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ETHANOL PRIMING: AN EFFECTIVE APPROACH TO ENHANCE GERMINATION AND SEEDLING DEVELOPMENT BY IMPROVING ANTIOXIDANT SYSTEM IN TOMATO SEEDS

Irfan Afzal[1], Fahad Munir[2], Chaudhry Muhammad Ayub[2], Shahzad Maqsood Ahmad Basra[1], Amjad Hameed[3], Fawad Shah[4]

[1]Seed Physiology Lab, Department of Crop Physiology, University of Agriculture, Faisalabad, Pakistan

[2]Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan

[3]Nuclear Institute for Agriculture and Biology, Jhang Road Faisalabad, Pakistan

[4]Commodity Inspection Division, Washington State Department of Agriculture, Yakima, WA-98902, USA

Abstract. Tomatoes reportedly have a positive response to seed priming. The present study evaluates the effects of ethanol priming on germination, seedling vigour and anti-oxidative responses of tomato seeds. Priming was achieved by exposing seeds of 'Roma' and 'Nagina' to 2, 4 and 6% aerated ethanol solutions for 24 h. Priming with low levels (2 and 4%) of ethanol improved seed germination, seedling vigour and enhanced antioxidative activity that results in better performance of tomato seeds. However, priming with 6% ethanol failed to improve seed germination and seedling development which relates to the decreased anti-oxidative activity in tomato seeds due to high ethanol level.

Key words: seed priming; dormancy, *Lycopersicon esculentum*, antioxidant enzymes, seedling development

ABBREVIATIONS

FGP – final germination percentage,
T_{50} – time taken to 50% germination,
MGT – mean germination time,
GI – germination index,
GE – germination Energy measured after 4th days of incubation,

Corresponding author: Irfan Afzal, Seed Physiology Lab, Department of Crop Physiology, University of Agriculture, Faisalabad, Pakistan, e-mail: iafzal@uaf.edu.pk

MET – mean emergence time,
FEP – final emergence percentage,
CAT – Catalase,
SOD – Superoxide dismutase.

INTRODUCTION

Tomato is an excellent source of many nutrients and secondary metabolites important for human health such as folate, potassium, vitamins C and E, flavonoids, chlorophyll, β-carotene and lycopene [Wilcox et al. 2003]. Increasing levels of dietary lycopene has been recommended by many health experts [Tonucci et al. 1995, Giovannucci 1999]. Lycopene has the ability to mitigate epithelial cancers, such as breast and prostate cancers, and also coronary disease. Tomato cultivars containing higher lycopene germinate and grow more slowly than traditional ones, and a high lycopene tomato contained high levels of abscisic acid (ABA) which is responsible for seed dormancy [Ramirez-Rosales et al. 2004]. Moreover, freshly harvested tomato seed often exhibit low germination due to primary dormancy. Dormancy persists for up to one year in tomato seeds [Liu et al. 1996] resulting in erratic and unacceptable seedling emergence.

Seed priming is a simple and low cost technique used to break dormancy and improve uniformity of radicle emergence [Liu et al. 1996]. Seed priming is a controlled hydration process that involves exposing seeds to low water potentials that restrict germination but permits pregerminative physiological and biochemical changes [Bradford 1986]. Priming enhances seed performance by increasing germination rate and uniformity resulting in faster and better seedling development in various crops [Taylor et al. 1998, Powell et al. 2000, Afzal et al. 2008]. Enhancement of seed germination by priming has also been associated with stimulation of antioxidant activities [Bailly et al. 1998, 2000, Chiu et al. 2002, Afzal et al. 2011]. Priming also reduces germination time rather than subsequent relative growth rate and is a valuable tool to improve seedling quality in rootstock tomato seedling production [Mavi et al. 2006].

Ethanol has been reported to stimulate germination of seeds of many plant species [Bewley and Black 1982]. Seed pretreatment of many species with ethanol or other anaesthetic-like substances during imbibition resulted in breakdown of dormancy [Taylorson and Hendricks 1979, Cohn et al. 1989, Hallett and Bewley 2002]. Due to ethanol's role in breaking seed dormancy, the germination performance and activities of antioxidant enzymes capable of scavenging free radicals and peroxides were investigated in tomato seeds after exposure to various levels of ethanol as a priming agent. This research was undertaken to provide insights in the relationship of ethanol priming and dormancy breakdown of tomato seeds by affecting antioxidant enzymes.

MATERIALS AND METHODS

Seeds of tomato (*Lycopersicon esculentum* Mill.) cv. Nagina and Roma were obtained from the Vegetable Research Institute, Faisalabad, Pakistan and had initial seed moisture contents of 8.23% and 8.13% respectively on a dry weight basis. The seeds were stored at 15°C for one year after harvest.

Priming protocol. Seeds (2 g) were soaked in aerated solution of 2%, 4% and 6% ethanol (100% undenatured) in 100 ml glass beakers for 24 h at 25°C. For hydropriming, seeds were soaked in distilled water under similar conditions. Hydroprimed and non primed seeds were used as controls. During priming, fresh air was supplied continuously. After ethanol priming, seeds were washed with distilled water [Bradford 1986] and dried back after spreading in a thin layer on dry filter papersat 27°C for 48 h. The seeds were then placed in polythene bags and stored in a refrigerator at 7°C for further studies.

Seed germination. Four replicates of 25 seeds each were placed in 9 cm diameter Petri dishes on Whatman No. 1 filter paper at 25°C in a growth chamber (Vindon, England) for 12 days. Five ml of distilled water was used to moisturize each Petri dish. Visible root protrusion was recognized as germination. Time to 50% germination (T_{50}) was calculated according to the formulae of Coolbear et al. [1984]. Mean germination time (MGT) was calculated according to Ellis and Roberts [1981]. Germination index (GI) was calculated as described in the Association of Official Seed Analysts [1983]. Energy of germination was recorded on the 4th day after planting. It is the percentage of germinating seeds on the 4th day after planting relative to the total number of seeds tested.

Seedling emergence. Control and treated seeds were sown in plastic trays (25 in each) containing moist sand, replicated four times and placed in a growth chamber (Vindon, England) maintained at 25°C under continuous fluorescent light for 2 weeks. Emergence was recorded daily according to the seedling evaluation following the Handbook of Association of Official Seed Analysts [1983]. Seedlings were harvested after two weeks of sowing and washed with deionized water. Washed seedlings were separated into root and shoot for fresh and dry weight determination. Dry weight was determined after oven drying at 65°C for 2 days.

Estimation of antioxidant enzymes. Tomato seeds (0.5 g), both ethanol primed or non primed were ground in 0.8 ml of 50 mM cold phosphate buffer (pH 7.8) in chilled mortar and pestles. The homogenate was then centrifuged at 15 000 *g* for 20 min at 4°C. The supernatant was removed and used for the determination of activities of enzymes.

The enzyme assay for estimation of catalase (CAT) contained 50 mM phosphate buffer (pH 7.0), 5.9 mM H_2O_2, and 0.1 ml enzyme extract. The reaction was initiated by adding the enzyme extract. The decrease in absorbance of the reaction solution at 240 nm was recorded after every 20 s with a spectrophotometer (Hitachi U-2100, Tokyo, Japan). An absorbance change of 0.01 unit min^{-1} was defined as 1 unit of CAT activity [Dixit et al. 2001]. Enzyme activities were expressed on a protein basis. Protein concentration of the enzyme extract was measured by the dye binding assay as described by Bradford [1976].

Superoxide dismutase (SOD) activity was assayed by measuring its ability to inhibit the photochemical reduction of nitrobluetetrazolium (NBT) following the method of [Dixit et al. 2001, Giannopolitis and Ries 1977] with some modification. The SOD reaction solution (3 ml) contained 50 µM NBT, 1.3 µM riboflavin, 13 mM methionine, 75 mM EDTA, 50 mM phosphate buffer (pH 7.8) and 50 µl enzyme extract. The tubes containing the reaction solution were irradiated under a light (15 W fluorescent lamps) at 78 $\mu mol \cdot m^{-2}\ s^{-1}$ for 15 min. The absorbance of the irradiated solution at 560 nm was

determined with a spectrophotometer (Hitachi U-2100, Tokyo, Japan). One unit of SOD activity was defined as the amount of enzyme which caused 50% inhibition of photochemical reduction of NBT.

Statistical analysis. All experiments were repeated twice in a completely randomized design; data recorded each time were pooled for statistical analysis by using software MSTATC to determine significance of variance ($P < 0.05$). The least significant difference test was used to compare the differences amongst treatment means.

RESULTS

Ethanol priming significantly ($P < 0.05$) affected germination and seedling development of both tomato cultivars (tab. 1). Priming with 2 and 4% ethanol resulted in lower T_{50} and MGT and higher FGP, GI, radicle and plumule lengths compared with untreated and hydroprimed seeds (tab. 1). However, priming with 6% ethanol failed to improve these parameters in both cultivars. In both cultivars, lowest T_{50} and MGT were noted in seeds primed with 2% ethanol that was followed by 4% ethanol (tab. 1). Maximum FGP, GI, radicle and plumule length was noted in seeds primed with 2 and 4% ethanolin both cultivars whereas GE was only improved in 2% ethanol primed and hydroprimed seeds (tab. 1).

Table 1. Effect of ethanol priming on the germination and seedling growth of two tomato (*Lycopersicon esculentum* Mill.) cv Roma and Nagina

	Treatments	T_{50} (days)	MGT (days)	FGP (%)	GI	GE(%)	Radicle length (cm)	Plumule length (cm)
Roma	control	6.48 a	9.34 a	66.67 d	10.54 c	19.10 e	1.19 b	4.63 d
	hydropriming	5.76 b	9.19 b	77.32 b	14.27 b	30.20 c	1.26 b	4.96 c
	priming with 2% ethanol	5.19 d	8.70 d	81.33 a	15.91 a	42.94 a	2.20 a	5.82 b
	priming with 4% ethanol	5.53 c	9.04 c	82.00 a	15.44 ab	37.66b	2.07 a	5.99 a
	priming with 6% ethanol	5.75 b	9.21 b	71.33 c	11.87 c	22.48d	0.67 c	4.88 c
	LSD at 0.05	0.07	0.12	1.63	1.47	1.82	0.24	0.19
Nagina	control	8.98 a	10.88a	22.67 e	2.059d	18.99a	2.17 c	2.60 c
	hydropriming	8.41 b	10.15 c	40.96 c	5.54 c	15.28 b	2.01 c	2.92 b
	priming with 2% ethanol	7.39 c	9.65 d	58.00 a	7.19 a	11.90 c	3.21 b	3.69 a
	priming with 4% ethanol	7.54 c	9.72 d	46.67 b	6.33 b	18.09a	3.39 a	3.77 a
	priming with 6% ethanol	8.42 b	10.38 b	31.33 d	5.78 c	10.41 c	1.49 d	2.91 b
	LSD at 0.05	0.07	0.18	0.45	0.09	0.58	0.052	0.19

For each tomato cultivar, means within a column followed by the same letters are not significantly different at $P \leq 0.05$

T_{50} – time taken to 50% germination, FGP – final germination percentage, MGT – mean germination time, GI – germination index, GE – germination energy measured after 4th days of incubation

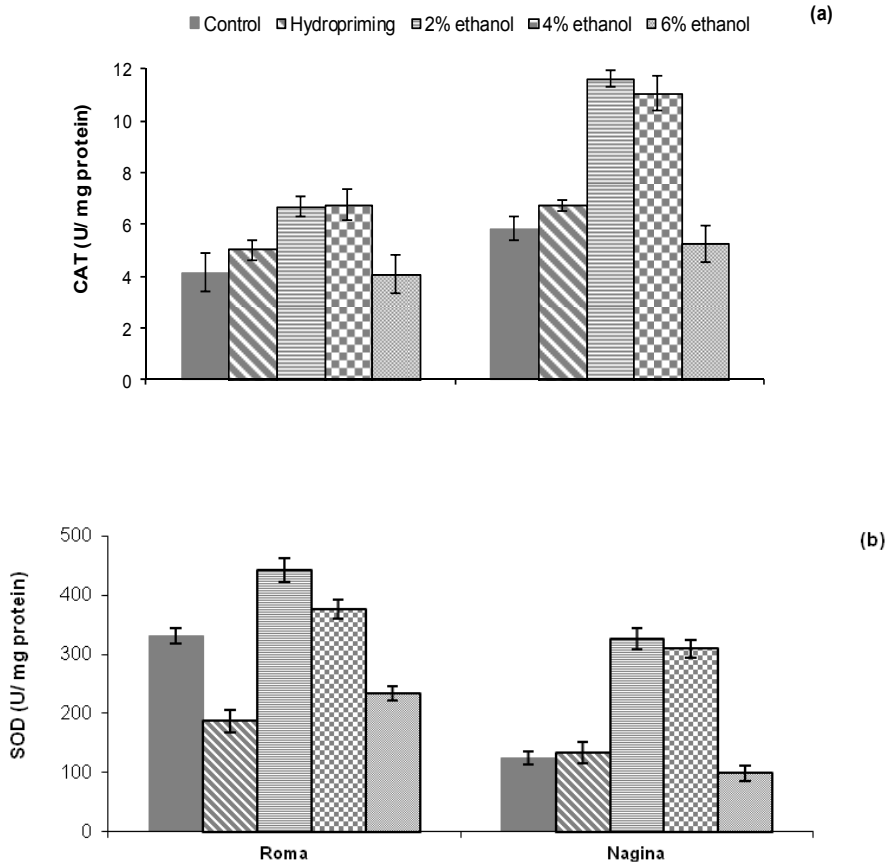

Fig. 1. Catalase (CAT) and superoxide dismutase (SOD) activities in seeds of 2 tomato cultivars Roma and Nagina after priming with 2, 4 and 6% ethanol. Data are the means ±SE of at least 4 different seed samples

All seed treatments resulted in lower MET compared with the control seeds (tab. 2). The highest FEP was recorded in seeds primed with 2 and 4% ethanol as compared to the control ones (non-primed and hydroprimed) and 6% ethanol primed seeds. In both cultivars, priming with 2 and 4% ethanol resulted in better seedling development i.e. higher root and shoot length, and seedling fresh and dry weight compared with control (tab. 2). Overall, hydropriming and 6% ethanol priming failed to improve seedling development of both cultivars (tab. 2).

Table 2. Effect ofethanol priming treatments on the seedling development of two tomato (*Lycopersicon esculentum* Mill.) cv Roma and Nagina

	Treatments	MET (days)	FEP (%)	Root length (cm)	Shoot length (cm)	Seedling fresh weight (mg)	Seedling dry weight (mg)
Roma	control	10.61 a	33.33 e	4.67 d	5.29 e	0.33 b	0.05 c
	hydropriming	10.14 a	57.00 c	4.89 c	6.08 d	0.40 b	0.09 b
	priming with 2% ethanol	9.63 c	69.33 a	6.57 a	7.41 a	0.89 a	0.14 a
	priming with 4% ethanol	9.88 b	60.00 b	6.39 a	7.03 b	0.65 ab	0.14 a
	priming with 6% ethanol	9.84 b	42.33 d	5.39 b	6.33 c	0.40 b	0.1 b
	LSD at 0.05	0.20	0.75	0.18	0.05	0.14	0.02
Nagina	control	11.61 a	47.33 d	5.23 d	4.09 c	0.29 b	0.04 c
	hydropriming	11.12 b	52.20 c	6.03 c	4.25 b	0.31 b	0.07 b
	priming with 2% ethanol	11.29 b	58.67 b	7.55 a	4.66 a	0.36 a	0.15 a
	priming with 4% ethanol	11.36 b	60.67 a	6.97 b	4.35 b	0.35 a	0.13 a
	priming with 6% ethanol	11.31 b	52.00 c	6.20 c	4.39 b	0.30 b	0.08 b
	LSD at 0.05	0.24	0.59	0.07	0.05	0.02	0.02

For each tomato cultivar, means within a column followed by the same letters are not significantly different at $P \leq 0.05$
MET – mean emergence time, FEP – final emergence percentage

There was a significant increase in SOD and CAT in both seeds primed with 2 and 4% ethanol as compared to untreated seeds in both cultivars. But SOD and CAT activities were negatively affected in seeds primed with water and 6% ethanol (fig. 1).

DISCUSSION

Ethanol priming overcame tomato seed dormancyand stimulated germination and seedling development of both cultivars tested. Responses were similar in earlier and synchronized germination and emergence observed with 2 and 4% ethanol compared with non-primed, hydroprimed and 6% ethanol treated seeds as depicted by lower MET, T_{50} and MGT, and higher GI, FEP and FGP. Moreover, ethanol priming further enhanced antioxidant defense mechanisms by improving SOD and CAT activities which were responsible for improving germination of both tomato seeds [Siadat et al. 2012]. The negative response of 6% ethanol indicates it may be toxic during priming due to excessive accumulation in imbibed seeds [Crawford1977].

Higher radicle and plumule lengths, root and shoot lengths as well as seedling fresh and dry weights observed in 2% and 4% ethanol primed seeds of both cultivars might be the result of earlier germination and emergence [Liu et al. 1996]. This earlier synchronized and faster emergence might be due to the enhanced synthesis of DNA, RNA and

protein [Bray et al. 1989] and activation of hydrolytic enzymes responsible for starch breakdown [Afzal et al. 2012] during priming. Earlier, Gallardo et al. [2001] also reported new proteins associated with priming. These results are also in accordance with findings of Mavi et al. [2006] who reported priming treatments increased tomato seedling (fresh and dry weight). Reduced radicle and plumule length of both cultivars represent the first indication of ethanol toxicity followed by reduced germination at higher ethanol concentration [Taylorson and Hendricks 1980/81].

The data presented here demonstrate clearly priming with 2 and 4% significantly increased SOD and CAT in both tomato cultivars. This supports the argument that SOD rapidly dismutes superoxide to H_2O_2, and inhibits hydroxyl radical production, and CAT helps in quenching H_2O_2 which ultimately protect seeds from its toxicity [McDonald 1999]. The positive role of ethanol in tomato seeds might increase the phospholipids head group spacing which in turn promotes germination by optimizing the binding and activation of an essential peripheral membrane protein [Hallett and Bewley 2002]. This increase in antioxidant activity is associated with enhancement of seed germination by priming [Bailly et al. 2000, Chiu et al. 2002]. Conversely, slow germination of seeds primed with 6% ethanol might be due to low antioxidant activity [Bailly et al. 2002].

CONCLUSIONS

Results show that tomato germination and seedling development can be enhanced by 2% and 4% ethanol priming through maintaining higher anti-oxidative mechanisms for eliminating excessive total peroxide. Therefore, ethanol priming with low concentration is an effective approach to enhance stand establishment of local tomato cultivars.

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POBUDZANIE ETANOLEM: SKUTECZNA METODA WZMAGAJĄCA KIEŁKOWANIE I ROZWÓJ SIEWEK POPRZEZ ULEPSZENIE SYSTEMU ANTYOKSYDACYJNEGO U NASION POMIDORA

Streszczenie. Uważa się, że pomidory wykazują pozytywną reakcję na pobudzanie nasion. Niniejsze badanie ocenia wpływ pobudzania etanolem na kiełkowanie, żywotność siewek oraz antyoksydacyjne reakcje nasion pomidora. Pobudzanie osiągnięto działając na nasiona odmian 'Roma" i „Nagina" 2, 4 i 6% napowietrzonymi roztworami etanoli przez 24 godziny. Pobudzanie za pomocą niskich poziomów (2 i 4%) etanolu poprawiało kiełkowanie nasion, żywotność siewek oraz wzmagało aktywność antyoksydacyjną, która daje lepsze wyniki w odniesieniu do nasion pomidora. Jednak pobudzanie 6% etanolem nie polepszyło kiełkowania nasion ani rozwoju siewek, co ma związek z obniżoną aktywnością antyoksydacyjną u nasion pomidora ze względu na wysoki poziom etanolu.

Słowa kluczowe: pobudzanie nasion, spoczynek, *Lycopersicon esculentum*, enzymy antyoksydacyjne, rozwój siewek

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