SHORT COMMUNICATION



Impact of Mother Plant Saline Stress on the Agronomical Quality of Pepper Seeds

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Abstract

Seed quality has been an important factor in achieving high germination and uniform growth rates in agricultural crops. Meanwhile, pepper plants are moderately sensitive to salt stress at electrical conductivity (EC) in the nutrient solution in the range of $1.2-3.0 \text{ dS m}^{-1}$. We are unaware of any studies regarding the effects of mother plant saline stress on the agronomical quality of pepper seeds. We assessed the effects of three levels of electrical conductivity of the nutrient solution used for mother plant fertigation (2.2, 3.5, and 4.5 dS m⁻¹) on the agronomical quality of pepper seeds (*Capsicum annuum* L. var. California Wonder). We have analyzed the following seed quality traits: (1) size and weight of seeds and number of seeds per fruit, (2) seed germination and vigor, and (3) chemical composition and histological features of mature seeds. The electrical conductivity treatment of 3.5 dS m⁻¹ caused a statistically significant reduction in the seed size and vigor, as well as partial histological damage to seed endosperm. Moreover, the electrical conductivity treatment of 4.5 dS m⁻¹ caused further reduction in the seed agronomical quality and generalized histological damage to seed endosperm. The electrical conductivity of the nutrient solution used for the fertigation of mother pepper plants should be below 3.5 dS m⁻¹. Future studies should be performed to better gauge the effect of nutrient solutions with electrical conductivity in the range of 2.2–3.5 dS m⁻¹ on the seed quality traits.

Keywords Salinity · Salt stress · Chilli · Chili · Germination · Seed vigor

1 Introduction

Seed quality has been an important factor in achieving high germination and uniform growth rates in agricultural crops (Hussain et al. 2018). Seed quality depends on many factors including seed physiology and chemical composition

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(Copeland and McDonald 2001). In particular, germination and vigor are largely determined by the amount of mineral nutrients, carbohydrates, proteins, and lipids in seeds (Andreasen et al. 2014). For example, the quality of pepper seeds (*Capsicum annuum* L.) hinges not only on environmental conditions such as temperature (Pagamas and Nawata 2007; Pagamas and Nawata 2008) but also on agronomic management factors, such as fruit load (Sanchez et al. 1993), seed development stage at harvest (Vidigal et al. 2009), and the nutrient profile of fertilizers applied during seed production (Xu and Kafkafi 2003; Xu et al. 2002).

Pepper plants are moderately sensitive to salt stress at electrical conductivity (EC) in the nutrient solution in the range of $1.2-3.0 \text{ dS m}^{-1}$ (Aktas et al. 2006; Baath et al. 2017). However, most studies on the effects of salinity on pepper plants generate salinity by adding sodium chloride to the nutrient solution. Such experimental approach does not reflect actual agricultural management practices where EC increase could occur due to the presence of other salts besides NaCl. Furthermore, the effect of salt stress on pepper plants has been mostly studied in terms of physiological and metabolic changes that it causes in different structures of the plant (Gunes et al. 1996; Lycoskoufis et al. 2005), as well as its impact on the plant's vegetative growth (Beltrano et al. 2013; Chartzoulakis and Klapaki 2000) and fruit quality (Azuma et al. 2010).

Finally, it is well known that various stresses experienced by mother plants during seed maturation have a profound effect on seed development (Gutterman 2000; Kranner et al. 2010; Kranner and Seal 2013). In particular, salt stress affecting mother plants during seed development may have a negative impact on the agronomical quality of seeds produced (Abdullah et al. 2001; Ghassemi-Golezani and Roozbeh 2011; Ungar 1988). Yet we are unaware of any studies regarding the effects of mother plant saline stress on the agronomical quality of pepper seeds.

Thus, we hypothesized that the agronomical quality of pepper seeds will diminish with rising salinity of the nutrient solution used for mother plant fertigation. Consequently, this work aims to assess the effects of three levels of EC on the agronomical quality of *Capsicum annuum* seeds. To this end, we have analyzed the following seed quality traits: (1) physical characteristics, such as size, weight, and number of seeds per fruit; (2) physiological variables, such as seed germination and vigor; and (3) chemical composition and histological features of mature seeds.

2 Material and Methods

2.1 Growing Conditions of Mother Plants

Our experiment was carried out in Quillota, Valparaíso Region, Chile (32° 50′ S; 71° 13′ W, 120 m.a.s.l.) in a 120- m^2 polyethylene greenhouse with the cover thickness of 200 µm. The greenhouse had a natural ventilation system and was equipped for fertigation with three levels of EC: 2.2 dS m⁻¹, 3.5 dS m⁻¹, and 4.5 dS m⁻¹ (Table 1). The details of the nutrient solutions that we utilized can be found elsewhere (Moya et al. 2017).

The seeds of pepper cv. California Wonder (Vikima, Denmark) were sown in pots with a 3:1 mixture of peat and perlite. At six true leaves, the plants were transplanted into 30-

L coir growth bags $(100 \times 30 \times 10 \text{ cm}, \text{L} \times \text{H} \times \text{W}; \text{Projar}$ Golden Grow Hydroponics Balance, Valencia, Spain). The bags were spaced apart in double rows $(0.4 \times 1.8 \text{ m}, 2.7 \text{ plants} \text{ m}^{-2})$. The experiment was conducted using a completely randomized block design with three replicates per treatment. The experimental unit was a bag containing six plants. Each bag was fertigated by three 4.0 L h⁻¹ drippers. Fertigation was controlled daily with a tolerance of $\pm 0.2 \text{ dS m}^{-1}$ of the target EC level. Irrigation water EC prior to fertilizer dissolution was $0.7 \pm 0.1 \text{ dS m}^{-1}$.

Auxiliary buds were removed to allow the plant to grow. Upon the formation of a base with three leading shoots, it was allowed to grow upwards. At 55 days post-transplant, 10 flowers were selected from the fourth and fifth forks and manually pollinated to produce 8 fruits per plant; other flowers and auxiliary buds were removed. The growth period lasted 120 days post-transplant until the physiological maturity of the seeds, i.e., 89–809 BBCH on the code scale of Feller et al. (1995). Thereafter, the seeds were manually removed, washed in water, and dried at room temperature for 48 h prior to storage; 1 month later, the seeds were analyzed.

2.2 Seed Analyses

The seed area was calculated in 200 dry seeds per treatment by imaging the planar side of the seeds with a digital camera (Canon EOS Rebel T3i) with subsequent processing using the program ImageJ (1.51 j 8 version). The dry weight of seeds was determined in 1500 seeds per treatment after drying at 65 °C for 72 h. Moisture content was established according to the methodology of the International Seed Testing Association (ISTA 2004). The total number of seeds was obtained from 15 fruits per treatment, and seed yield was assessed using 12 plants per treatment. For yield, all the seeds were extracted, dried at room temperature for 48 h, and visually classified into intact and damaged seeds using wet flotation test (Daneshvar et al. 2017).

The germination rate was determined using 200 seeds per treatment, which were sown on filter paper saturated in distilled water and incubated for 14 days at 25 °C \pm 0.1 in darkness. Both germination and seedling classification (normal, abnormal, or dead) were based on the International Seed

Table 1Nutrient solutions withincreased electrical conductivity(EC) used for the fertigation ofmother plants of Capsicumannuum L. (cv. CaliforniaWonder) under distinct treatments

Treatment EC (dS m^{-1})	pН	Concentration (mmol L^{-1})						
		K ⁺	Ca ²⁺	Mg ²⁺	NO_3^-	$\mathrm{H_2PO_4}^-$	2O_4^2	
2.2	5.8	5.0	5.0	1.8	12.5	2.0	1.8	
3.5	5.8	8.3	5.1	2.1	16.2	3.0	2.4	
4.5	5.8	11.5	7.4	2.1	21.4	4.0	2.5	

Testing Association guidelines (ISTA 2018). Further, the vigor test was carried out in accordance with Van der Burg et al. (1994), but with one modification of testing 120 seeds per treatment. Seeds were sown in polyethylene pots (288 cell polyethylene seedling tray, 35 mL per cell) with substrate mixture of peat and perlite (3:1 ratio). Trays were watered daily, and no fertilizers or pesticides were used. Seedlings were cultivated in greenhouse conditions at 24 °C ± 2 for 60 days; seedling emergence was evaluated at 14 days after sowing, whereas seedling height and dry mass were evaluated at 60 days after sowing. Dry matter content was measured in roots, stems, and leaves of seedlings, which were dried at 65 °C for 72 h, using 15 samples per structure and per treatment.

For histological observations, the seeds were cut longitudinally and dried at 70 °C for 72 h prior to analysis (Xu and Kafkafi 2003). The observations were carried out using a magnifying glass and a scanning electron microscope (Hitachi, model SU3500 Tokyo, Japan). Further, the content of mineral elements on the embryo surface was determined using the energy dispersive X-ray analysis of the scanning electron microscope. Three specific measuring points of the embryo were used for the analysis (Supplementary Fig. S1), which was carried out in five seeds per treatment.

Variance analysis (ANOVA) was carried out using the Minitab Statistical Software 17 package (Minitab Inc., State College, PA). The differences among treatments were compared using the Tukey test ($P \le 0.05$). Percentage-expressed variables were analyzed after the arcsine transformation.

3 Results and Discussion

An increase in the EC of the nutrient solution used for fertigation of mother plants caused a statistically significant reduction in seed area ($F_{2,599} = 4.07$; P = 0.000) and seed dry weight ($F_{2,44} = 12.22$; P = 0.000) (Table 2). The seed area was more sensitive to the EC treatments, with the first evidence appearing already at the EC treatment of 3.5 dS m⁻¹. Such

weight reduction of the seeds may have to do with limited flow of nutrients and photoassimilates to the seeds due to the stress experienced by the mother plant at seed formation (Ambika et al. 2014; Bewley et al. 2012). On the other hand, it is well known that larger seeds have better agronomical performance than smaller seeds (Ambika et al. 2014; Ghassemi-Golezani and Roozbeh 2011; Peñaloza and Durán 2015). In summary, the highest salinity treatment had a negative effect on seed quality given the observed reduction in seed weight and seed area (Table 2).

The number of seeds per fruit and germination percentage were unaffected by the treatments (Supplementary Table S1). However, the treatments caused a drop in the number of intact seeds ($F_{2,8} = 13.80$; P < 0.01) and an increase in the number of damaged seeds ($F_{2,8} = 25.69$; P < 0.01) at higher EC rates during the treatments (Table 2). Furthermore, the vigor test showed statistically significant differences between the treatments with regard to seed emergence, as well as seedling height and weight (Table 2). These results are in agreement with Matthews et al. (2012), who suggested that seeds characterized by lower emergence negatively affect seedling performance. The most sensitive endpoints to the EC treatments were seedling height and weight, which demonstrated the negative effects of the EC treatment already at 3.5 dS m⁻¹.

Importantly, our study revealed that stem and leaves had greater sensitivity to salt stress than roots (Table 2), which conforms with the results of some other studies where plant leaves and roots also had dissimilar responses to salt stress. However, the patterns vary in distinct crops (Bouthour et al. 2015; Hela et al. 2011; Hu et al. 2012; Ji et al. 2016; Liu et al. 2014; Reyes-Perez et al. 2016). Even distinct genotypes of *Capsicum annuum* exhibit dissimilar leaf and root responses to saline stress (Javed et al. 2020; Tehseen et al. 2016) making it impossible to draw general conclusions about the relative sensitivity of plant leaves and roots to salt stress.

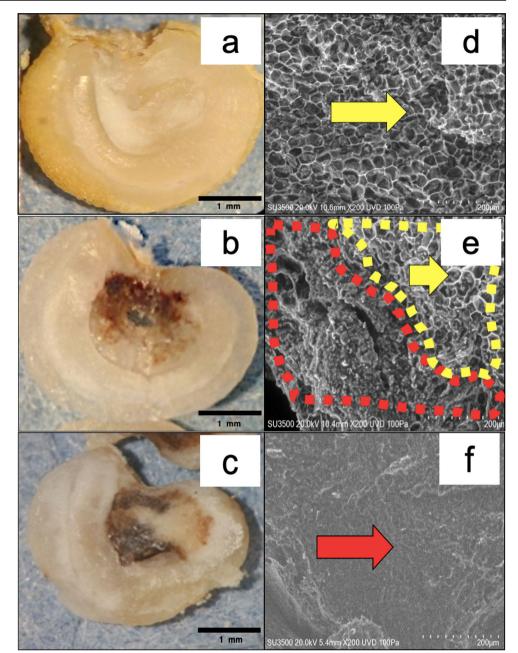
Moreover, histological observations suggest that seed tissues were healthy at the EC treatment of 2.2 dS m^{-1} (Fig. 1a and d). However, with an increase in the EC of the nutrient solution used for fertigation of mother plants, endosperm

 Table 2
 Observed attributes of Capsicum annuum L. (cv. California Wonder) seeds under treatments with increased electrical conductivity (EC) used for the fertigation of mother plants

Treatment EC (dS m ⁻¹)	Seeds					Seedlings			
	Area (mm ²)	Dry weight (mg 100 seeds ⁻¹)	Intact (mg fruit ⁻¹)	Damaged (mg fruit ⁻¹)	Emergence (%)	Height (mm)	Dry weight (mg)		
							Root	Stem	Leaves
2.2	15 ± 21^a	659 ± 4^a	174 ± 5.6^{a}	137 ± 15^{b}	64 ± 2.3^a	59 ± 20^a	32 ± 2.6^a	17 ± 1.4^{a}	33 ± 1.4^a
3.5	12 ± 14^b	631 ± 3^{a}	183 ± 8.7^a	152 ± 7.8^{b}	58 ± 5.2^{a}	53 ± 8.6^b		12 ± 0.98^{b}	
4.5	11 ± 11^{c}	596 ± 4^{b}	147 ± 11^{b}	200 ± 7.8^a	42 ± 3.5^{b}	49 ± 20^{b}	26 ± 1.7^{b}	13 ± 1.9^{b}	26 ± 2.6^{b}

Letters indicate statistically significant differences on the basis of the Tukey test ($\alpha = 0.05$). NS non-significant

Fig. 1 Magnifying glass observations of Capsicum annuum L. (cv. California Wonder) seeds under distinct electrical conductivity treatments: **a** 2.2 dS m⁻¹, **b** 3.5 dS m⁻¹, and **c** 4.5 dS m⁻¹. Scanning electron microscopy micrographs of Capsicum annuum L. (cv. California Wonder) seeds under distinct electrical conductivity treatments: d 2.2 dS m⁻¹, e $3.5~\text{dS}~\text{m}^{-1}\text{,}$ and $\textbf{f}~4.5~\text{dS}~\text{m}^{-1}\text{.}$ Red arrows indicate endosperm damage (tissues with progressing cellular lysis are visible), whereas yellow arrows indicate healthy tissue with undamaged cell walls. Red dotted zone indicates damaged tissues, whereas yellow dotted zone indicates healthy tissues



damage occurred. Specifically, we observed tissues with cellular lysis in progress in treatments with increased EC. This damage was partial in the EC treatment of 3.5 dS m^{-1} (Fig. 1b and e) and generalized in the EC treatment of 4.5 dS m^{-1} (Fig. 1c and f). However, no embryo damage was observed in any of the treatments.

Finally, regarding the chemical composition of the seeds, we found that element concentrations at the treatments of 3.5 and 4.5 dS m⁻¹ were statistically indistinguishable from those at the treatment of 2.2 dS m⁻¹ (Supplementary Table S2). Although the supply of mineral nutrients available to the mother plant affects the chemical composition of the seeds (Fenner 1992), our study demonstrated that greater

concentrations of mineral elements in the nutrient solution do not necessarily produce greater accumulation of these elements in the seeds. This may be due to the selective absorption of certain ions by the plant under salt stress conditions (Volkmar et al. 1998).

4 Conclusion

Our results support the hypothesis that the agronomical quality of pepper seeds diminishes with rising salinity of the nutrient solution used for mother plant fertigation. These findings are important because escalating soil and water salinization diminishes crop yields, thus posing a major threat to food security (Saddiq et al. 2020; Wang et al. 2020).

Future studies should be performed to better gauge the effect of nutrient solutions with distinct electrical conductivity on the seed quality traits, such as physical and physiological characteristics, chemical composition, and histological features.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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