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Effect of Controlled Hydration Treatment on Germination and Seedling Growth under Salt Stress during Development in Tomato Seeds

I. Demir and S. Ermis
(University of Ankara, Faculty of Agriculture, Department of Horticulture, Ankara, Turkey)

Summary

Tomato (*Lycopersicum esculentum* Mill.) seed lots harvested 60, 70, 80 and 90 and 50, 60, 70 and 80 DAA (days after anthesis) in 1998 and 1999, respectively, were treated with controlled hydration at 25 °C for 26 h. Following drying, treated and untreated seeds were incubated (at 25 °C) and emerged (in peat moss) in 100 mM NaCI in order to assess the effect of the treatment on germination (total, normal and abnormal germination) and seedling growth (emergence, mean emergence time, seedling fresh and dry weight, hypocotyl and cotyledon length) in saline conditions. Na and Cl contents of the treated and untreated seedlings were also determined. The treatment enhanced normal germination, emergence, mean germination time, seedling fresh and dry weights, hypocotyl and cotyledon length, compared with untreated seeds. Treated seeds produced seedlings that had higher Na and Cl contents compare with control under salt stress. Results indicated that controlled-hydration treatment increased resistance to salt stress in all developmental stages.

Zusammenfassung

Wirkung einer kontrollierten Hydratisierungsbehandlung auf Keimung und Sämlingswachstum bei Tomaten unter Salzstress. Saatgut von Tomaten (*Lycopersicum esculentum* Mill.), das 1998 60, 70, 80 oder 90 und 1999 50, 60, 70 oder 80 DAA (Tage nach Blütenöffnung) geerntet worden war, wurde 26 Stunden lang bei 25 °C einer kontrollierten Hydratisierungsbehandlung unterzogen. Nach der Trocknung wurden behandelte und unbehandelte Samen bei 25 °C inkubiert und zum Auflaufen (in Sphagnum) in 100 mM NaCI-Lösung verbracht, um die Wirkung der salinen Bedingungen auf die Keimung (Gesamt-, normale und abnorme Keimung) und das Keimlingswachstum (Keimrate, durchschnittliche Keimdauer, Keimlingsfrisch- und -trockengewicht, Hypokotyl- und Kotyledonenlänge) zu untersuchen. Außerdem wurde der Na- und Cl-Gehalt der behandelten und unbehandelten Sämlinge bestimmt.
Die Vorbehandlung steigerte im Vergleich zur Kontrolle die normale Keimung, die Keimrate, die durchschnittliche Keimdauer, das Keimlingsfrisch- und -trockengewicht sowie die Hypokotyl- und Kotyledonenlänge. Das vorbehandelte Saatgut brachte unter Salzstress Sämlinge mit höheren Na- und Cl-Gehalten hervor. Die hier dargestellten Ergebnisse weisen darauf hin, dass die kontrollierte Hydratisierungsbehandlung die Resistenz der Sämlinge gegen Salzstress in allen Entwicklungsstadien erhöht.

Key words. Tomato – hydration treatment – germination – salt stress

Introduction

Developed transplant production is a prerequisite for successful vegetable production. Salinity stress is a major environmental constraint to irrigated agriculture in arid, semi-arid and coastal regions in direct sowing or transplant production and might severely affect germination and seedling growth (PASSAM and KAKOURIOTIS 1994). Although, salt tolerance may vary according to developmental stage of the plant and species, the first exposure of the crop to salinity stress would occur in germination phase (ALMANSOURI et al. 2001; GHOULAM and FARES 2001) and tomato seeds were found to be vulnerable to salinity stress during germination (FOOLAD and LIN 1997).

Some of the earlier studies reported that pre-sowing seed treatments (priming, hydration, etc.) alleviated adverse seed bed conditions and improved germination and seedling growth at low temperatures (SACS 1977), matric stress (FRETT and PILL 1989) and salinity (PILL et al. 1991; PASSAM and KAKOURIOTIS 1994). One of which is named as aerated or controlled hydration treatment in which seeds were imbibed in aerated water at appropriate temperature long enough to proceed pre-germinative physiological and biochemical activities but to prevent radicle emergence and were found effective on improvement of germination and seedling growth in various vegetable species (POWELL et al. 2000; DEMIR 2002).

One of the determining factors of seed performance under saline conditions may also be developmental stage of the seed, since it was reported that the more mature the seed is the higher performance at temperature and water stress in tomato (DEMIR and SAMIT 2001), osmotic water stress in *Brassica* (STILL and BRADFORD 1998) and stressful storage conditions (high moisture and temperature) in bean (SANHEWE and ELLIS 1998). Harvesting less mature, fully mature and over mature seeds together and mix-up in the same batch in most of the continuously flowering species including tomato (fruits are formed in clusters in various nodes) might affect overall germination and seedling growth performances of seed lots under saline conditions.

The present study was then undertaken to investigate whether controlled hydration can enhance the germination and seedling growth of sequentially harvested tomato seeds under saline conditions. That will help to produce uniform, rapid and developed transplants in saline conditions in the case of sowing in salty peat moss or unavoidable use of salty irrigation water and in turn to increase overall performance of the seed lots.

Materials and Methods

Plant husbandry and seed harvest

Plant husbandry and fruit harvests were as described in DEMIR and SAMIT (2001). Namely, fruits of the processing tomato cultivar 'Rio Grande' (*Lycopersicum esculentum* Mill.) were harvested 60, 70, 80 and 90 in 1998 and 50, 60, 70 and 80 days after anthesis (DAA) in 1999 and seeds were extracted by using commercial hydrochloric acid HCI (37%) for arranging slurry to pH2 and keeping 10 minutes at room temperature in both years. Seeds were then washed in running tap water and dried in the shade for 3 days. Seeds with 10% of final moisture content were put in the glass jars and stored in the fridge (4 °C) until use.

Controlled hydration treatment

Controlled hydration treatment was carried out according to procedure of DEMIR (2002). Namely, 400 seeds of each harvest were put in glass tube (11 cm height, 3 cm diameter) containing distilled water. Eight glass tubes then, placed in tube holder and kept in water bath at 25 °C for 26 hours in the dark. Each tube was aerated by a syringe and all syringes were connected through plastic pipes (0.2 mm) to an aquarium pump (1 l min^{-1}). Following hydration treatment, seeds were dried on filter papers for 48 h at 20 °C to their original moisture contents.

Seed germination

Germination of treated and untreated seeds were carried out in Petri dishes (9 cm diameter) containing two Whatman (No. 1) filter paper imbibed with either 5 ml of distilled water (control) or the saline solution of 100 mM NaCl (concentration that was used to rank tomato seed lots according to salinity resistance) (SRINIVAS 2001). Corresponding electrical conductivity of solution was recorded as 10.2 mS cm^{-1} at 25 °C. Four replicates of 50 seeds were germinated for each harvest in treated and untreated batches. In order to prevent evaporation, edges of Petri dishes were covered with impermeable colourless parafilm. Seeds were allowed to germinate at 25 °C in the dark for 14 days. Germination percentages (total/normal/abnormal) were recorded every 24 hours during this period according to ISTA rules (ANONYMOUS 1996).

Seedling growth (emergence, hypocotyl and cotyledon length, seedling fresh and dry weight)

Seeds (three replicates of 40 treated and untreated seeds in each harvest) were sown 1 cm deep in a mixture of riverbed sand and compost (Plantaflor, Humus-Verkaufs GmbH, Germany) in ratio of 1 : 2 in seedling trays (11×8×5 cm). Electrical conductivity of the saturated extract of compost (MIYAMOTO 1989) was 0.8 mS cm^{-1} as measured using a conductivity meter (Schott-Geräte GmbH, Hofheim) before sowing. The trays were placed on a bench in an unheated greenhouse, watered with saline solution (100 mM) or distilled water (control) and covered with paper until emergence. Minimum and maximum temperatures during seedling growth period were recorded as 14 °C and 34 °C, respectively. Trays were inspected daily and emergence recorded as the appearance of the hypocotyl in the surface. Additional solution was added as required. Experiment was terminated 25 days after sowing. Seedlings were counted; hypocotyl length and the lengths of the cotyledon pairs were measured and expressed as cm $plant^{-1}$. Mean emergence time was calculated according to ELLIS and ROBERTS (1980). Total seedling fresh and dry weights were also determined per harvest for treated and control seeds and expressed as mg $plant^{-1}$. Dry weight was determined after 24 h at 80 °C.

Na and Cl determination

Seedlings after dry weight determination were ashed in muffle furnice at 500 °C for 24 h, dissolved in 5 ml of 2 M HNO_3 and finally diluted to 25 ml reverse osmosis water. Extracts were filtered and stored in plastic vials until analysed. Na was measured by flamephotometer (Jenway PFP7, ELE Instruments Co. Ltd.) and water extractable Cl was determined by potentiometric titration with $AgNO_3$ as described by LAMBERT and DUBOIS (1971).

Means of the treatment were compared with LSD values at 5% level.

Results

Seed germination

All seeds germinated (radicle emerged) regardless of harvests and treatment in 100 mM NaCl. While, normal germination percentages differed not only between harvests but control and treated seeds. The lowest normal germination percentages of control seeds were obtained in seeds harvested 60 DAA in 1998 as 56% and 50 DAA as 68% in 1999. Normal germination reached maximum by 70 DAA and 60 DAA in the control seeds and remained stable thereafter (1999) or declined gradually (1998). Controlled hydration treatment promoted normal germination compared to control seeds in all harvests and both years. Differences between control and treated lots varied between 6 and 25% in 1998, 1 and 15% in 1999 and maximum in seeds harvested at 60 and 50 DAA as 25% and 15% in 1998 and 1999, respectively (Table 1). Similarly, maximum decrease in abnormal germination by the treatment occurred in early harvests as 25% in both years.

Seedling Growth

Normal emergence changed between 80% and 91% in control, while corresponding values in treated seeds were 89% and 100% in harvests of the year 1998. The advantage of the treatment in normal seedling emergence percentages in seeds harvested at 60, 70, 80 and 90 DAA were 15, 9, 9 and 0%, respectively. Controlled hydration treatment reduced time to germination between 6 and 31 hours varying according to the harvest. In 1998, interactive effect of harvest x treatment was significant ($P<0.05$) on normal emergence percentages, mean emergence time and cotyledon length. But factors were individually effective on seedling fresh and dry weights and hypocotyl length (Table 2).

Controlled hydration significantly increased seedling fresh and dry weight and hypocotyl length in all harvests in 1998 ($P<0.05$). The differences were greatest in seeds of 60 DAA as 24.5 mg $plant^{-1}$ in fresh weight, 0.5 mg $plant^{-1}$ in dry weight and 1.8 cm $plant^{-1}$ in hypocotyl length. Fully expanded cotyledon length measurements showed that treated seeds produced at least 0.8 cm (90 DAA) and longer cotyledons compare with control seeds under salt stress (Table 2). The more mature the seeds were the less difference in hypocotyl and cotyledon lengths between hydrated and untreated seeds.

Results of seedling growth in lots of 1999 showed that treatment improved emergence percentages, mean emergence time, seedling fresh weight and dry weight, hypocotyl and cotyledon lengths. Similar to 1998 it was more prominent in seeds harvested earlier (50 DAA) stages of development than later (80 DAA). Interactive effects of harvest x treatment on all seedling characteristics were significant ($P<0.05$) in 1999. Treatment increased emergence percentage (9%) and decreased mean time to germination (26 hours) in seeds harvested at 50 DAA. But these were 6% and 12 hours at 80 DAA. Seedlings produced by the treated seeds of 50 DAA had 9.1 mg $plant^{-1}$ higher fresh and 0.2 mg $plant^{-1}$ dry weights, and 1.7 cm longer hypocotyl and 0.5 cm cotyledon lengths compare with those of control (Table 3). These differences were comparatively smaller in mature seeds (Table 3).

Na and Cl contents

Na content of treated seedlings in 1998 was higher than control in all lots, except 60 DAA. Na content of seedlings changed between 11.30 and 20.89 g kg^{-1}. Seedlings produced by treated seeds had higher Na content that was minimum of 3 g kg^{-1} Na in seeds of 90 DAA and maximum at 80 DAA as about 9 g kg^{-1}. Results in lots of 1999 also showed that seedlings of treated seeds had higher Na content than control except earliest harvest (50 DAA) in which difference was little and insignificant ($P>0.05$).

Cl content changed between 6.80 and 9.65% in control and 8.50 and 9.30% in treated seedlings in 1998. The difference between treated and control seeds was statistically significant at all harvests ($P<0.05$). Control seedlings in 1999 lots had significantly lower Cl content in all harvests. Seeds harvested 60 and 70 DAA had the minimum of 6.25% of Cl. Seedlings obtained from treated seeds had Cl contents between 8.30 and 8.75% (Table 4).

Table 1. Effect of controlled hydration treatment on normal and abnormal germination of tomato seeds in 100 mM NaCl at 25 °C.

Year	Treatment	Days after anthesis (DAA)				
		50	60	70	80	90
1998		Normal germination (%)				
	Control	–	56	81	72	64
	Treated	–	81	87	90	85
	LSD(int): 17.61					
		Abnormal germination (%)				
	Control	–	44	19	28	36
	Treated	–	19	13	10	15
	LSD(int): 17.76					
1999		Normal germination (%)				
	Control	68	80	77	87	–
	Treated	83	86	84	88	–
	LSD(treat): 5.42					
	LSD(harv): 7.42					
		Abnormal germination (%)				
	Control	32	20	23	11	–
	Treated	7	14	19	13	–
	LSD(treat): 6.50					
	LSD(harv): 9.20					

Table 2. Effect of controlled hydration treatment on normal emergence percentage, emergence time (h), seedling fresh and dry weight (mg plant^{-1}), hypocotyl and cotyledon length (cm plant^{-1}) of tomato seeds in the year 1998.

Year	Treatment	Days after anthesis (DAA)			
		60	70	80	90
1998		Normal emergence (%)			
	Control	85	91	80	90
	Treated	100	100	89	90
	LSD(int): 11.05				
		Mean emergence time (h)			
	Control	98	93	89	87
	Treated	67	77	77	81
	LSD(int): 17.2				
		Seedling fresh weight (mg plant^{-1})			
	Control	36.0	56.1	50.7	56.2
	Treated	60.5	67.3	67.0	68.0
	LSD(Treat): 3.06				
	LSD(Harv): 4.32				
		Seedling dry weight (mg plant^{-1})			
	Control	2.8	3.2	3.0	3.4
	Treated	3.3	3.5	3.6	3.7
	LSD(Treat): 0.11				
	LSD(Harv): 0.15				
		Hypocotyl length (cm)			
	Control	3.23	5.52	5.40	5.10
	Treated	5.01	6.31	5.87	5.52
	LSD(Treat): 0.28				
	LSD(Harv): 0.40				
		Cotyledon length (cm)			
	Control	3.5	3.8	4.0	4.1
	Treated	5.2	5.1	5.2	4.9
	LSD(int): 0.784				

Discussion

Stress conditions such as salinity can depress germination and emergence in both direct field sowing (cereals, legumes) or transplant production (vegetables). This can be due to osmotic or ionic effects of the saline germination media. However, evidence exists that both low water potential (ALMANSOURI et al. 2001) and ionic toxicity (GHOULAM and FARES 2001) can be limiting factors.

Various seed treatments (priming, osmoconditioning, hydration etc.) were used for improving the rate and uniformity of seeds under environmentally adverse conditions (BRADFORD 1995). It has recently been demonstrated that priming with $NaNO_3$ and Mannitol improved germination of asparagus and tomato (PILL et al. 1991), and cucumber (PASSAM and KAKOURIOTIS 1994) seeds in saline media, respectively. Despite we did not use the same procedure of the treatment, the results obtained in this study indicated that controlled hydration increased germination and seedling growth under salt stress in tomato seeds harvested at different developmental stage. The effect of the treatment was more prominent in early harvests of 60 DAA (1998) and 50 DAA (1999) compare with the other stages (Tables 1, 2 and 3). Physiological basis of the beneficial effect of hydration treatments were reported to occur through embryo enlargement (HEGARTY 1978), DNA replication (POWELL et al. 2000) and increase in enzyme activities and protein structures (SMITH and COBB 1992).

Tomato seeds harvested at 70 DAA were indicated to be the most resistant to water stress simulated by PEG (DEMIR and SAMIT 2001). Similarly, control tomato seeds of 70 DAA had higher germination and seedling growth than those of earlier and later harvests under salt stress in this work (Table 1, 2 and 3). However, hydration treatment advanced not only germination and seedling growth compare with untreated but reduced the seed quality differences between developmentally different seed lots via obtaining greater improvement in early harvests in both years under salt stress. This has great importance in obtaining uniform and developed seedling from mechanically harvested commercial lots (seeds at various developmental stages mixed up) under salt stress. These findings are in agreement with results of previous studies in tomato and cucumber seeds despite different treatment method was used (PILL et al. 1991; PASSAM and KAKOURIOTIS 1994).

Seeds that had high germination and seedling growth in nonstress were also tended to do well under stress conditions. JONES (1986) reported that genetic and physiological parameters that facilitate high germination and emergence in nonstress are advantageous to high germination under salt stress in tomato. FOOLAD (1996) indicated germination responses at different salt stress in tomato was genetically controlled and selection for rapid germination and emergence at one salt concentration would result in progeny with improved germination at diverse salt stress levels. Agreeing with that conclusion SRINIVAS (2001) found that tomato germplasm accessions can be ranked according to their salt tolerance by germinating at 100 mM NaCl.

Table 3. Effect of controlled hydration treatment on normal emergence percentage, emergence time (h), seedling fresh and dry weight (mg plant^{-1}), hypocotyl and cotyledon length (cm plant^{-1}) of tomato seeds in the year 1999.

Year	Treatment	Days after anthesis (DAA)			
		50	60	70	80
1999		Normal emergence (%)			
	Control	82	84	86	74
	Treated	91	90	95	80
	LSD(int): 17.97				
		Mean emergence time (h)			
	Control	127	103	103	96
	Treated	101	89	89	84
	LSD(int): 14.2				
		Seedling fresh weight (mg plant^{-1})			
	Control	42.8	46.5	47.6	52.5
	Treated	51.9	49.9	54.6	51.9
	LSD(int): 4.61				
		Seedling dry weight (mg plant^{-1})			
	Control	2.7	2.8	2.9	3.1
	Treated	2.9	2.9	3.2	3.0
	LSD(int): 0.45				
		Hypocotyl length (cm)			
	Control	3.2	3.6	4.4	4.1
	Treated	4.9	5.2	5.3	4.3
	LSD(int): 0.66				
		Cotyledon length (cm)			
	Control	3.2	3.9	3.7	3.9
	Treated	3.7	4.2	4.3	4.2
	LSD(int): 0.97				

Table 4. Na (g kg^{-1}) and Cl (%) content of tomato seedlings produced from seeds harvested different days after anthesis in 1998 and 1999 after controlled hydrated or not.

Year	DAA	Treatment	Na (g kg^{-1})	Cl (%)
1998	60	Control	18.591 a	8.20 a
		Treated	14.610 b	8.50 b
	70	Control	11.308 b	6.80 b
		Treated	15.688 a	8.60 a
	80	Control	11.402 b	7.00 b
		Treated	20.899 a	9.30 a
	90	Control	17.363 b	9.65 a
		Treated	20.332 a	9.15 b
1999	50	Control	12.497 a	7.10 b
		Treated	11.868 a	8.30 a
	60	Control	10.836 b	6.25 b
		Treated	13.735 a	8.75 a
	70	Control	12.426 b	6.25 b
		Treated	15.358 a	8.30 a
	80	Control	11.344 b	7.75 b
		Treated	14.284 a	8.60 a

The tolerance of seed germination to salinity is a measure of the seed ability to withstand the effects of high concentration of soluble salts in the medium. The seeds then, should generate required osmotic potential to improve the water status of the embryo permitting protrusion of the radicle (HEGARTY 1978). Results in this experiment proved that treated seeds in both years are more capable of improving water status of embryo since they showed lower mean emergence time and consequently higher seedling growth at NaCl stress (Table 1, 2 and 3). One reason is that when seeds were sown at stressful environment treated seeds had already completed first two stages of imbibition and brought to the point close to radicle emergence during hydration treatment proceeded. However, untreated seeds should go through first two stages (POWELL et al. 2000).

The contents of Na and Cl were significantly higher in seedlings obtained from treated than untreated seeds when plants were grown at NaCl. It appears to be then that accumulation of inorganic ions in seedlings of treated seeds might play a role in arranging osmotic potential and accumulation of more water (water content = seedling fresh weight – dry weight). MARCONI et al. (2001) found that more tolerant potato species accumulated more water than less tolerant ones at 100 mM NaCl.

FOOLAD (1996) proposed that salt tolerance is a developmentally regulated stage specific phenomenon and should be evaluated separately for every different stage of ontogeny of the plant. The results of the present experiment showed that seedling stage was found to be

more sensitive indicator of salt stress compare with total germination in tomato. Despite the viability is widely accepted component of seed quality, seedling growth (emergence, fresh and dry weight, cotyledon and hypocotyl lengths) can be more discriminative in ranking the seed lots regarding performance under stress (SRINIVAS 2001). In agreement with that conclusion, seed vigour traits such as germination rate seedling growth, dry weight and emergence in squash (FRANCOIS 1985), carrot (SCHIMIDHALTER and OERTLI 1991), cucumber (PASSAM and KAKOURIOTIS 1994), ryegrass (MACAR 1987) and sugar beet (GHOULAM and FARES 2001) have been reported to be more sensitive to salinity comparing total germination. However, controlled hydration upgraded seedling emergence, fresh weight, dry weight, hypocotyl and cotyledon length, which allows obtaining, developed and uniform seedlings under salt stress. Longer and stronger hypocotyl will also allow obtaining developed seedlings in deep plantings.

In conclusion, the present study revealed that controlled hydration alleviated the effect of salt stresses regarding germination and emergence processes in serially harvested tomato seed lots during development. Treated seed lots were found to be more capable of producing higher normal germination, seedling growth (cotyledon, and hypocotyl length, seedling weight) at the NaCl stress compare with untreated. Treated seeds of all developmental stages had higher performance so that it can be used to improve uniformity and obtain developed seedling production under salt stress.

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Addresses of authors: I. Demir and S. Ermis, University of Ankara, Faculty of Agriculture, Department of Horticulture, 06110 Ankara, Turkey, e-mail: demir@agri.ankara.edu.tr.