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Responses of tomato seeds to hydro- and osmo-priming, and possible relations of some antioxidant enzymes and endogenous polyamine fractions

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ABSTRACT

The efficiency of hydropriming seeds of Casle rock tomato or osmopriming with polyethyleneglycol (PEG) or K_2HPO_4 was studied. Sowing was also carried out after 0, 2, and 4 weeks following ambient air drying of the primed seeds. The results showed that 8-hour hydropriming or osmopriming in PEG or K_2HPO_4 had non significant effects, which would minimize the influence of hydropriming. But, a progressively increased enhancement was evident with lapse of osmopriming duration for 3 and 7 days, with a better potential of PEG than K_2HPO_4. Seed germination, subsequent growth of seedlings, and uniformity of transplants (45-day-old) showed best results on osmopriming for 7 days, ambient drying, and then direct sowing (zero dry storage). The activities of catalase and peroxidase enzymes were progressively increased with lapse of time of priming with a higher responsiveness to K_2HPO_4 than with PEG. The recorded number and relative band concentrations of peroxidase isozymes were also in agreement with the assayed peroxidase activities in the differently treated seeds. A noticeable decrease of malondialdehyde (MDA) concentration was also observed with increasing osmopriming duration from 8 hours to 7 days, particularly with PEG. Determination of endogenous polyamines (PAs) showed progressive decrease of higher polyamines (spermidine and spermine), especially spermine, with the increase in the duration of osmopriming by PEG and K_2HPO_4.

Keywords: Tomato; Priming; Catalase; Peroxidase; Lipid Peroxidation; Polyamines.

INTRODUCTION

Seed priming is used as a technique to enhance seed performance, notably with respect to rate and uniformity of germination (Taylor *et al.* 1998), thereby improve seedling stand and enable better crop establishment (Job *et al.* 2000). The strategy of seed priming is to perform a controlled water uptake by the seeds during the rapid water imbibitional phase and up to the end of the following lag period (germination *Sensu stricto*) phase (Bewley 1997). It appears likely that priming enhances seed performance by initiating the early events of germination up to but still below the threshold of cell division (Gurusinghe *et al.* 1999). Metabolic changes that take place during priming are not enough to induce radicle protrusion (McDonald 2000). Enhancement of germination percentages was achieved in response to seed priming in some vegetable crops (Suzuki and Khan 2000) and flower species (Cantliffe 1997).

The rate of germination (inverse of time to germinate) and improvement of seedling stands were also accelerated as a result of seed priming in tomato (Corbineau *et al.* 2000), pepper (Lee *et al.* 1997), as well as carrot, lettuce, and onions (Jeong *et al.* 2000b). Variation in the results depended on temperature, priming duration, concentration of the priming chemical, and the crop type (Jeong *et al.* 2000 c&d).

An important factor is to determine how long the benefits last during dry storage of seeds, following priming (McDonalds 2000). However, the general rule in this connection is that primed seeds should be considered vigorous but without prolonged storage periods. This rule was obvious with many plants such as leek (Maude *et al.* 1994), pepper (Lanteri *et al.*

1997), and sweet corn (Chang & Sung 1998). Drew *et al.* (1997) demonstrated that while the percentage germination was not affected by dry storage, seedling growth was adversely affected and the number of abnormal seedlings increased with storage duration.
The opportunity to gain best results with seed priming has inspired investigation into the physiological principles controlling this process. Considerable evidence exists that repair of DNA, RNA, protein, membranes and enzymes occur during imbibition (McDonalds 2000). An interesting point in this respect is the possibility that priming increase the activity of enzymes that counteract the effects of lipid peroxidation. Thus, Jeng & Sung (1994) found that free-radical scavenging enzymes were increased by increasing hydration of peanut seeds. Chiu *et al*. (1995) also showed that priming-enhanced membrane repair in watermelon seeds could be ascribed to evoked activities of several lipid peroxide-scavenging enzymes. A similar conclusion was also reached by Chang & Sung (1998) with sweet corn seeds. Another important subject is the possible cell damage with the increase in time of dry storage, following seed priming (Drew *et al*. 1997). Other studies, however, suggest that the maximum beneficial effects of priming are achieved during the drying phase, when enzymes and antioxidants are afforded sufficient time to affect repair and physiologically stabilize the seed (Dell' Aquila & Tritto 1991).

Thus, the present work intended to adjust optimum treatments for priming of tomato seeds. A central objective was to manipulate the duration of the priming treatment and dry storage in order to gain benefits of maximum germination potential as well as uniformity and satisfactory seedling stands. The activity levels of catalase and peroxidase, as well as peroxidase isozymes were studied together with lipid peroxidation levels. This trial, for gaining further refinement of the physiology priming, was also devoted to determine changes in endogenous polyamine fractions. To our knowledge, studies in this connection concerning the role of polyamines, if any, are scanty.

MATERIALS AND METHODS

Seeds of tomato (*Lycopersicum esculentum* cv. Castle Rock) were obtained from the Department of Vegetable Crop Research, Horticultural Institute, Agriculture Research Center, Ministry of Agriculture, Giza, Egypt.
On the bases of preliminary experiments, we selected two osmopriming solutions: Polyethylene glycol-6000 (20%) and K_2HPO_4 (200 mM), beside hydropriming in pure water. Constant numbers of seeds were primed in Petri-dishes (12 cm d.) on filter paper (Whatman No. 1), each containing a constant volume of the priming solution.
To avoid fungal growth during the priming process, a fungicide topsin (1g / L) was added to the priming solutions. The Petri-dishes were placed in a controlled-temperature cabinet (germinator) at 25 ℃ in darkness. Seeds were left in the different solutions for 8 hours, 3 days, and 7 days (during this period germination took place in the hydroprimed seeds). At the end of each priming period, the seeds were rinsed thoroughly in water, surface dried with filter paper, then divided into two groups.
Seeds of the first group were directly sown (0 storage), whereby seeds of the second group were stored in paper envelopes at room temperature for two and four weeks, and then were afterwards planted. Sowing of the above mentioned seeds of both groups and the control seeds (normal dry lot) was carried out in a glass green house, using special trays, with 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 were planted, for each treatment. Sowing was carried out so that each cell of the tray had one intact seed covered with 0.5 cm of the mixture. Fertilizer solution was applied with irrigation water. The trays

were cheked daily, and newly emerged plumules were recorded until this process was completed. Daily temperature was recorded (maximum 24± 2 ℃ and minimum 15 ± 2 ℃) during the experimental period.

The germination rate was calculated according to Edmond and Drapala (1958), as the mean number of days required for germination. At least 10 randomly choiced transplants (45-day-old), from each treatment, were taken for measurements of different growth criteria. Statistical analysis was done, using the least significant difference (L.S.D.) from the control (Snedecor & Cochran 1967). Determination of photosynthetic pigments was carried out in the youngest fully expanded leaves from the top, according to the method of Metzner *et al*. (1965).

Assaying the activities the enzymes catalase and peroxidase, detection of peroxidase isozymes, and quantification of lipid peroxidation and polyamine fractions were carried out in extracts of the primed and non-primed seeds as follows:

Catalase and peroxidase activities: Preparation of the crude extracts of catalase and peroxidase and assaying their activities was done according to the method of Kar & Mishra (1976) with some modification as described by Iturbe-Ormaetxe *et al*. (1998). Catalase activity was expressed as µmol H_2O_2 decomposed / g d. wt. equivalents min^{-1}. Peroxidase activity was expressed as change in optical density at 430 nm as a result of oxidation of substrate.

Peroxidase isozymes: The method described by Nehra *et al*. (1991) was followed to isolate peroxidase isozymes with some variation as follows: 0.5g fresh sample were grinded with liquid nitrogen in a mortar, and then 1.5 ml extraction buffer (Tris-borate: 0.125M at pH 8.9) were added. Samples were transferred to fresh tubes and kept at 4 ℃ for half an hour. The samples were centrifuged at 12.000 rpm at 4 ℃ for 20 min. The supernatants were used to fractionate the isozyme by PAGE as mentioned by Stegmann *et al*. (1985). A volume of 40µl extract of each sample was mixed with 10µl glycerol and 10µl bromophenol blue. Then, 50 µl of this mixture was loaded on the gel. The method described by Larsen & Benson (1970) was followed for the appearance of the bands of peroxidase.

Lipid peroxides: Lipid peroxides were extracted and determined according to the method described by Minotti & Aust (1987). Lipid peroxides were quantified using TBARS assay kit. 100µl of sample or standard were added to 100µl SDS solution, 2.5 ml TBA buffer, then the reaction mixture was incubated at 95 ℃ for one hour. The samples were removed from incubation and cooled to room temperature in an ice bath for 10 min., followed by centrifugation at 3000 rpm for 15 min. The absorbance of the supernatant solution was recorded at 532 nm. Malondialdehyde (MDA) was used as standard.

Polyamines: The extraction procedure was essentially that of Flores & Galston (1982), as adopted by Daebes (2000). Standards and plant extracts were dansylated according to Daebes (2000). For measurement of the different PA fractions: putrescine (Put), spermidine (Spd), and Spermine (Spm) by HPLC, the parameters described by Smith (1985) were applied.

RESULTS AND DISCUSSION

The effects of hydropriming tomato seeds for 8 hours or osmopriming with PEG or K_2HPO_4 for 8 hours, 3 days, and 7 days, are dealt with herein. Responses are also discussed with sowing after 0, 2, and 4 weeks after drying the primed seeds. In case of hydropriming, increased duration over 8 hours induced seed germination, so application of the priming treatment for 3 and 7 days was only restricted to the two used osmotica (PEG and K_2HPO_4).

Performance of germination and transplants

The results presented in Figure (1) show that 8-hour hydropriming and osmopriming in PEG or K_2HPO_4 did not significantly affect the germination percentage of Tomato seeds. A progressively statistical increase was evident with lapse of the priming duration to 3 and 7 days. In this respect, PEG gave better results than K_2HPO_4. Highly significant enhancement of seed germination was recorded when seeds of the above mentioned treatments were planted directly (0 storage) or after dry storage for 2 or 4 weeks. An inverse relation was obvious between the germination percentage and the longevity of dry storage following priming. Figure (1) shows that the rate of germination was also more or less similarly affected. Thus, it could be concluded that best results were obtained on priming with PEG for one week, followed by direct sowing (without dry storage).

The results in Figure (2) further indicated that 8-hours hydro-or osmopriming with PEG or K_2HPO_4 did not significantly affect the shoot and root length of tomato transplants (45-day old), as compared to those of non-primed seeds. These parameters generally showed a highly significant increase by increasing the duration of priming to 3 and 7 days, with a better performance of the latter. The values of standard deviations indicated that the uniformity of transplants showed higher superiority in priming for 7days, with a slightly better performance of PEG than K_2HPO_4, whereby direct sowing of the dried primed seeds or after 2 or 4 weeks showed no variation. The increase of beneficial effect of priming with lapse of time during priming was also found by many workers. For e.g., Ozbingol *et al*. (1998) mentioned that priming potential in tomato was maximum after 5-7 days, as compared to shorter periods. McDonalds (2000) interpreted this relation on the basis of the following equation:

$$GR_{50} = GR_i + k\,(\psi - \psi_{min})\,t_p$$

Where, GR_{50} is the median germination rate of primed seeds, GR_i is the initial median germination rate before priming, and t_p is the duration of priming treatment at water potential ψ. The linear proportionality constant k can be defined as the inverse of a hydropriming time constant that relates accumulated time in a prehydrated condition to a subsequent increase in germination rate (McDonalds 2000).

The results in (Figure 2) showed higher enhancement in the mean number of leaves per plant as a result of osmopriming with PEG or K_2HPO_4 on giving the treatment for 3 or 7 days. It was obvious that priming with PEG for 7 days followed by direct sowing (0 post priming drying) induced highest increase in this growth criterion.

Figure (2) also shows that the fresh and dry weights per transplant did not show significant differences from the control as a result of hydropriming for 8 hours. A highly significant increase was recorded, on the other hand, as a result of osmopriming with PEG or K_2HPO_4 at different durations of priming and post priming drying periods (0, 2, and 4 weeks). Maximum dry matter accumulation was recorded in response to priming for 7 days, followed by zero time drying, particularly on using PEG. This result was markedly in alliance with concomitant enhancement of the number of leaves (Figure 2) and the different photosynthetic pigments (Figure 3) of these plants. Changes in photosynthetic pigments showed inconsistent results regarding hydropriming or osmopriming with K_2HPO_4 for 8 hours. Longevity of the dehydration time after priming is generally known to be inversely related to germination potential and establishment of seedling stands in many plants (Lanteri *et al*. 1997, Chang & Sung 1998). However, Dell' Aquila & Tritto (1990) showed that optimum effects of wheat seed osmopriming were achieved 2 weeks after drying.

On the bases of the above mentioned results it could be concluded that priming with PEG (20%) for one week, followed by direct sowing after drying (zero dry storage) provided

best performance of germination, as well as transplant uniformity and stand of Castle Rock tomato. The performance of K_2HPO_4 solution followed that of PEG, whereby hydropriming was least efficient. This conclusion generally agrees with those of other research workers on tomato (Ozbingol *et al*. 1998, Corbineau *et al*. 2000). In contrary, Mauromicale & Cavallaro (1997) mentioned that priming tomato seeds with inorganic salt solutions was superior to priming in PEG. However, McDonalds (2000) reported that in instance of seed priming, PEG would be the preferred osmoticum because it is inert and its large molecular size precludes it from being taken up by the embryo, thus minimizing adverse effects on germination and subsequent seedling growth.

Catalase & peroxidase activities and peroxidase isozymes, in relation to lipid peroxides: The results in Table (1) show different enhancement levels of both catalase and peroxidase in response to hydropriming and osmopriming, using PEG and K_2HPO_4, for 8 hours, as well as 3 and 7days. In this experiment, seeds were farther left in the hydropriming medium (H_2O) for 3 and 7 days, as done with osmopriming, in order to follow the activities of the enzymes studied during early germination. It is evident that the activity of both enzymes was more or less similar after 8 hours in seeds of all treatments. Progressive enhancements were recorded with lapse of time in the different priming solutions. The magnitude of this change was more pronounced with respect to catalase than peroxidase. This increase was also relatively higher in response to priming with K_2HPO_4 solution, as compared to that of PEG.

The assayed activities of peroxidase were found to be consistent with the number and relative band concentration of peroxidase isozymes in the differently treated seeds (Table 2). These results showed a relatively higher number of bands (3) of peroxidase isozymes, in response to hydropriming and osmopriming by K_2HPO_4, as compared to priming with PEG (2 bands). It should also be added that the number of bands was increased from 2 to 3, only in treatment with K_2HPO_4.

It is generally accepted that repair of seeds deteriorated by lipid peroxidation occurs during hydration, mainly via production of antioxidants and repair enzymes. In this connection, many authors (e.g. Dell' Aquila & Tritto 1990, Jeng & Sung 1994, Chiu *et al.* 1995, Chang & Sung 1998, McDonalds 2000) stated that membrane repair could be ascribed to evoked activities of free-radical scavenging enzymes. Thus, the markedly enhanced levels of catalase and peroxidase activities with time, in the hydroprimed seeds, might indicate a higher deterioration rate, lower repair efficiency, or both, compared to corresponding osmoprimed seeds. This presumption could be further verified by the results in Table (1), showing quantified lipid peroxides as estimated by the values of endogenous malondialdehyde (MDA), in response to different priming treatments. These results further supported the above mentioned conclusion that osmopriming treatments with PEG and K_2HPO_4 were concomitant with a noticeable decrease of MDA concentration, with lapse of priming duration from 8 hours to 7 days. The levels recorded in the osmoprimed seeds, particularly with PEG, were markedly lower than those in corresponding hydroprimed seeds. Thus, it might be concluded that both hydropriming and osmopriming might start more or less similarly during the first 8 hours of treatments. But, at increased durations, it seems likely that transcripts of potentially active proteins in the osmoprimed seeds are retained during subsequent drying and are quickly reactivated when germination is allowed. The uniformity of seedlings of osmoprimed seeds might be ascribed to that their transcripts would be culminating in more rapid and uniform completion of germination than in hydroprimed or non-primed seeds.

The last part of this work focused on the changes in endogenous polyamines (PAs): putrescine (Put), spermidine (Spd), and spermine (Spm), as well as their total values. The results obtained (Table 3) showed a marked progressive drop of Spd and Spm, particularly

the latter, from 8 hours and afterwards till 7 days, in both the hydroprimed and osmoprimed seeds. But, in the hydroprimed seeds, especially after 8 hours and 3 days, total PAs were obviously higher than corresponding contents in the osmoprimed seeds. It should be noted that during this period (after 8 h and upwards), hydroprimed seeds showed obvious germination and radicle extension, whereby this did not occur in case of osmopriming solutions.

Thus, higher PAs (Spd and Spm) were assumed to interfere with switching on of germination processes during continuous hydropriming and its inhibition in osmoprimed seeds. As far as we are aware, there is no work that directly relates seed priming and PAs, but strong evidence exists that Spd and Spm (particularly Spm) are required for the G_1 to S transition during cell division (Kumar *et al*. 1997, Crozier *et al*. 2000). Another assumed prediction is the possible interference of PAs with seed repairing mechanisms during hydration, as discussed above, due to their polycationic nature (Tiburcio *et al*. 1997).

Although the part we have devoted to connect PAs and seed priming is premature, other possibilities of PA interactions still in mind. Polyamines were rather recently proved to interfere with embryogenesis (Crozier *et al*. 2000), so the question herein is: Do they participate in protecting the seed transcripts in some way during priming as does ABA during embryogenesis? The last point is based on the sharing of both PAs with ethylene in a common precursor S-adenosyl-L-methionine (Kumar *et al*. 1997). On the basis that ethylene is a candidate of germination (Obroucheva & Antipova 2000), so PAs might be tentatively assumed to interfere with the signal transduction cascade of ethylene during germination, and silencing during seed priming. However, extensive work is needed to verify the above mentioned presumptions.

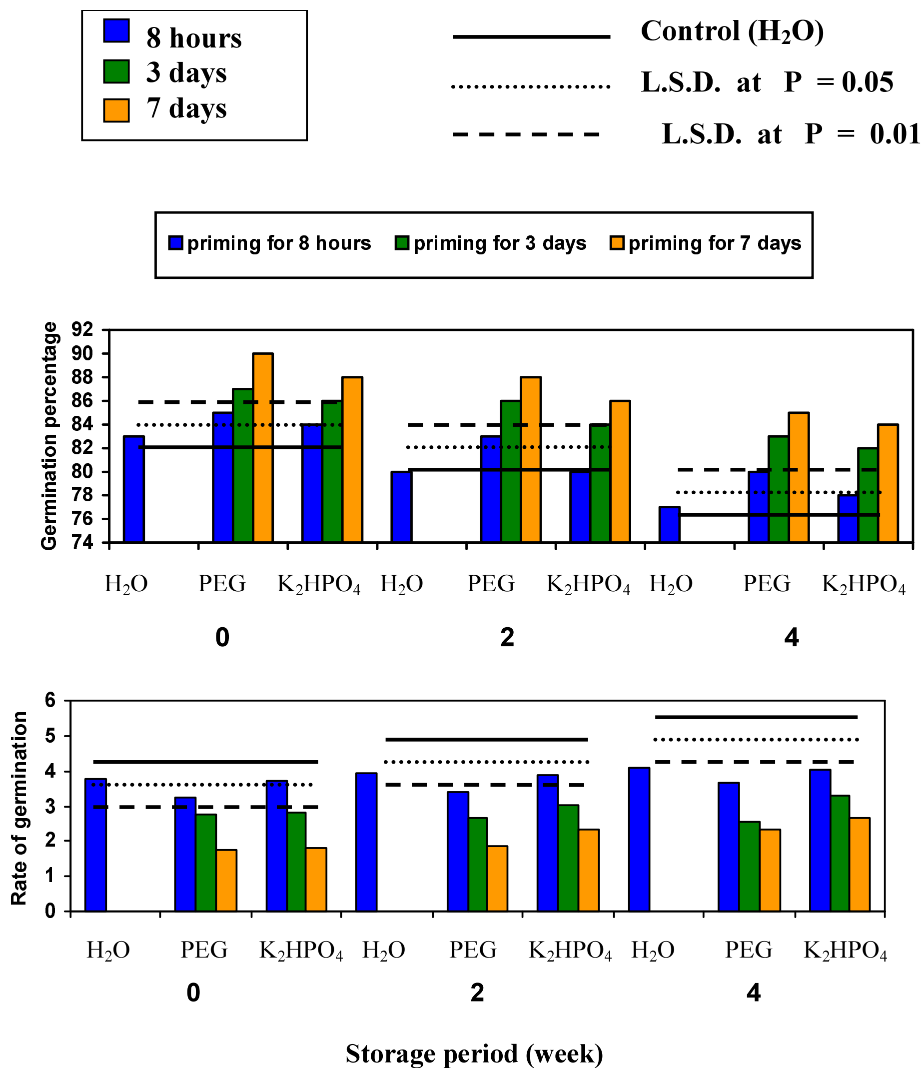

Figure (1): Percentage and rate of germination of Castle rock tomato seeds hydroprimed for 8 hours or osmoprimed in PEG (20%), or K_2HPO_4 (200 mM) for 8 hours, 3 days and 7 days, then dry stored for 0, 2 and 4 weeks .

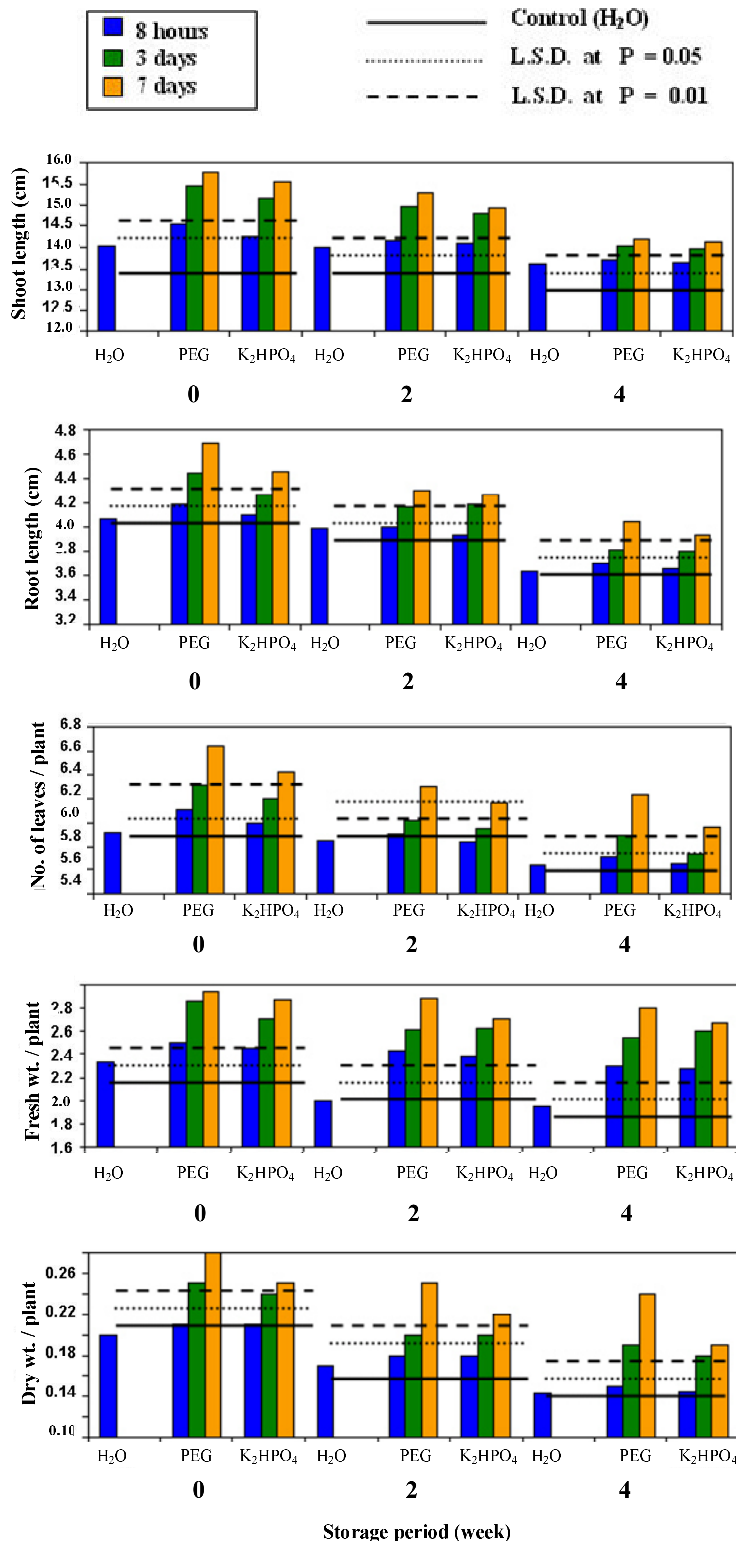

Figure (2): Growth parameters of Castle rock tomato transplants (45-day-old) from seeds hydroprimed for 8 hours or osmoprimed in PEG (20%), or K_2HPO_4 (200 mM) for 8 hours, 3 days and 7 days, then dry stored for 0, 2, and 4 weeks.

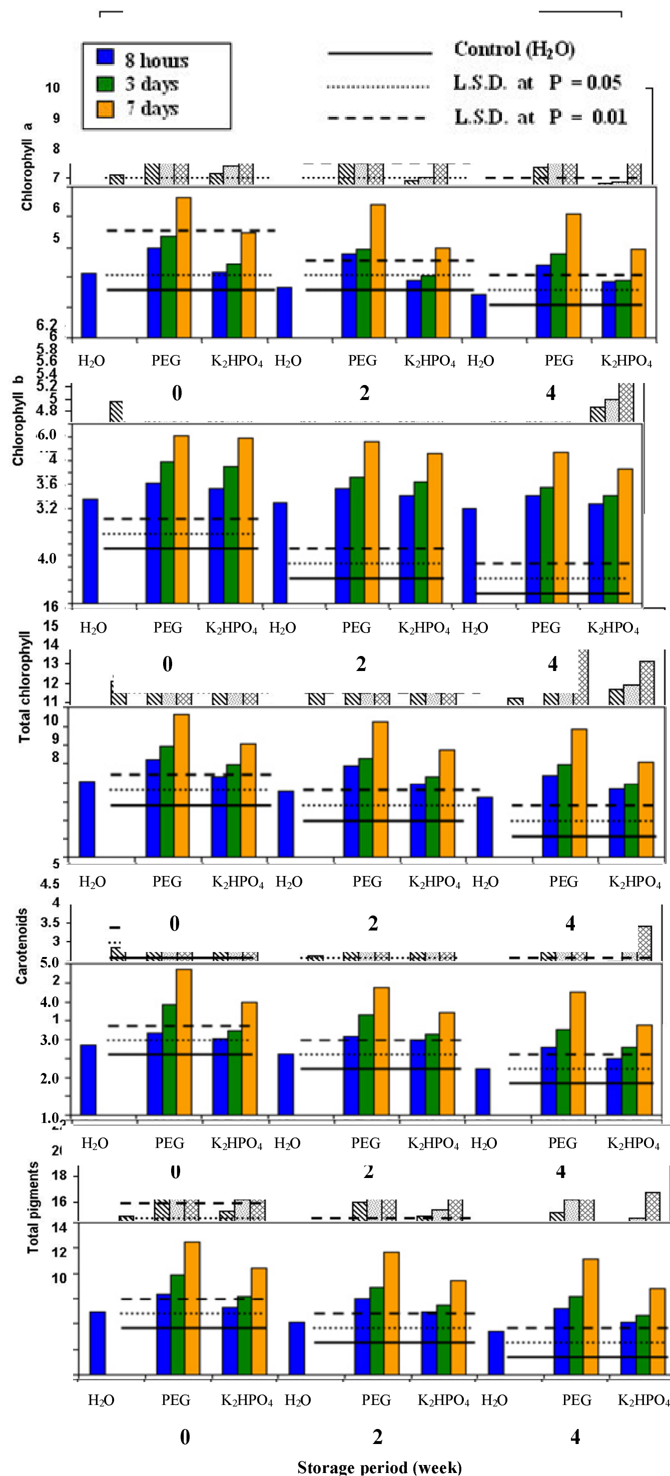

Figure (3): Photosynthetic pigments of leaves of Castle rock tomato transplants (45-day-old) from seeds hydroprimed for 8 hours or osmoprimed in PEG (20%), or K_2HPO_4 (200 mM) for 8 hours, 3 days and 7 days, then dry stored for 0, 2, and 4 weeks.

Table 1: Activities of catalase, (μ M H_2O_2 decomposed/g d.wt. equiv. min^{-1}) and peroxidase (change in optical density as a result of oxidation of substrate) and concentrations of malondialdehyde (MDA) (n m/g d.wt. equiv.) of hydro-and osmoprimed Castle rock tomato seeds.

Priming Solution	Time of priming	Enzyme activity		MDA
		Catalase	Peroxidase	
H_2O	0	360	300	2379
	8h	857	411	2454
	3d	6146	936	2995
	7d	3429	3857	5242
PEG (20%)	8h	857	434	2442
	3d	1180	689	787
	7d	1423	749	286
K_2HPO_4 (200 mM)	8h	943	611	1336
	3d	2387	916	1261
	7d	4246	1094	273

Table 2: Electrophoretic protein-banding patterns of peroxidase isozymes of hydro-and osmoprimed Castle rock tomato seeds.

Priming Solution	Time of priming	RF								No. of bands
		0.0	16	26	34	100	139	183	205	
H_2O	0	18.28	_	_	_	_	_	81.72	_	2
	8h	35.53	_	_	_	_	37.61	26.86	_	3
	3d	26.34	_	18.07	_	_	_	_	55.59	3
	7d	52.17	_	_	_	_	19.19	_	28.64	3
PEG (20%)	8h	34.17	_	_	_	_	_	65.83	_	2
	3d	30.5	_	_	_	69.5	_	_	_	2
	7d	27.35	_	_	_	_	_	72.65	_	2
K_2HPO_4 (200mM)	8h	21.49	_	_	_	_	_	78.51	_	2
	3d	10.94	_	_	14.06	_	_	75.01	_	3
	7d	11.92	_	_	_	41.95	_	46.13	_	3

Table 3: HPLC analysis for endogenous concentrations (mg/100 g d.wt equiv.) of Putrescine (Put), Spermidine (Spd), Spermine (Spm), and total polyamines (TPAs) of hydro-and osmoprimed Castle rock tomato seeds.

Priming Solution	Time of priming	Concentrations (mg/100 g d.wt. equiv.)			
		Put	Spd	Spm	TPAs
H_2O	0	5.7	31.88	N.S.	37.58
	8h	5.16	14.62	12.11	31.89
	3d	6.35	5.04	11.54	22.93
	7d	4.37	1.69	0.28	6.34
PEG (20%)	8h	3.66	10.43	1.42	15.51
	3d	5.02	8.55	1.80	15.37
	7d	4.50	1.63	N.S.	6.13
K_2HPO_4 (200 mM)	8h	0.42	1.60	1.59	3.61
	3d	1.12	0.43	0.86	2.40
	7d	0.38	0.94	N.S.	1.32

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الملخص العربى

استجابة بذور الطماطم للتهيأة المائية والأسموزية للإنبات وعلاقاتها الممكنة ببعض الإنزيمات المضادة للأكسدة وبمحتوى عديدات الأمين

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تمت دراسة كفاءة عمليتى التهيأة المائية و الأسموزية لبذور الطماطم (كاسيل روك) على نسب و معدلات الإنبات ونمو وتماثل الشتلات باستخدام كل من محلول بولىاثيلين جليكول وفسفات البوتاسيوم أحادى الإيدروجين. كما درس أيضا تأثير زراعة البذور مباشرة أو بعد أسبوعين أو أربعة عقب تجفيف االبذور فى الهواء بعد انتهاء فترة المعاملة. وأوضحت النتائج أن التهيأة المائية أو الأسموزية بكل من المحلولين المستخدمين قليلة الجدوى فى حالة المعاملة لمدة ثمان ساعات مما يقلل من أهمية التهيأة المائية نظرا لأن استمرار البذور فى الماء لأكثر من ثمان ساعات يؤدى الى انباتها. وقد تزايدت كفاءة المعاملات الأسموزية معنويا مع مد فترة المعاملة من ثلاثة الى سبعة أيام، حيث كان تأثير البولى اثيلين جليكول أفضل من فسفات البوتاسيوم. واتضح من النتائج أن أفضل المعاملات هى تلك الناتجة من الزراعة المباشرة للبذور بدون فترة تخزين. وقد لوحظ أيضا عند استمرار المعاملات لثلاثة أو سبعة أيام تزايد أنشطة إنزيمى الكاتاليز والبيروكسديز بصورة مضطردة، وبخاصة مع المعاملة بفسفات البوتاسيوم وقد تطابقت نتائج أنشطة انزيم بيروكسيديز مع عدد الحزم وتركيز البروتين لمتشابهاته الإنزيمية (Isozymes) المفصولة بالتفريد الكهربى. وعلاوة على ذلك، سجلت تركيزات مالوندايلدهايد (Malondialdelyde) انخفاضا ملحوظا مع تزايد فترة التهيأة الأسموزية من ثمان ساعات حتى سبعة أيام وعلى وجه الخصوص باستخدام محلول بولى اثيلين جليكول.

وأوضحت النتائج أيضا انخفاضا مضطردا واضحا فى عديدات الأمين وبخاصة الأعلى منها (سبرميدين وسبرمين) وخصوصا السبرمين وذلك مع تزايد فترات التهيأة بكل من فسفات البوتاسيوم والبولى إيثلين جليكول.