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The Effect of Priming on Tomato Rootstock Seeds in Relation to Seedling Growth

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Abstract: This study was carried out to determine the effect of primig treatments (2% KNO_3, 1% NaCl, 500 ppm GA_3) on seedling emergence and seedling growth of two tomato rootstock seeds for two experimental runs. Although all treatments were superior to control, KNO_3 was found the best treatment. Treatments were not significantly effective on final emergence but reduced significantly mean emergence time, T_{50} time to 50% emergence, $T_{1.5}$ time to reach 1.5 mm hypocotyl thickness and increased seedling size (fresh and dry weight). Although all treated ones had remarkably effective but the greatest reduction in coefficient of variation (CV) in KNO_3 treated seeds indicated that treatment also reduced plant to plant variation among the seedlings. Results indicated that priming is rather effective through reducing germination time than subsequent relative growth rate and a valuable tool to improve seedling quality in turn obtaining thicker and well-developed seedlings in rootstock tomato seedling production.

Key words: Tomato rootstock seeds, priming, seedling emergence

INTRODUCTION

Grafting in vegetables was first performed in Korea and Japan in the late 1920's and has been a widely used technique in vegetable seedling production in order to get the advantages of rootstock such as resistance to diseases, robust growth, nematode resistance, higher yield and plant development (Yetisir and Sari, 2003; Passam *et al*., 2005). Tomato is one of the major species in which grafting is performed intensively to increase the growth and robustness of the grafted cultivars. Estimated grafted tomato seedling production in 2006 is about 15 million in Turkey. This value gradually increases since grafted plants gave higher yield and resistant to adverse growing conditions (i.e., drought, salt, high temperature) (Hasan Ünal, Grow Seedling, Antalya, Turkey). Incompatibility, requirement of better conditions for grafted plants, time, space and the appropriate technique to be used are common problems that are encountered in grafted seedling production. However, one common phenomenon in grafted seedling production is slow and erratic emergence of rootstock seeds. Delayed emergence causes larger differences in thickness of rootstock that resulted in incompatibility, lowers the efficacy of grafting, reduces the efficiency of machine grafting and non-uniform grafted seedlings. Therefore, rapid, timely and synchronous germination of rootstock seeds is a necessity for ease rootstock-scion compatibility, fast and efficient grafted seedling production.

Priming is a presowing seed treatment in which seeds are exposed to an external water potential low enough to restrict germination by various means (i.e., PEG, inorganic salts, matric material, hydration) and yet permit pre-germinative physiological and biochemical activities (Bradford, 1986; Thornton and Powell, 1995; Taylor *et al*., 1998). Priming enhances seed performance by increasing germination rate and uniformity which will resulted in faster and better seedling development that was reported in various crop seeds (Pill, 1995; Warren and Bennet, 1997; Taylor *et al*., 1998; Powell *et al*., 2000). However, rootstock seeds are generally hybrid which are rather smaller than cultivated ones. It has also been known that pre-sowing treatment effects may change between species and even different lots within the same species (Parera and Cantliffe, 1994). Enhanced emergence, of tomato rootstock seeds will increase the quality (higher fresh and dry weight, thicker hypocotyls) of grafted seedling production. In turn this will allow the producers to save more energy, less time for grafting, ease the use of automation and efficient use of glasshouse space.

The objective of this study was then to determine the effect of priming treatment on emergence percentage, rate and seedling growth in tomato rootstock seeds.

MATERIALS AND METHODS

Two cultivars of hybrid tomato rootstock seeds (*L. esculentum* Mill. cvs. Kemerit F1 and Yedi F1) were

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obtained from Grow Seedling Company Antalya/Turkey. Seed moisture content was determined by the high-temperature oven method (130°C, 1 h) (ISTA, 1996) and was 8.1%. Seeds were kept at 5°C in air proof laminated foil packets until use.

Three hundred seeds of each cultivar were primed on top of filter paper moistened with 8 mL either of 2% KNO_3, 1% NaCl, 500 mg L^{-1} GA_3 solution and kept at 20±0.3°C for 4 days in the dark in 9 cm petri dishes (Bradford, 1985; Demir and Mavi, 2004). During the priming treatment, dishes were covered with cling film to prevent loss of liquid and priming solution was renewed everyday in order to prevent fungal infection and maintain the concentration of the solutions. At the end of the treatment, seeds were washed under tap water for 2 min, surface water was taken by filter papers and they were dried to the original moisture content (8±0.3%) at 20±2°C and 40-45% RH. Treatments were repeated twice (Run 1 and Run 2).

Emergence tests were conducted within 2 days after drying and set up with three replicates of 50 seeds in each treatment and run. Seeds were sown 1 cm deep in compost (Plantaflor, Humus Verkaufs, GmBH, Germany) in sandwich boxes (18×11×8 cm). Seedlings were grown in the growing cabinet at 20±2°C for 30 days. Light was provided by cool fluorescent lamps at the rate of 78 μmol m^2 s^{-1}. for 12 h day^{-1} by cool fluorescence lamps (Philips) on the seedling level. Relative humidity in the cabinet was maintained above 75% throughout the experiment to minimise water loss from the boxes. Watering was done with the equal amount of water and the same time of the day for each box (20 mL day^{-1}). In the growing cabinet, boxes were rotated every day to obtain uniform temperature during emergence. Appearance of hypocotyl hook on the compost surface was used as an emergence criterion and emerged seedlings were recorded daily (at the same time of the day). To determine seedling growth rate, following number of emerged seedlings stabilized, destructive harvests were taken on randomly selected and normally developed 20 seedlings of each replicate at 18, 22, 25 and 29 days after sowing. Individual seedling was cut above ground, cleaned, weight and hypocotyl thickness was measured 1 cm above the compost surface and expressed as mm/plant. Regressions were applied to these values in order to find out the effect of the treatments on time to reach seedlings to 1.5 mm hypocotyl thickness which is the optimum graftable level in rootstock seedlings ($T_{1.5}$). Immediately after seedlings were cut, fresh and subsequently dry weights were determined and expressed as mg/plant. Seedling dry weight was determined by drying at 80°C for 24 h.

In order to find out the effect of the treatments on uniformity, days to 50% of final emergence percentage (T_{50}) (Korkmaz *et al*., 2004) and mean emergence time (MET) was calculated (Ellis and Roberts, 1981). Both were expressed as days.

Duncan multiple range test was performed using SPSS as a randomized complete block design ($p<0.05$) in order to compare the means of the treatments. Moreover, regressions (R^2) were performed to find out the relationship between hypocotyl growth and days after sowing along with relationship between seedling fresh weight and days after sowing.

RESULTS

Although final percentages were not significantly differed, the primed seeds emerged earlier and maintained at higher level of emergence throughout the emergence period than control (Table 1 and Fig. 1). Control seeds started to emerge by 10-11 days while treated ones started to emerge by 6-8 days, being earliest in KNO_3 treatment (Fig. 1). GA_3 treatment gave the slowest emergence among all treated seeds. KNO_3 and NaCl treated ones had rather similar emergence pattern. In many of the counts emergence percentages of the cultivars did not differ significantly ($p>0.05$). Kemerit showed slightly higher emergence percentage than Yedi. This was conspicuous in NaCl and GA_3 treated seeds run 1 and counts between 9 and 17 days (Fig. 1). But this difference was little or none in final stages of emergence period (after 17 days) in all cases.

The lowest mean seedling emergence time was observed in KNO_3 treatment as 8.4, 8.1 in run 1 and 9.2 and 9.8 days in run 2 in Kemerit and Yedi cultivars, respectively. Mean emergence time was significantly lower in KNO_3 treatment than the rest in all cases except one, cv. Yedi, run 2 in which salt treatments did not differ significantly among each other (Table 1). The mean emergence time of NaCl and GA_3 treated seeds in general were inferior to that of KNO_3. The highest mean emergence time was recorded in control seeds in all cases (Table 1). Similarly, T_{50} (time to 50% emergence) was the

Table 1: Mean emergence time and final seedling emergence percentages of treated and control seeds of two tomato rootstock (Kemerit and Yedi) in the compost in two experimental runs

	Run 1				Run 2			
	Kemerit		Yedi		Kemerit		Yedi	
Treatments	FEP	MET	FEP	MET	FEP	MET	FEP	MET
GA_3	89a	10.7c	81a	11.8c	81a	11.5c	82a	11.9b
NaCl	81ab	9.6b	73b	10.6b	87a	10.5b	83a	10.0a
KNO_3	80ab	8.4a	83a	8.1a	85a	9.2a	86a	9.8a
Control	73b	14.1d	81a	14.1d	84a	14.6d	82a	15.0c

Means with the different letter (s) in the same line in each cultivar and run are significant ($p<0.05$), FEB: Final Emergence Percentage, MET: Mean Emergence Time, $p<0.05$

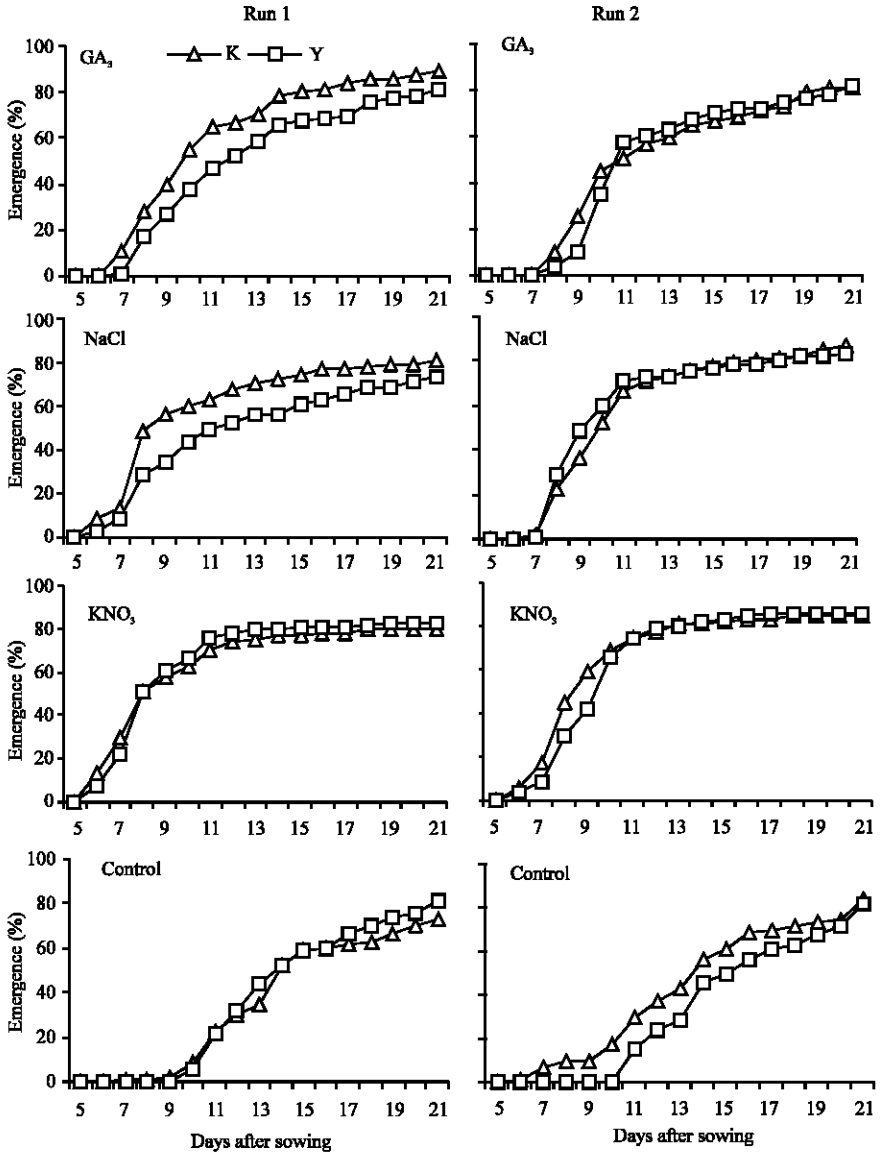

Fig. 1: Cumulative seedling emergence of primed (GA_3, NaCl, KNO_3) and control seeds of two tomato rootstock varieties (Kemerit: Δ Yedi: □) for two experimental runs.

lowest in KNO_3 treated seeds, varied between 5.9 and 6.7 days, which was reduced about 50-55% compared to control (10.3 and 11.8 days) (Table 2).

Destructive seedling harvests at four stages (18, 22, 25, 29 days after sowing) indicated that treatments increased seedling fresh weight significantly ($p<0.05$) compared to control except a few cases (Kemerit run 1, 25 and 29 days, Yedi, run 1, 25 and 29 days, run 2, 25 days) out of sixty four. Inorganic salt treatments gave higher seedling fresh weight but KNO_3 appeared to be slightly superior to NaCl treatment. At the final destructive harvest, maximum seedling fresh weight was reported in KNO_3 treated seeds in both cultivars with only one exception. But, differences between salt primed seeds was insignificant at the final stage ($p>0.05$). Similar trend was observed in seedling dry weight. Salt primed seeds gave higher dry weight compared to control and GA_3 but this was not significant in all cases. Particularly KNO_3 was

Table 2: Changes in T_{50} (time to 50% emergence) values in treated and control seeds of two tomato rootstock varieties (Kemerit and Yedi) in two experimental runs

	T_{50}			
	Run 1		Run 2	
Treatments	Kemerit	Yedi	Kemerit	Yedi
GA_3	7.4	9.2	9.1	9.2
NaCl	6.9	9.6	7.7	7.4
KNO_3	5.9	5.8	6.0	6.7
Control	11.5	10.9	10.3	11.8

better than NaCl. KNO_3 treated seeds produce seedlings that 21-25 mg higher dry weight content than NaCl (Table 3). Response of the cultivars to the treatments showed a similarity. Fresh and dry weight of control seedling did not change significantly. Moreover, in both cultivars the optimum treatment appeared to be KNO_3.

$T_{1.5}$, time to seedlings to become 1.5 mm thickness, reaching the graftable size, was reduced significantly by the treatments. KNO_3 treated seeds reached to this stage within 18.7-20.8 days while corresponding values were between 21.5 and 24.0 days and between 24.8 and 31.1 days, in NaCl and GA_3 treated seeds, respectively (Table 4). However, control seeds took between 31.9 and 36.5 days to reach the same hypocotyl thickness. KNO_3 treatment reduced the $T_{1.5}$ minimum of 12 days compared to control.

Hypocotyl thickness in Fig. 2 showed a linear increase throughout the development. Although treated seeds produced thicker hypocotyl seedlings than control in all harvests the maximum values among treated ones were observed in KNO_3 treatment. Regression (R^2) values between consecutive destructive harvests and hypocotyl thickness were changed between 0.80 and 0.99 but it was very high (>0.91) in thirteen cases (Table 5).

There was a correlation between mean emergence time and seedling fresh weight in tomato rootstock seeds (Fig. 3). Relations were significant and changed between 0.91 and 0.99 ($p<0.25$, 2 d.f.). Control seeds that emerged later or had longer MET produced smaller and weak seedlings. Contrarily, early emergers, (treated seeds) had lower MET values and produced heavier seedlings than control seeds. When Table 4 and Fig. 3 are compared it

Table 3: Changes in seedling fresh and dry weight of treated and control seeds of two tomato rootstock vareties (Kemerit and Yedi) for two experimental runs

	Kemerit				Yedi			
Days	GA_3	NaCl	KNO_3	Control	GA_3	NaCl	KNO_3	Control
Seedling fresh weight (mg/plant)								
	Run 1							
18	311b	377b	515a	138c	353b	398b	544a	126c
22	821bc	1025ab	1046ab	308d	626c	821bc	1271a	343d
25	810cd	1314b	1499ab	441d	834cd	1077 bc	1917 a	424d
29	1427bc	1936a	1861ab	749e	1291cd	1528abc	1958a	877de
	Run 2							
18	212c	305b	296b	119d	200c	324b	438a	111d
22	745c	791c	829b	223d	664c	1118a	1014ab	246d
25	672cd	1023ab	1033ab	355d	778bc	1310a	1145a	528cd
29	1303c	1761b	1855b	839d	1685b	2400a	2630a	1150cd
Seedling dry weight (mg/plant)								
	Run 1							
18	33b	36b	52a	13c	34b	38b	53a	11c
22	84ab	103a	98a	31c	62b	86ab	109a	34c
25	106c	153bc	184ab	52d	113c	137bc	201a	48d
29	194bc	275a	250ab	93d	156c	234ab	255a	100d
	Run 2							
18	22c	31b	30b	10d	20c	34ab	40a	11d
22	69bc	74bc	86ab	21d	61c	103a	93ab	24d
25	81bc	116ab	122a	43d	83bc	136a	120a	62cd
29	153de	226bcd	196cde	136e	244abc	287ab	312a	131e

Seedlings were destructively harvested after 18, 22, 25 and 29 days after sowing. Means with different letter(s) among the treatments and destructive harvests within the same cultivar and run are significantly different ($p<0.05$)

Table 4: Changes in $T_{1.5}$ (time 1.5 mm thickness) values as affected by priming treatment in tomato rootstock seeds in two cultivars (*L. sculentum* cvs. Kemerit and Yedi) and two experimental runs

Cultivar	Treatments	Run 1	Run 2
	GA_3	25.8c	31.1d
Kemerit	NaCl	22.5ab	24.0c
	KNO_3	20.8ab	18.7a
	Control	31.9d	36.5e
	GA_3	26.0c	24.8c
Yedi	NaCl	24.0b	21.5ab
	KNO_3	19.3a	19.8b
	Control	32.5e	32.0d

Means with different letter (s) in the same column and run in each cultivar are significant ($p<0.05$)

Table 5: R^2 values for the relationship between days after sowing and changes in hypocotyl thickness in tomato rootstock seedlings shown in Fig. 2 in relation to treatments

	Yedi		Kemerit	
Treatments	Run 1	Run 2	Run 1	Run 2
GA_3	0.985	0.856	0.932	0.920
NaCl	0.914	0.993	0.979	0.983
KNO_3	0.816	0.808	0.925	0.961
Control	0.948	0.981	0.962	0.968

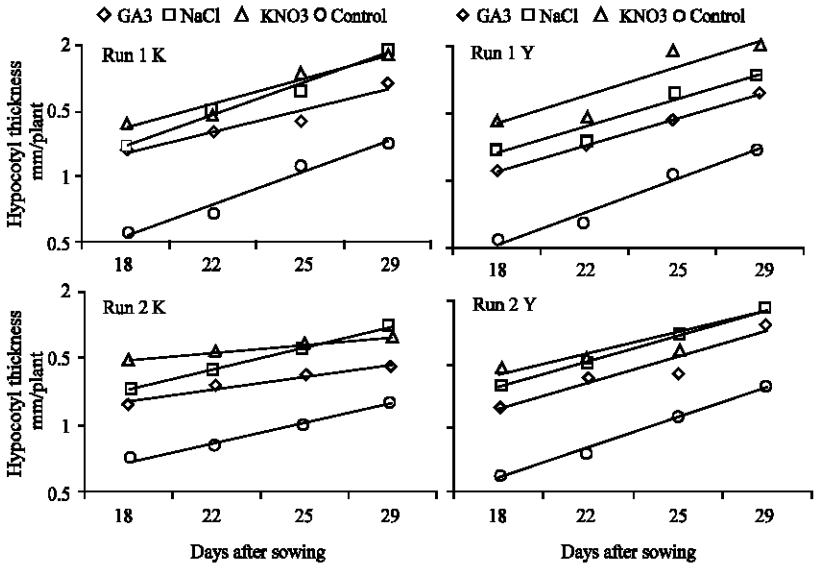

Fig. 2: Relationship between days after sowing and hypocotyl thickness (mm/plant) in primed and untreated rootstock tomato seeds (□: NaCl, ◇: GA_3, Δ: KNO_3, ○: Control). Measurements were taken in destructive harvests after 18, 22, 25 and 29 days after sowing. Experiment was conducted two times (Run 1 and 2) and on two cultivars (K: Kemerit, Y: Yedi). R^2 values were given in Table 5

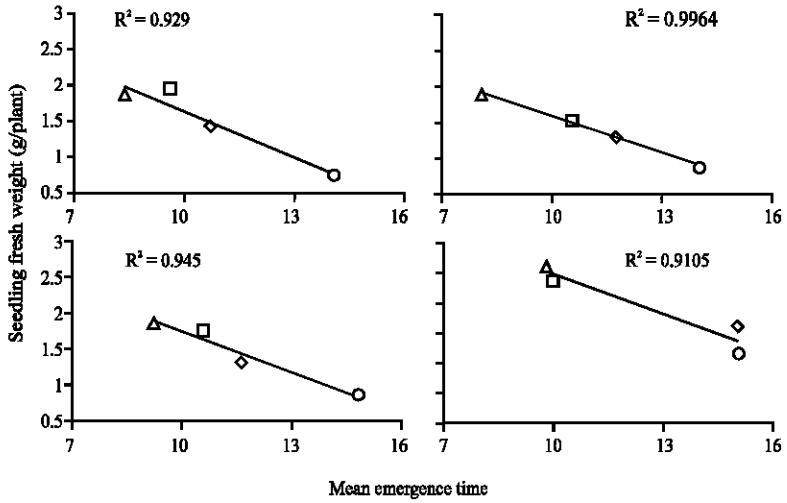

Fig. 3: Relationship between mean emergence time (day) and seedling fresh weight (g/plant) in treated and untreated tomato rootstock seeds. (□ : NaCl,◇: GA_3, Δ : KNO_3, ○: Control)

Table 6: Relationship between CV of seedling fresh weight 29 days after sowing and treatment types

Treatments	Control	GA_3	NaCl	KNO_3
Run 1				
Kemerit	30.25	5.07	2.97	2.37
Yedi	22.87	1.81	1.80	0.81
Run 2				
Kemerit	22.00	6.58	2.75	0.53
Yedi	13.49	1.73	1.86	0.47

can be concluded that earlier emerging seedlings produced not only high fresh weight and larger seedling size but also thicker hypocotyls.

KNO_3 treatment gave the lowest coefficient of variation (C.V.), between 0.47 and 2.37, compared with 1.80 and 2.97 in NaCl, 1.73 and 6.58 in GA_3, 13.49 and 30.25 in control which obviously shows that treatment increased uniformity and reduced the plant to plant difference within the population (Table 6).

DISCUSSION

We found in this work that priming enhanced tomato rootstock seedling growth through increasing faster seedling emergence, higher seedling fresh and dry weight and hypocotyl thickness compared to that of control. KNO_3 treated seeds gave better performance than NaCl or GA_3 treatments in our work (Fig. 1). However, all three treatments were superior to untreated ones. Rapid emergence and strong early seedling growth are crucially important to get a better and timely field establishment in direct sown species (Parera and Cantliffe, 1994; Taylor *et al*., 1998; Massawe *et al*., 1999) and developed transplant production (Nascimento and West, 1999) in open field and protected vegetable cultivation.

Results of the present research showed that priming treatments are promising to obtain well developed rootstock seedlings which can be used for better grafted seedling production. Grafting of vegetable species has been increasingly employed in over recent years due to the number of advantages on crop performance (Yetisir and Sari, 2003; Passam *et al*., 2005). These include increased growth and development, higher rate of nutrient uptake increased tolerance to stressful conditions and diseases (Rıvero *et al*., 2003; Passam *et al*., 2005). In Japan 60% of seedlings of fruit bearing vegetables are grafted (Oda, 1999), annual rate of increase in grafting 100% in Italy, 3% of total tomato and aubergine seedlings in Greece is grafted (Passam *et al*., 2005). In Turkey grafted tomato seedling production has enormously increased over last a few years and is about 15 million in 2005. Success in grafting depends on number of factors but rapid germination and strong rootstock seedling structure is a prerequisite for success in grafting. The more uniform and fast developed rootstock seedlings that obtained by the treatment, reduction in T_{50}, lower C.V. and MET (Table 2 and Table 4), are not only to ease grafting process but also to use automation in grafting since it is an expensive and time consuming process (Oda *et al*., 1997). Moreover, when seedlings reached to the graftable level in a shorter time (earlier emergence and fast development) the glasshouse space are to be used more profitably. Uniform transplants also reflect in simultaneous (less variation among plant size) plant growth, lower CV of fresh weight (0.47-2.37 in KNO_3 compared with 13.49-30.25 in control in our work) and early fruit maturity (Gray, 1976; Currah, 1978; Cantliffe, 1998). This is of interest to greenhouse growers who use the grafted seedlings and aim mainly at early, timely and more lucrative tomato production. One of the advantages of this is to increase efficacy of machine use and glasshouse space in grafting (Passam *et al*., 2005).

Findings on enhancing effect of priming on seedling quality i.e., reduced time to reach graftable thickness, increased seedling size (fresh and dry weight) in both tomato rootstock seeds were in agreement with previous studies in which the rate and emergence percentages of many vegetables and field crops seeds were improved by pre-hydration treatments (Bradford, 1986; Parera and Cantliffe, 1994; Taylor *et al*., 1998). However, the effect of the treatments may change in response to species, treatment duration and method, temperature even seed lots in the same cultivars (Parera and Cantliffe, 1994; Taylor *et al*., 1998). In this study, KNO_3 was found the better treatment than NaCl and GA_3 in rootstock tomato seeds in both runs. It was reported that stimulating effect of nitrate on seed germination might be through dormancy breakage (Hilhorst, 1990) stimulation of oxygen uptake (Hilton and Thomas, 1986) and as a cofactor of phytochrome (Hilhorst, 1990). Extended embryonic growth was also reported by Nerson and Govers (1986) following KNO_3 treatments.

Slow and erratic emergence of tomato rootstock seeds could be due to their small seed size compared to cultivated varieties. A thousand weights of these seeds are about 2-2.3 g. Whereas, cultivated species is about 3.5-4.0 g. Taylor and Ten Broeck (1988) reported that energy content of a given seed depends on the amount of stored material within the seed. Higher amount of stored material in cultivated tomato seeds than that of rootstock would be used to generate more energy to split seed coat and to overcome the resistance exerted by overlying growth media during seedling emergence. This was also observed in diploid watermelon seeds (large size) which had large seedlings compared to triploid ones (small size) (Sung and Chiu, 1995). Undeveloped embryo of the rootstock tomato seeds may also be another reason since seeds are incapable of the radicle protrudes through

seed coat. Hydration treatments have been reported as promoting on both embryonic development and softening the seed coat (Welbaum and Bradford, 1991; Taylor *et al*., 1998).

Positive correlation between mean emergence time and seedling fresh weight indicated that much of the variation in seedling size resulted from differences in times from sowing to emergence (Fig. 3). Destructive harvests showed that relative growth rates of seedlings were similar among the treatments and control. In other words, the effect of the treatments on seedling size basically originated from the time differences from sowing to germination. Parera and Cantliffe (1994) indicated that the basic enhancing effect of the treatment stems from reduced germination time rather than subsequent growth rate. This conclusion tallies with the numerous studies in which differences in seed quality (resulting from a variety of causes i.e., ageing, seed developmental differences) have been shown not to affect seedling relative growth but emergence time (Currah, 1978; Brocklehurst *et al*., 1984; Argerich and Bradford, 1989; Ellis, 1989; Demir and Ellis, 1993). Faster emergence also provided the thicker hypocotyls which are more appropriate for grafting. This observed in reduction of $T_{1.5}$ in treated samples (Table 4). Following priming, seeds have completed phase I (hydration) and phase II (lag phase) of germination and only require a favourable water potential gradient for water uptake in order to begin radicle growth (Pill, 1995; Powell *et al*., 2000). During the process of priming, endosperm loosening, softening the seed coat, hydrolysis of starch that causes of synthesis of proteins and enzymes are known to occur (Taylor *et al*., 1998, Powell *et al*. 2000). Higher emergence percentage and seedling emergence might have resulted from these positive effects of priming.

In conclusion, this study has shown that priming increased emergence percentage and rate and seedling fresh and dry weight of tomato rootstock seeds. This will provide uniform and well developed rootstocks seedlings and in turn enhance grafted tomato transplant production.

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