Seed priming and sulfur effects on soybean cell membrane stability and yield in saline soil

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Abstract – The objective of this work was to determine the effects of seed priming and sulfur application on cell membrane characteristics, seedling emergence, chlorophyll content and grain yield of soybean (*Glycine max*) in saline soil. A complete-block design in 4x3 factorial arrangement with three replicates was used to test four types of seed priming (water, auxin, gibberellin and non-priming) and three levels of sulfate availability (0, 70 and 140 kg ha^{-1} K_2SO_4). The soil had a silty loam texture with an electrical conductivity of 3.61 ds m^{-1}, a pH of 8.2 and a saturation percentage of about 46%. Seed priming had significant effects on mean emergence rate (MER), emergence percentage, relative water content (RWC) of leaves, relative chlorophyll content, time of maturity, shoot length and grain yield. The highest values for these variables were observed in the priming treatments, except for the time of maturity. Sulfur application had significant effects on MER, shoot length, RWC, membrane injury index and grain yield. Priming treatments provide greater emergence rates and grain yields and interact sinergicaly with sulfur rates.

Index terms: *Glycine max*, chlorophyll content, environmental stress, injury index, seedling emergence.

Condicionamento osmótico das sementes e disponibilidade de enxofre na estabilidade da membrana celular e produtividade de soja em solo salino

Resumo – O objetivo deste trabalho foi determinar os efeitos do condicionamento osmótico das sementes e da disponibilidade de enxofre sobre características da membrana celular, emergência de plântulas, conteúdo relativo de clorofila e produtividade de soja (*Glycine max*) em solo salino. O experimento foi conduzido em delineamento de blocos ao acaso, em arranjo fatorial 4x3, com três repetições, para avaliar quatro tipos de condicionamento osmótico das sementes (água, auxina, giberelina e ausência de condicionamento) e três níveis de disponibilidade de enxofre para as plantas (aplicação ao solo de 0, 70 e 140 kg ha^{-1} de K_2SO_4). O solo utilizado apresentava textura média, com condutividade elétrica de aproximadamente 3,61 ds m^{-1}, pH de 8,2 e percentagem de saturação em torno de 46%. O condicionamento osmótico das sementes teve efeito significativo sobre a taxa média de emergência (MER), percentagem de emergência, conteúdo relativo de água das folhas (RWC), conteúdo relativo de clorofila, época de maturação, comprimento da parte aérea e produtividade de grãos. Os maiores valores para essas variáveis, exceto época de maturação, foram observados nos tratamentos com condicionamento osmótico. A aplicação de enxofre teve efeito significativo sobre MER, comprimento da parte aérea, RWC, índice de dano à membrana celular, e produtividade de grãos. O condicionamento osmótico proporciona maiores taxas de emergência e produtividade de grãos e interage significativamente com a aplicação de enxofre.

Termos para indexação: *Glycine max*, conteúdo de clorofila, estresse ambiental, índice de dano, emergência de plântulas.

Introduction

A major impact of environmental stress on plants is cellular membrane modification due to salt stress, expressed in increased permeability and leakage of ions. Consequently, the relative water content (RWC) in the leaves decreases with increasing concentrations of salt solution (Kocheva & Georgiev, 2003). The accumulation of active oxygen species under stress conditions may damage many cell compounds, such as lipids, proteins, carbohydrates and nucleic acids (Bewley & Black, 1994), and deplete the chlorophyll pool (McDonald, 2000) as a result of cell-membrane lipids peroxidation (Soeda et al., 2005).

Germination and emergence rates are important factors for the successful establishment of crops. Harris et al. (2001) demonstrated that on-farm seed priming (overnight seed soaking, surface drying and sowing) markedly improved establishment and early vigor of upland rice, maize and chickpea, resulting in faster development, earlier flowering and maturity and higher yields. This simple, low-cost and low-risk intervention also has positive impacts on extensive farming systems and their economy (Harris et al, 2001).

Sulfur has an essential role in the synthesis of proteins and of a wide variety of metabolites that are critical for plant growth (Ali et al., 1990). Sulfur availability is partially related to soil moisture (Itanna, 2005). Sardans et al. (2006, 2008) reported a negative correlation between leaf sclerophylly and leaf S concentration in a Mediterranean forest. The amino acid profile of soybean meals could be improved with greater amounts of S-containing amino acids, methionine and cysteine. Increasing the amount of these amino acids in soybean meals would enhance their market value (McVey et al., 1995). Sulfur is predominantly absorbed from the soil solution as sulfate anion (SO_4^{2-}) by plants and transported to chloroplasts in expanding leaves, where most S reduction is reported to occur (Anderson, 1990).

The objectives of this study were to determine the effects of different seed primings and of sulfur fertilization on soybean cell membrane characteristics, emergence rate, chlorophyll content and grain yield.

Materials and Methods

The field experiment was done in Ardabil (38º15"N; 48º15"E; altitude of 1,350 m), northwest Iran, at a research site of University of Mohaghegh Ardabili, in 2007. The experimental area is located in the country's semiarid temperate zone, which features cold winters and moderate summers. The average annual rainfall is about 400 mm, and most rainfall is concentrated between winter and spring (January to June). The soil used was an Entisoil with silty loam texture, with an electrical conductivity (EC) of 3.61 ds m^{-1}, a pH of 8.20 and a saturation percentage (SP) of about 46%.

The experimental design was of randomized complete blocks in a 3x4 factorial arrangement with three replicates. The treatments were: K_2SO_4 fertilizer rates at 0, 70 and 140 kg ha^{-1} (S_1, S_2 and S_3 respectively), seed priming using distilled water, auxin and gibberellin as pre-sowing treatments, and a control treatment without priming.

Soybean seeds (cv. Williams, maturity group III) were washed with distilled water, dipped in 0.1% mercuric chloride for 5 min and then washed thoroughly with distilled water. The washed seeds were divided into four lots: two were fully immersed in aerated auxin and gibberellin solutions (auxin and gibberellin-primed); the third was immersed in aerated water (1:2 w/v) (water-primed); and the fourth served as control non-primed seeds. Seeds in pre-sowing treatments were kept in an incubator for 12 h at 25±1°C. The seeds were then washed with distilled water and dried on filter papers at room temperature (25°C). Seeds were sown in May 2007. Each plot had five 3.2 m rows with a 40 cm space between rows and 5 seeds m^{-1}. Potassium sulfate was applied before sowing, at a depth of 8 cm and a distance of 5 cm from the seeds. Data from border rows were not taken.

The parameters determined were: shoot length (cm), emergence (%), mean emergence rate (MER, emerged seedlings per day), time of maturity (days), grain yield (kg ha^{-1}), leaves relative water content (RWC), membrane cell injury index (%) and relative chlorophyll content (SPAD index).

Relative water content was estimated according to Turner (1981): RWC = (FW - DW)/(TW - DW), where FW is the fresh weight of the leaves; TW is the weight at full turgor, measured after floating the leaves for 24 hours in water under light and at room temperature; and DW is the estimated weight of leaves after drying until constant weight, at 75±5ºC.

Leaf cell membrane stability (CMS) was measured by polyethylene glycol (PEG) test according to Premachandra (1992). Leaf samples (2 g) were taken at the second leaf stage and immersed in 30% PEG 6000 (Kimya Garan Co., Tehran, Iran) for 24 hours. For non-desiccated control, the leaf segments were immersed in equal volume of distilled H_2O. After washing with distilled water, the leaf segments were submerged in 30 mL deionized water for 24 hours. Solution conductivities were measured and the samples were autoclaved for 15 min, cooled to room temperature and then measured once more. The electrolyte leakage was measured using a conductimeter. The CMS and injury index of the samples were calculated according to the equations: CMS (%) = 1 - (1 - T1/T2)/(1 - C1/C2) × 100 and injury index (%) = (100 - CMS), where T1 and T2 are the first and second conductivity measurements of the desiccated treatment after autoclaving, C1 and C2 represent the first and second conductivity measurements of the control after autoclaving. Leaves relative chlorophyll content was measured with a portable chlorophyll meter (SPAD-502, Minolta, Japan). Daily emergence counts for seedlings that were visible above the soil level with a minimum height of 1.0 cm were taken for 28 days. The mean emergence rate (MER) was estimated according to Wang et al. (2003): $MER = \frac{\sum n}{\sum Dn}$, where n is the number of emerged seedlings, and D is the sampling day.

Analysis of variance was performed using SAS software package (SAS Institute, 1998) after normality test. The main effects and interactions were tested using Duncan's multiple range test at 5% probability.

Results and Discussion

Interaction between treatments was observed in emergence rate and grain yield (Table 1). The highest emergence rate was achieved in S_3 with auxin and gibberellin priming, and the lowest was achieved in non-primed treatments and in S_3 water priming. Research evidence has indicated that priming soybean seeds before sowing, particularly in low-vigor seeds, improves germination (Wartidiningsih et al., 1994). Priming has been developed and used extensively to improve seed germination and seedling emergence in a wide range of crop species (McDonald, 2000).

Grain yield increased with sulfur (S_2 and S_3) and priming (water, auxin and gibberellin) treatments in comparison with control plots (S_1 and non-priming). The highest grain yield was observed in the interaction between S_2 and S_3 with auxin, gibberellin and water priming respectively. The lowest grain yield was achieved at S_1 with non-priming (Table 1). Similar results were reported for priming by Rashid et al. (2004) and for sulfur by Sexton et al. (1998). Sulfur deficiency has been reported to decrease seed yield in the field by up to 20% (Sexton et al., 1998).

Emergence test showed that seed priming significantly increased emergence percentage (Table 2). Seed emergence of many species is stimulated by plant growth regulators and inhibited by salt stress (Sparks, 2005). These results are in agreement with Foti et al. (2008), who observed faster development, earlier flowering and maturity, and higher yields of maize under different seed priming types. Sedghi et al. (2008) demonstrated that priming with gibberelline increases the rate and percentage of seedling emergence in comparison with auxin in medicinal pumpkin (*Cucurbita pepo*). They concluded that gibberelline induces the production of the hydrolytic enzymes responsible for endosperm degradation, and causes faster germination. According to Tiryaki et al. (2004), cytokinin and ethylene secretion is stimulated by seed priming, and ethylene – or its precursor, 1-aminocyclopropane-1-carboxilic acid (ACC) – is the simplest unsaturated hydrocarbon that regulates diverse metabolic and developmental processes in plants, including seed germination.

No significant differences were observed in injury index among priming treatments (Table 2). Relative water and chlorophyll contents were greater in

Table 1. Mean emergence rate (MER) and grain yield of soybean submitted to four priming treatments and three sulfur (K_2SO_4) application rates (kg ha^{-1})[(1)].

Sulfur application rates	Nonprimed	Water priming	Auxin priming	Gibberellin priming
	Mean emergence rate (no. day^{-1})			
0	1.2g	2.5def	3.7cd	4.2bc
70	1.3fg	3.0cde	4.0c	3.8c
140	1.8efg	2.0efg	5.5a	5.3ab
	Grain yield (kg ha^{-1})			
0	1,378.3f	1,431.7e	1,477.3d	1,480.7d
70	1,540.0c	1,576.7ab	1,588.3a	1,590.0a
140	1,551.7bc	1,580.7ab	1,588.7a	1,590.7a

[(1)]Means followed by equal letters within the evaluated variable do not differ by Duncan's multiple range test, at 5% probability.

Table 2. Soybean studied traits at three different seed priming treatments and sulfur (K_2SO_4) application rates (kg ha^{-1})[(1)].

Priming Treatments	Emergence (%)	Injury index (%)	Relative water content (%)	Chlorophyll Content (SPAD index)	Shoot length (cm)	Time of maturity (days)
Nonprimed	63.60b	27.44a	74.55b	22.66b	28.22b	121.44a
water	85.77a	26.44a	76.33ab	26.12ab	32.55a	105.33b
Auxin	89.00a	25.88a	76.44ab	30.02a	34.00a	105.22b
Gibberellin	89.33a	25.55a	78.33a	29.40a	33.88a	104.66b
Sulfur application rates						
0	81.08a	33.83a	64.83c	27.21a	30.25b	107.91a
70	82.58a	24.08b	80.02b	27.80a	31.66b	110.16a
140	82.16a	21.08c	83.33a	26.14a	34.58a	109.41a

[(1)]Means followed by equal letters do not differ by Duncan's multiple range test, at 5% probability.

gibberellin primed plants than in non-primed plants. The overall growth (shoot length and time of maturity) in primed plants was higher than in non-primed plants. At 100 days after sowing, an 18% increase in shoot length in primed plants was observed. Sulfur application showed no difference in the emergence percentage. Increasing the sulfur application rate decreased injury index in cell membrane and improved RWC. No significant differences in chlorophyll content and time of maturity were observed between the sulfur application treatments but shoot length was increased by S_3 treatment.

Conclusions

1. On-farm seed priming improves emergence and early growth of soybean in fast drying and salty soils.
2. In general, priming treatments provide greater emergence rates and grain yields, and interact sinergically with sulfur application.

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