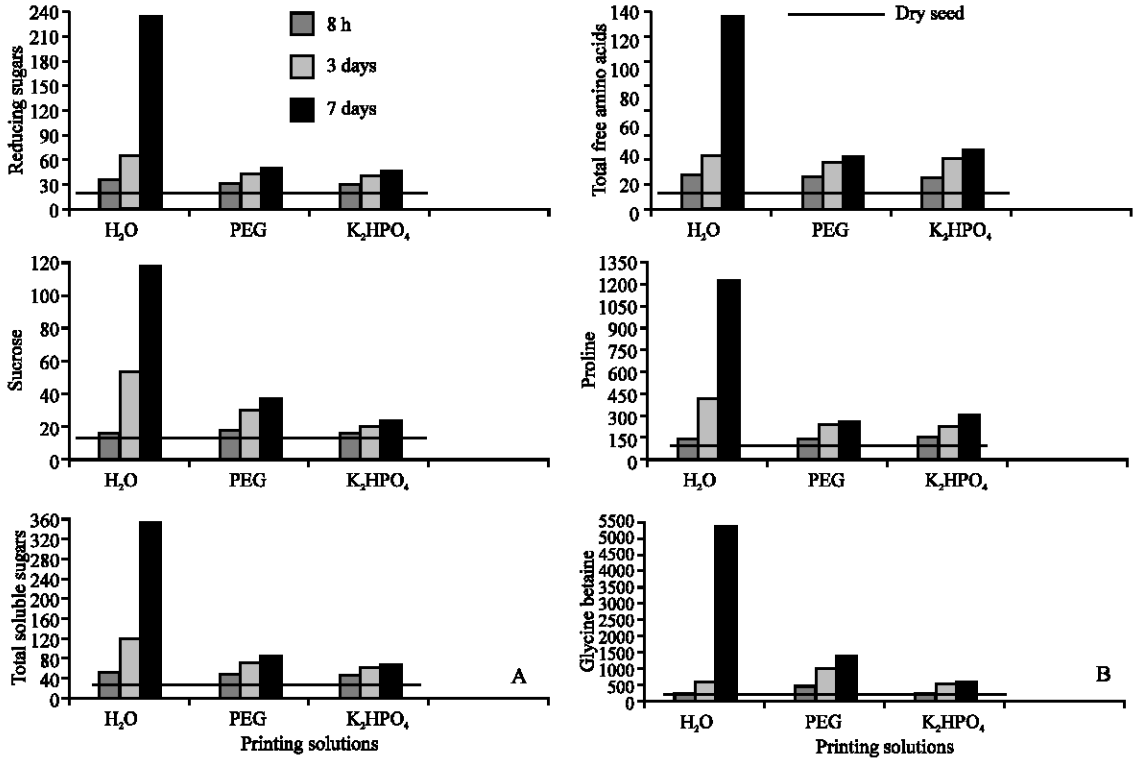

Fig. 3: Endogenous concentrations (mg/100 g d.wt.) of reducing sugars, sucrose and total soluble sugars [A] and Endogenous concentrations (mg/100 g d.wt) of total free amino acid, proline and glycine betaine [B] of tomato seeds variety castle rock hydroprimed and osmoprimed in PEG (20%) and K_2HPO_4 (200 mM) for 8 h, 3 and 7 days. Each value is the mean of 3 replicates

rock showed better responsiveness than those of Super strain B, with regard to priming effects. In addition, PEG followed by K_2HPO_4 solution achieved better performance than that of KNO_3. Consequently, the effect of long storage periods was investigated with Castle rock seeds, osmoprimed for 7 days in either PEG 6000 (20%) or K_2HPO_4 (200 mM) solution. This was carried out in order to detect the critical time of dry storage at which the beneficial effects of priming are still maintained. Thus, the germination potential of seeds and growth parameters of transplants (45-day-old) were recorded after 2, 4 and 6 months and compared with those planted directly (0 storage) after the priming treatment. In this respect, storage was carried out at room temperature in paper envelops which is an easy way that can be followed by common growers. The results obtained showed a progressive decrease in the percentage of germination of the PEG-primed seeds with lapse of time of dry storage (Table 3). The decrease in germination percentage in treatment with PEG, was 5.53, 13.12 and 16.60% after 2, 4 and 6 months storage, as compared with corresponding values unsubjected to storage (0 time). In case of osmopriming Castle rock seeds in K_2HPO_4 solution, the decrease in germination percentage after 2, 4 and 6 months was 6.72, 12.48 and 16.67%, respectively. The rate of germination was also continuously retarded with increased longevity of dry storage duration from zero to 2, 4 and 6 months, particularly on seed priming in K_2HPO_4 solution (Table 3).

The data in Table 4 show that the length of shoot and root of transplants was also statistically decreased as a result of storage of the seeds osmoprimed with PEG or K_2HPO_4 for 4 and 6 months. Similar trends were also obtained with regard to the number of leaves and the fresh and dry weights per transplant (Table 4) as well as their photosynthetic pigment contents (Fig. 2). Thus, it is recommended not to store air dried osmoprimed Castle rock tomato seeds for more than two months in order to maintain priming benefits, putting into consideration that maximum results are attained after direct sowing of the primed seeds, i.e. without storage (0 storage). In this connection, McDonald[4] stated that even though primed seeds are subsequently redried, it is at a more advanced physiological point than before priming and more porne to deterioration. Gradual loss of priming benefits during dry storage for different periods was also recorded in many plants[29-32]. From another point of view, it could also be concluded in the present study that the dry storage

Table 4: Growth criteria of transplants (45-day-old) resulting from tomato seeds variety Castle rock primed for 7 days in 20% Polythylen Glycol (PEG) K_2HPO_4 (200 mM) and KNO_3 (250 mM), primed seeds were subjected to dry storage for 0, 2, 4 and 6 months. Seeds of the control are normal (unprimed) dry lots. Each value is the mean of 10 replicates±SD

Storage time (months)	Priming solution	Length of shoot (cm)	Length of root (cm)	No. of Leaves/plant	Fresh weight (g)/plant	Dry weight (g)/plant
0	Control (0.0)	14.60±1.20	6.30±0.58	7.33±0.57	2.01±0.36	0.20±0.09
	PEG (20%)	18.55±0.17	7.66±0.23	8.80±0.28	3.77±0.12	0.37±0.03
	K_2HPO_4 (200 mM)	18.39±0.19	7.50±0.28	8.75±0.28	3.43±0.14	0.33±0.03
2	PEG (20%)	18.20±0.21	7.60±0.19	8.76±0.29	3.29±0.13	0.33±0.03
	K_2HPO_4 (200 mM)	18.15±0.20	7.43±0.22	8.70±0.31	3.03±0.19	0.32±0.05
4	PEG (20%)	14.80±0.30	6.38±0.28	7.30±0.44	2.50±0.21	0.23±0.06
	K_2HPO_4 (200 mM)	14.50±0.36	6.30±0.39	7.20±0.49	2.29±0.20	0.22±0.06
6	PEG (20%)	12.90±0.45	6.09±0.61	6.95±0.65	1.88±0.41	0.19±0.18
	K_2HPO_4 (200 mM)	12.66±0.68	6.00±0.59	6.90±0.65	1.69±0.43	0.18±0.18
LSD	5%	0.86	0.53	0.3	0.66	0.037
	1%	1.16	0.73	0.41	0.9	0.05

period which followed the first hydration treatment phase did not comprise an effective requirement for the priming process in tomato seeds. In contrary, some types of seeds complete their priming efficiencies during dry storage. For example, Dell'Aquila and Tritto[47] revealed that optimum effects of osmopriming wheat seeds were achieved 2 weeks after dry storage.

Changes in free sugars, amino acids, proline and glycine betaine during priming: There are different arguments in the literature with respect to how the osmoprimed seeds are kept active under the stressful effects of osmotica. In this connection, seeds can be hydroprimed by misting or soaking in water and redrying them before they complete germination[4]. This is different from osmopriming, where the seeds remain quiescent in the priming solutions. Castle rock tomato seeds were found in the present work to achieve complete germination (radicle is visual) after 3 days, whereby after 7 days, the radicle showed measured length. Consequently, the tomato seeds were considered to be hydroprimed if left in water for 8 h, followed by air drying. If the seeds are afterwards left in water for 3 and 7 days they undergo normal germination. It is generally known that osmopriming increases Osmotic Pressure (OP) in the cell sap[33] and one of the ways in which cells protect themselves against loss of water is by accumulation of molecules which increase OP[34]. The results obtained in the present study showed a steep elevation, from 8 h to 3 and 7 days, in the percentage of soluble sugars (monosaccharide, sucrose and total soluble sugars) in hydroprimed and germinated seeds (Fig. 3A), as well as in free amino acid contents (Fig. 3B). In osmoprimed seeds, a gradual increase was shown in these sugar fractions and free amino acids, throughout the duration of the experiment (Fig. 3A and B). Thus, the sharp increase in soluble sugars and free amino acids in the hydroprimed seeds were supposed to resemble products of the metabolic machinery in the embryonic cells channeled to germination. On the other side, the increase in the osmoprimed seeds was assumed to participate in increasing the OP values and meanwhile to indicate signs of restricted metabolic activity, which would be below the threshold permitting germination. In this respect, osmopriming was found to be associated with some biochemical processes and enhancement of respiration[48,49], where energy metabolism was found to increase with osmotic treatment, mainly due to increased number of mitochondria[50,51]. Increased sugar contents in osmoprimed tomato seeds were also found by Cayvela *et al.*[52]. Min *et al.*[53] also revealed increments of both soluble sugars and free amino acids in tomato seeds in response to osmopriming in PEG, where sugar elevation was attributed to enhanced α-amylase activity. Lee and Kim[54] reached a similar conclusion in rice seeds. Other researchers[55-58] stated that the increased sucrose contents (at the expense of oligosaccharides) decreases the intracellular glass stability, thus increases the mobility of molecules in the cytoplasm. Thus, the drawback of reduced longevity of primed seeds[5] might be attributed to increased sucrose: oligosaccharide ratios, where oligosaccharides are known to play a key role in the acquisition of seed desiccation tolerance[58]. Thus, it was assumed in the present study that soluble sugars and free amino acids are produced, at different levels, in primed as well as germinated seeds where a checkpoint exists which controls the transition from potential to actual germination. At such point when hydroprimed seed is dried it inters a quiescent state similar to osmoprimed seed.

Free proline and glycine betaine (Fig. 3B) showed approximately comparable enhanced concentrations in both hydroprimed and osmoprimed (in PEG or K_2HPO_4) seeds after 8 h imbibition. These contents were progressively elevated in seeds under germination and gradually increased in the primed seeds throughout the priming duration. At the end of experimental period (7 days), the folds increases in proline (over the values at 8 h) were 8.4, 1.6 and 2 in H_2O, PEG and K_2HPO_4, respectively. Corresponding increases in glycine betaine were 19.8, 2.5 and 2.3 in the same sequence. The evoked

levels in the germinated seeds might be a concomitant of increased number of cells due to obvious germination. However, the role of proline and glycine betaine as compatible solutes in protection against dehydrative damage is well established[59,60].

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