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# Soybean Performance under Salinity Stress

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## 1. Introduction

Soybean seed is a major source of high-quality protein and oil for human consumption (Katerji *et al.* 2001). The unique chemical composition of soybean has made it one of the most valuable agronomic crops worldwide (Thomas *et al.* 2003). Its protein has great potential as a major source of dietary protein. The oil produced from soybean is highly digestible and contains no cholesterol (Essa and Al-ani 2001). Growth, development and yield of soybean are the result of genetic potential interacting with environment. Soybean seed production may be limited by environmental stresses such as soil salinity (Ghassemi-Golezani *et al.* 2009). Minimizing environmental stress will optimize seed yield (Mc Williams *et al.* 2004).

Soil salinity, resulting from natural processes or from crop irrigation with saline water, occurs in many arid and semi-arid regions of the world (Meloni *et al.* 2004). The UNEP (United Nations Environment Program) estimates that 20% of the agricultural land and 50% of the cropland in the world is salt-stressed (Yan 2008). Most of the salt stresses in nature are due to Na<sup>+</sup> salts, particularly NaCl (Demirel 2005). High salinity lowers water potential and induces ionic stress, and results in secondary oxidative stress. It severely limits growth and development of plants by affecting different metabolic processes such as CO<sub>2</sub> assimilation, oil and protein synthesis (Nasir khan *et al.* 2007).

Plants vary tremendously in their ability to tolerate salinity (Bischoff and Warner 1999). The term halophyte means “salt tolerant plant” but is used specifically for plants that can grow in the presence of high concentration of Na<sup>+</sup>. Plants that can not grow in presence of high concentration of Na<sup>+</sup> salts are called glycophytes (Brevedan and Egli 2003). Soybean is classified as moderately salt sensitive instead of moderately salt tolerant (Katerji *et al.*, 2000). Salt tolerance of plants may be dependent on growth stage, varieties, nutrition and environment (Bischoff and Warner 1999).

Netondo *et al.* (2004) reported that photosynthetic activity decreases when plants are grown under saline conditions leading to reduced growth and productivity. The reduction in photosynthesis under salinity can be attributed to a decrease in chlorophyll content (Jamil *et al.* 2007) and activity of photo-system II (Ganivea *et al.* 1998). Salinity can affect chlorophyll content through inhibition of chlorophyll synthesis or an acceleration of its degradation (Reddy and Vora 1986). Fluorescence of chlorophyll reflected the photochemical activities of photo-system II (Ganivea *et al.* 1998). Photochemical efficiency of photo-system II (fv/fm) could be reduced by salinity stress (Jamil *et al.* 2007; Netondo *et al.* 2004).

Plants have evolved complex mechanisms that contribute to the adaptation to osmotic stress caused by high salinity (Meloni *et al.* 2004). Osmotic adjustment has undoubtedly gained considerable recognition as a significant and effective mechanism of salinity tolerance in crop plants (Pakniyat and Armion 2007). In salt stressed plants, osmotic potential of vacuole decreased by proline accumulation (Yoshiba *et al.* 1997). Several possible roles have been attributed to supra-optimal level of proline including osmoregulation under salinity, stabilization of proteins and prevention of heat denaturation of enzymes and conservation of nitrogen and energy for a post-stress period (Aloni and Rosenshtein 1984).

Final seed weight is the result of seed filling rate during the linear phase and the duration of this period. Seed filling rate was described as the accumulation of seed dry matter per unit time, which varied among varieties and had positive correlation with final seed weight (Guffy *et al.* 1991). Researchers showed that environmental stresses may hasten the seed filling rate and decrease grain filling duration (Yazdi-Samadi *et al.* 1977). This can influence final yield of all grain crops such as soybean. Seed filling period is under genetic control and it is sensitive to salt stress (Brevedan and Egli 2003). Soybean seed protein and oil contents may be also influenced by environmental factors such as salinity (Nakasathien *et al.* 2000). Oil and protein syntheses occur during seed filling (Yazdi-Samadi *et al.* 1977). Approximately 18% to 21% of soybean seed dry weight is oil in the form of triacylglycerol. From 24 to 40 days after flowering, oil percentage increases rapidly and by the end of this period accounts for approximately 30% of the total oil of the mature seed. The remaining 70% is synthesized during 40 to 64 days after flowering, also a period of seed desiccation (Hajduch *et al.* 2005). The objective of this study is to evaluate the performance of soybean cultivars in response to different levels of NaCl salinity.

## 2. Materials and methods

Seeds of three soybean cultivars (Williams, Zan and L<sub>17</sub>) were obtained from Agricultural Research Institute, Moghan, Iran. Two experiments with factorial arrangements on the bases of randomized complete block (RCB) with three replications were conducted in 2007 (Tabriz, Iran) and 2008 (Uremia, Iran) to investigate changes in chlorophyll content index (7 weeks) and fluorescence of chlorophyll (4 times) in leaves and to determine proline content and grain yield of three soybean cultivars under a non-saline (control) and three saline (3, 6 and 9 dS/m NaCl) conditions. Six seeds were sown 3 cm deep in each pot, filled with 900 g perlite, using 144 pots in each experiment. Pots were then placed in the greenhouse. The temperature variation in the greenhouse was 17-34°C and 13-28°C during the first and second experiments, respectively. Tap water and saline solutions were added to the pots in accordance with the treatments to achieve 100% FC.

After emergence, seedlings were thinned to keep 4 plants in each pot. During the growth period, the pots were weighed and the losses were made up with Hoagland solution (EC = 1.3 dS/m). Perlites within the pots were washed every 25 days and non-saline and salinity treatments were reapplied in order to prevent further increase in electrical conductivity (EC), due to adding the Hoagland solution.

Leaf chlorophyll content index (CCI) was measured by a chlorophyll meter (CCM-200, Opti-Science, USA) in weekly intervals for seven weeks. After seedling establishment, a plant was marked in each pot and CCI of upper, middle and lower leaves was measured at each stage. Subsequently, mean CCI for each treatment and replicate at each developmental stage was calculated.

The chlorophyll fluorescence induction parameters were measured in leaves by a chlorophyll fluorometer (OS-30, OPTI-SCIENCES, USA) every 10 days from 30 to 60 days after sowing. Fluorescence emission was monitored from the upper surface of the leaves. Dark-adapted leaves (30 min.) were initially exposed to the weak modulate measuring beam, followed by exposure to saturated white light to estimate the initial ( $F_0$ ) and maximum ( $F_m$ ) fluorescence values, respectively. Variable fluorescence ( $F_v$ ) was calculated by subtracting  $F_0$  from  $F_m$ . The  $F_v/F_m$  ratio measures the efficiency of excitation energy capture by open PSII reaction centers, representing the maximum capacity of light-dependent charge separation in PSII (Rizza *et al.* 2001).

The proline content was determined spectrophotometrically according to Bates *et al.* (1973). 200 mg leaf samples were powdered in liquid nitrogen and were homogenized in 5 ml sulphosalicylic acid. 2 ml acid ninhydrine and 2 ml glacial acetic acid were added to the extract. The samples were heated at 100 °C. The mixture was extracted with toluene and the free toluene was quantified spectrophotometrically at 520 nm.

During grain filling, four harvests were made at 10 days intervals, beginning 75 days after sowing. Grain yields of two experiments for the same replicates and treatments were mixed, in order to provide enough grains for the measurement of protein and oil. Percentages of oil and protein for each sample were measured, using a seed analyzer (model: Zeltex ZX-50). Subsequently, Protein and oil yields per grain and per plant were calculated. A regression model was used to describe the seeds oil and protein accumulation. The following equation was applied to calculate the rates of protein and oil accumulation in soybean grains under different treatments:

$$\text{Accumulation rate (mg d}^{-1}\text{)} = \text{Maximum weight (mg)}/\text{Filling duration (day)}$$

At maturity, plants of each pot were separately harvested and grains were detached from the pods. Finally, grains were weighed and grain yield per plant for each treatment at each replicate was determined.

MSTATC software was used to analyze the data for CCI and chlorophyll fluorescence as factorial split plot and those for proline and grain yield as factorial. Means of the traits were compared at  $p \leq 0.05$ . SAS software was used for regression analysis of the grain filling data and Excel software was applied to draw figures.

### 3. Results

The results of analysis of variance showed highly significant ( $P \leq 0.01$ ) effects of year, cultivar, salinity and time on both chlorophyll content index (CCI) and fluorescence of chlorophyll. Means of CCI and  $f_v/f_m$  in 2007 were higher than those in 2008. The CCI and fluorescence of chlorophyll in soybean leaves decreased with increasing salinity. L17 and Zan had the highest and the lowest CCI and  $f_v/f_m$ , respectively (Table 1).

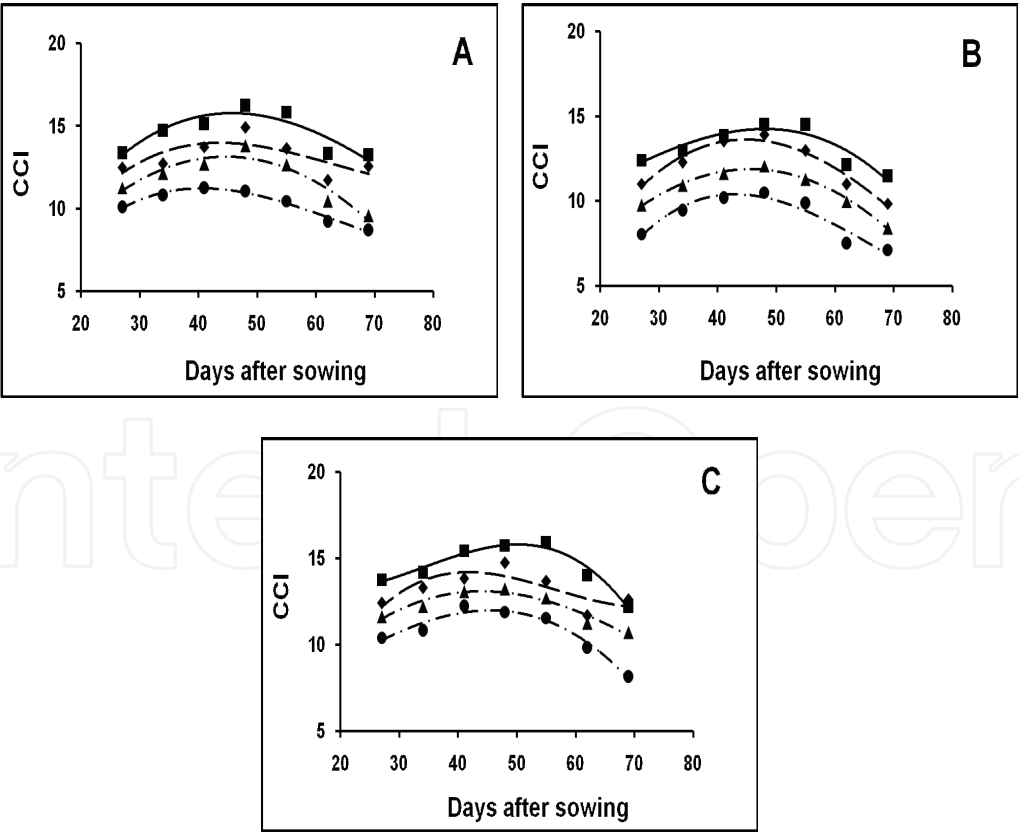
Means of CCI and chlorophyll fluorescence of soybean cultivars increased with progressing plant growth up to the points where maximum values were achieved under non-saline and saline conditions (Figures 1 and 2). Maximum CCI of all cultivars under salinity treatments was obtained earlier than that under non-saline treatment (Figure 1), but maximum chlorophyll fluorescence under all treatments was achieved at almost similar stage (Figure 2). Thereafter, due to senescing of leaves, CCI and chlorophyll fluorescence started to decrease. Means of CCI and chlorophyll fluorescence at all developmental stages decreased

as the salinity increased. In general, L17 and Williams had more CCI and chlorophyll fluorescence at different stages of growth and development, compared with Zan (Figures 1 and 2).

Treatment		CCI	fv/fm
year	1	13.66 <sup>a</sup>	0.779 <sup>a</sup>
	2	10.50 <sup>b</sup>	0.728 <sup>b</sup>
Salinity (dS m <sup>-1</sup> )	0	14.06 <sup>a</sup>	0.792 <sup>a</sup>
	3	12.81 <sup>b</sup>	0.768 <sup>b</sup>
	6	11.50 <sup>c</sup>	0.742 <sup>c</sup>
	9	9.97 <sup>d</sup>	0.713 <sup>d</sup>
Cultivar	L <sub>17</sub>	12.63 <sup>a</sup>	0.764 <sup>a</sup>
	Zan	11.19 <sup>b</sup>	0.739 <sup>b</sup>
	Williams	12.43 <sup>a</sup>	0.758 <sup>a</sup>

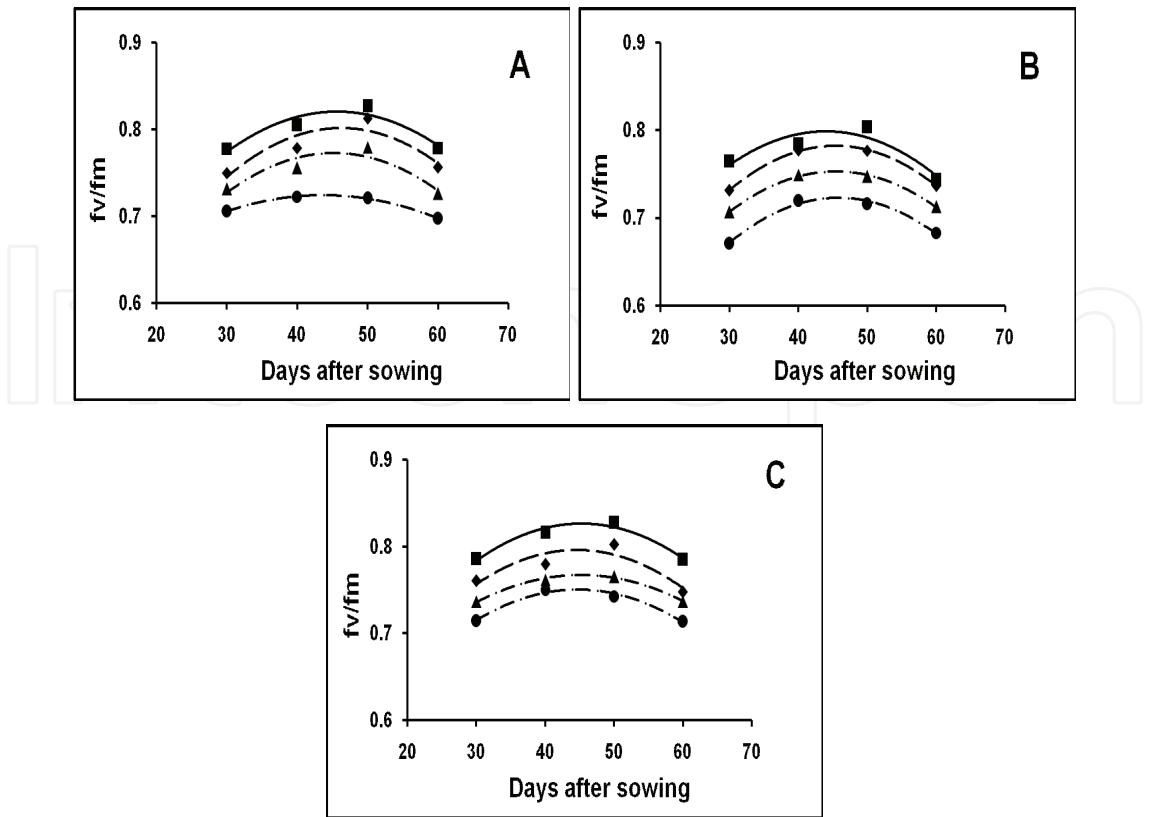
Different letters in each column indicate significant difference at p≤0.05.

Table 1. Comparison of means chlorophyll content index (CCI) and fluorescence of chlorophyll (fv/fm) of three cultivars of soybean under salinity stress



A: Williams    B: Zan    C: L17, — 0 dS/m    - - 3 dS/m    . . . 6 dS/m    - · - · 9 dS/m

Fig. 1. Changes in chlorophyll content index (CCI) of soybean cultivars under non-saline (control) and Saline conditions (means of two years).



A: Williams    B: Zan    C: L17, — 0 dS/m    - - 3 dS/m    . . . 6 dS/m    - . . . 9 dS/m

Fig. 2. Changes in chlorophyll fluorescence (fv/ fm) of soybean cultivars under non-saline (control) and Saline conditions (means of two years).

Leaf proline content and grain yield per plant were significantly ( $P \leq 0.01$ ) affected by cultivar and salinity, but cultivar  $\times$  salinity interaction was not significant for these traits ( $P \geq 0.05$ ). Leaf proline content of soybean increased with increasing salinity. Proline content of Zan was significantly higher than that of Williams and L<sub>17</sub>. However, proline content of the latter cultivars was similar (Table 2). Grain yield per plant significantly decreased as salinity increased. Zan had the lowest grain yield per plant, but there was no significant difference in grain yield of L<sub>17</sub> and Williams (Table 2).

Treatment		Proline content (Mm/g)	Grain yield per plant(g)
Salinity (dS m <sup>-1</sup> )	0	19.40 <sup>d</sup>	1.250 <sup>a</sup>
	3	26.12 <sup>c</sup>	0.892 <sup>b</sup>
	6	39.28 <sup>b</sup>	0.516 <sup>c</sup>
	9	45.89 <sup>a</sup>	0.274 <sup>d</sup>
Cultivar	L <sub>17</sub>	31.71 <sup>b</sup>	0.782 <sup>a</sup>
	Zan	35.36 <sup>a</sup>	0.651 <sup>b</sup>
	Williams	30.96 <sup>b</sup>	0.766 <sup>a</sup>

Different letters in each column indicate significant difference at  $p \leq 0.05$ .

Table 2. Comparison of means of proline content and grain yield per plant of three cultivars of soybean under salinity stress



Protein percentage of soybean cultivars under all saline and non-saline conditions increased with increasing grain filling period up to a point where maximum value was achieved. Maximum protein percentage for Zan and L<sub>17</sub> was attained about 10 days earlier than Williams (Figure 3). Protein percentage of all soybean cultivars at different stages of seed development decreased, as the salinity increased. However, this reduction was higher for Williams, compared with Zan and L<sub>17</sub>. In contrast, oil percentage of all cultivars increased with increasing salinity. Oil percentage decreased as protein percentage increased with proceeding of grain filling (Figure 3).

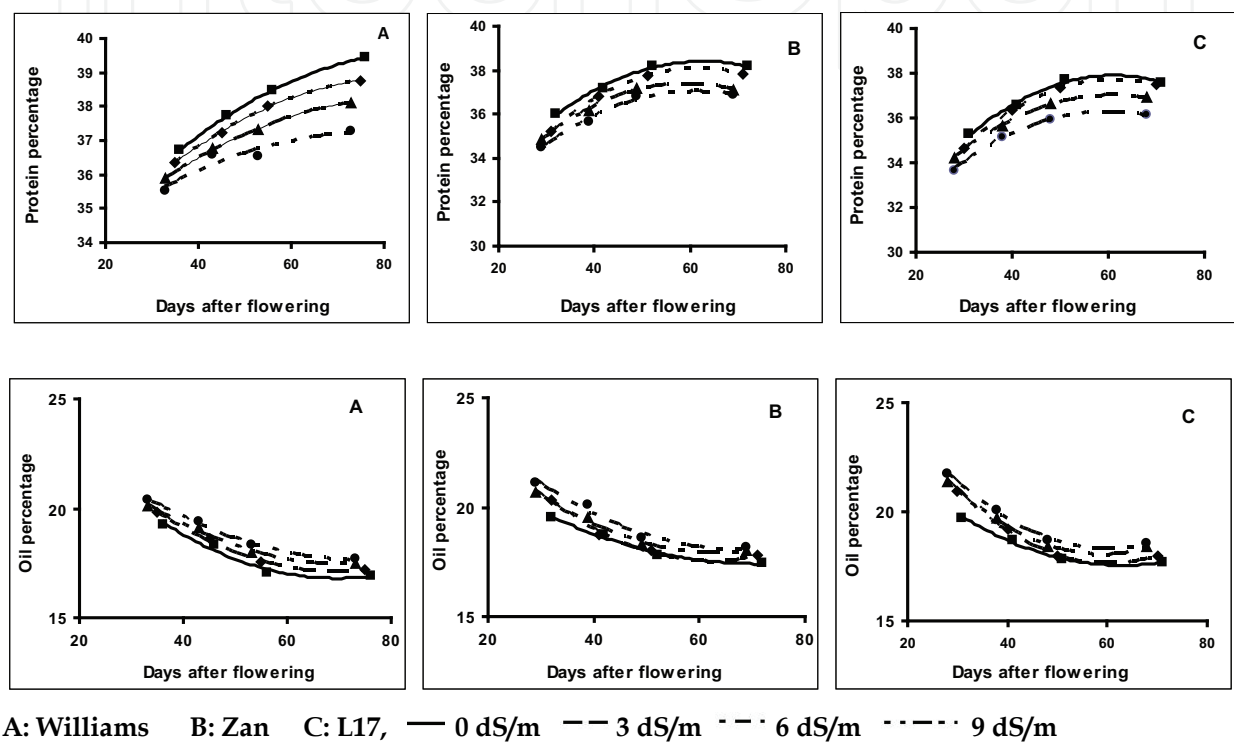


Fig. 3. Changes in seed protein and oil percentage of soybean cultivars under non-saline (control) and Saline conditions

Protein and oil contents per grain of soybean cultivars under non-saline and all saline conditions increased with progressing seed development up to 50-65 days after flowering, depending on cultivar and salinity level (Figure 4). Maximum protein and oil contents per seed under salinity stress were achieved earlier than those under non-saline conditions. Although both protein and oil per seed decreased with increasing salinity, protein content per grain at different stages of seed development was much higher than oil content under all treatments (Figure 4).

Effects of salinity on rate of protein accumulation, duration of protein and oil accumulation, grain yield per plant and protein and oil yields per plant were significant ( $P \leq 0.01$ ), but its effect on rate of oil accumulation was not significant ( $P \geq 0.05$ ). All these traits, except rate of protein accumulation and the duration of oil accumulation, were also significantly affected by cultivar (Table 3).

Means of all the traits, except rate of oil accumulation, decreased with increasing salinity. Despite this reduction, Rate of protein accumulation for control and 3 and 6 dS/m NaCl

salinity was statistically similar (Table 3). Williams had the highest rate and duration of protein accumulation and rate of oil accumulation, but L<sub>17</sub> had the highest grain yield per plant. The lowest grain, protein and oil yields were obtained for Zan, while differences in protein and oil yields between Williams and L<sub>17</sub> were not significant (Table 3).

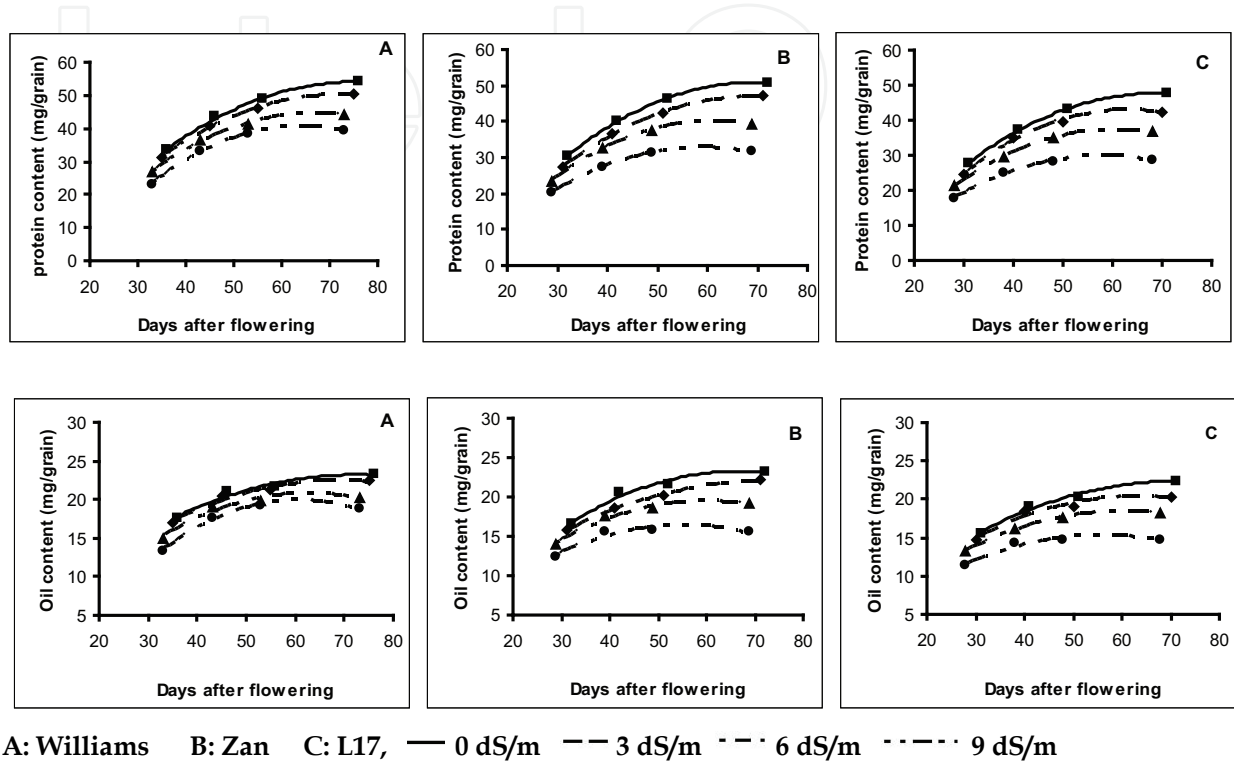


Fig. 4. Changes in seed protein and oil content of soybean cultivars under non-saline (control) and saline conditions

Treatment		Rate of protein accumulation (mg/day)	Duration of protein accumulation (day)	Rate of oil accumulation (mg/day)	Duration of oil accumulation (day)	Grain Yield per plant (g)	Protein yield per plant (mg)	Oil yield per plant (mg)
Salinity (dS m <sup>-1</sup> )	0	0.851 a	59.8 a	0.390 a	59.2 a	1.250 a	478.41 a	216.18 a
	3	0.846 a	55.6 b	0.398 a	55.4 b	0.892 b	338.70 b	157.27 b
	6	0.806 a	50.7 c	0.409 a	48.2 c	0.516 c	192.54 c	92.38 c
	9	0.712 b	47.2 d	0.388 a	44.6 d	0.275 d	101.12 d	49.84 d
Cultivar	Williams	0.841 a	55.3 a	0.415 a	52.8 a	0.766 a	296.65 a	131.65 a
	ZAN	0.791 a	53.8 a	0.389 ab	52.4 a	0.651 b	245.12 b	114.72 b
	L <sub>17</sub>	0.779 a	50.7 b	0.384 b	50.3 a	0.782 a	291.31 a	140.38 a

Different letters in each column indicating significant difference at p≤0.05.

Table 3. Comparison of means of rate and duration of protein and oil accumulation in grains of three cultivars of soybean under salinity stress



#### 4. Discussion

Decreasing chlorophyll content index (CCI) of soybean leaves with increasing salinity (Table 1, Figure 1) could be related to increasing the activity of chlorophyll degrading enzyme: chlorophyllase (Jamil *et al.* 2007), and the destruction of the chloroplast structure and the instability of pigment protein complexes (Singh and Dubey 1995). Similar results were reported for tomato (Lapina and Popov 1970), pea (Hamada and El-Enany 1994), alfalfa (Winicov and Seemann 1990), sunflower (Ashraf 1999), sorghum (Netondo *et al.* 2004), and wheat (El-Hendawy *et al.* 2005). Differences in CCI among cultivars (Table 1, Figure 1) indicate that this trait can be also influenced by genetic constitution.

Reduction in  $f_v/f_m$  due to salinity stress (Table 1, Figure 2) is possibly related to the damage of chlorophyll under saline conditions (Ganieva *et al.* 1998). Ashraf (2004) found that ionic imbalance can also cause the reduction in  $f_v/f_m$  under high salinity conditions. Nasir Khan *et al.* (2007) reported that the decrease in chlorophyll content and PS II activity have adverse effect on growth and grain yield of treated plants.

Increasing leaf proline content under salinity stress (Table 2) might be caused by the induction or activation of proline syntheses from glutamate or decrease in its utilization in protein syntheses or enhancement in protein turnover. Thus, proline may be the major source of energy and nitrogen during immediate post stress metabolism and accumulated proline apparently supplies energy for growth and survival, thereby inducing salinity tolerance (Gad 2005). Zan had the highest proline content (Table 2) and the lowest CCI and  $f_v/f_m$  (Table 1). Gad (2005) also reported that proline content was much higher in sensitive cultivar of tomato than in salt-tolerant.

Large reductions in grain yield per plant clearly show that soybean is a salt sensitive crop, but the extent of this sensitivity varies among cultivars (Table 2). Salinity can severely limit crop production because high salinity lowers water potential and induces ionic stress and results in a secondary oxidative stress (Shanon 1998). Reductions in grain yield as a result of salt stress have also been reported for some other crop species (Ashraf 2004; Katerji *et al.* 1992; Sohrabi *et al.* 2008). These reductions are closely related with low CCI and PS II activity (Table 1) and high leaf proline content (Table 2) in soybean cultivars.

Oil and protein are the most important constituents of soybean grain. These are synthesized and deposited in the grain during pod filling (Yazdi-Samadi *et al.* 1977). Decreasing protein percentage and content with increasing salinity (Figures 3 and 4) could be attributed to the disturbance in nitrogen metabolism or to inhibition of nitrate absorption. It has been stated that the reduction in nitrogen under saline conditions might be due to the reduction of absorbed water and a decrease in root permeability (Strogonov *et al.* 1970). Medhat (2002) reported that salinity stress induce changes in the ion content of plant cell which intern induce changes in the activity of certain metabolic systems that might have serious consequences for protein.

The effect of salinity on oil percentage of soybean cultivars was opposite to that on protein percentage (Figure 4), suggesting that oil percentage increases as protein percentage decreases in response to salinity stress. Hobbs and Muendel (1983) reported similar results for soybean seeds under moisture stress. However, protein and oil contents of individual grains produced under non-saline conditions were higher than those produced under saline

conditions (Figure 4). This was associated with production of larger grains under non-saline conditions (Ghassemi-Golezani *et al.* 2009).

Salinity had little effect on rate of protein and oil accumulation in soybean grains. Therefore, decreasing oil and protein yields per plant with increasing salinity mainly resulted from the large reductions in durations of protein and oil accumulation and grain yield per plant under saline conditions (Table 3). Although, duration of protein accumulation and rate of oil accumulation for L<sub>17</sub> were slightly lower than those for other cultivars, the lowest protein and oil yields of Zan were strongly associated with the lowest grain yield per plant of this cultivar (Table 3). The greater grain, protein and oil yields per plant of L<sub>17</sub> and Williams were due to production of comparatively more grains per plant by the former and larger grains by the latter cultivars as previously reported by Ghassemi-Golezani *et al.* (2009).

## 5. Conclusion

Salinity stress can considerably reduce chlorophyll content index and PS II activity and consequently grain yield per plant in soybean cultivars. These reductions enhance with increasing salinity. In contrast, leaf proline content increases due to NaCl salinity. Oil percentage of soybean grains increases as protein percentage decreases under salinity stress. However, both protein and oil contents of individual grains under non-saline conditions are higher than those under saline conditions. Oil and protein yields per plant of soybean cultivars decrease with increasing salinity as a result of reductions in durations of protein and oil accumulation and grain yield per plant in response to salinity stress. In general, soybean is a sensitive crop to salinity stress, but the extent of this sensitivity varies among cultivars.

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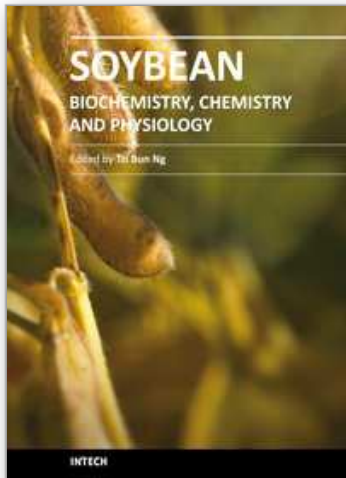
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