

Oil and Protein Accumulation in Soybean Grains under Salinity Stress

Kazem GHASSEMI-GOLEZANI¹⁾, Minoos TAIFEH-NOORI²⁾, Shahin OUSTAN¹⁾

Mohammad MOGHADDAM¹⁾, Sadjad SEYYED-RAHMANI³⁾

¹⁾ *University of Tabriz, Faculty of Agriculture, Department of Agronomy and Plant Breeding, Iran; golezani@gmail.com (corresponding author)*

²⁾ *Islamic Azad University of Maragheb, Pasdaran Ave., 9 Neyestan Str., Iran*

³⁾ *Mazandaran's Agricultural Insurance Fund (Governmental Organization), Sari, Iran*

Abstract

Two factorial experiments based on randomized complete block design (RCBD) with three replications were conducted in 2007 and 2008, to evaluate grain development (four harvests) and rate and duration of oil and protein accumulation in three soybean cultivars ('Williams', 'Zan' and 'L17') under a non-saline (control) and three saline (3, 6 and 9 ds/m NaCl) conditions. Six seeds were sown in each pot filled with 900 g perlite, using 144 pots for each experiment. After emergence, seedlings were thinned and 4 plants were kept in each pot. Rate of oil accumulation up to maturity was not significantly affected by salinity. With increasing salinity, rate and percentage of protein accumulation, duration of oil and protein accumulation and oil and protein content per grain decreased, but oil percentage increased. Oil and protein yields per plant decreased as salinity increased. These reductions were mainly attributed to the short duration of protein and oil accumulation and grain yield per plant under saline conditions. 'Williams' had the highest rate and duration of protein accumulation and rate of oil accumulation, but 'L17' had the highest grain yield per plant. Consequently, differences in protein and oil yields per plant between these two cultivars were not statistically significant. However, 'Zan' had the lowest protein and oil yields, due to the lowest grain yield per plant.

Keywords: oil, protein, salinity stress, soybean

Introduction

Soybean seed is a major source of high-quality protein and oil for human consumption (Katerji *et al.*, 2001). Growth, development and yield of soybean are the result of genetic potential interacting with environment. Minimizing environmental stress will optimize seed yield (McWilliams *et al.*, 2004).

Salinity is a worldwide problem, affecting about 95 million hectares worldwide. 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⁺ 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 CO₂ 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⁺. Plants that can not grow in the presence of high concentration of Na⁺ salts are called glycophytes (Brevedan and Egli, 2003). The 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).

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 vary among varieties and had a positive correlation with seed's final 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 approximately 30% of the total oil of the mature seed. The remaining 70% is synthesized during the next 40 to 64 days after flowering, also a period of seed desiccation (Hajdouch *et al.*, 2005). The objective of this study was to evaluate oil and protein accumulation of soybean cultivars in response to different levels of NaCl salinity.

Materials and methods

Two experiments were carried out in 2007 and 2008. Seeds of three soybean cultivars ('Williams', 'Zan' and 'L17') were obtained from Agricultural Research Institute, Moghan, Iran. In each year, a factorial experiment based on randomized complete block (RCB) design with three replications was carried out to evaluate the accumulation of oil and protein in grains (four harvests) 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 general. Pots were then placed in a greenhouse. The temperature in the greenhouse varied between 20 and 32°C in 2007 and between 15 and 28°C in 2008. 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 Hoagland solution. During grain filling, four harvests were made at 10 days intervals, beginning 75 days after sowing. Grains of each sample were oven-dried at 70°C for 48 hours and then grain dry weight was determined.

Grain yields of the 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). Subsequent-

ly, 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:

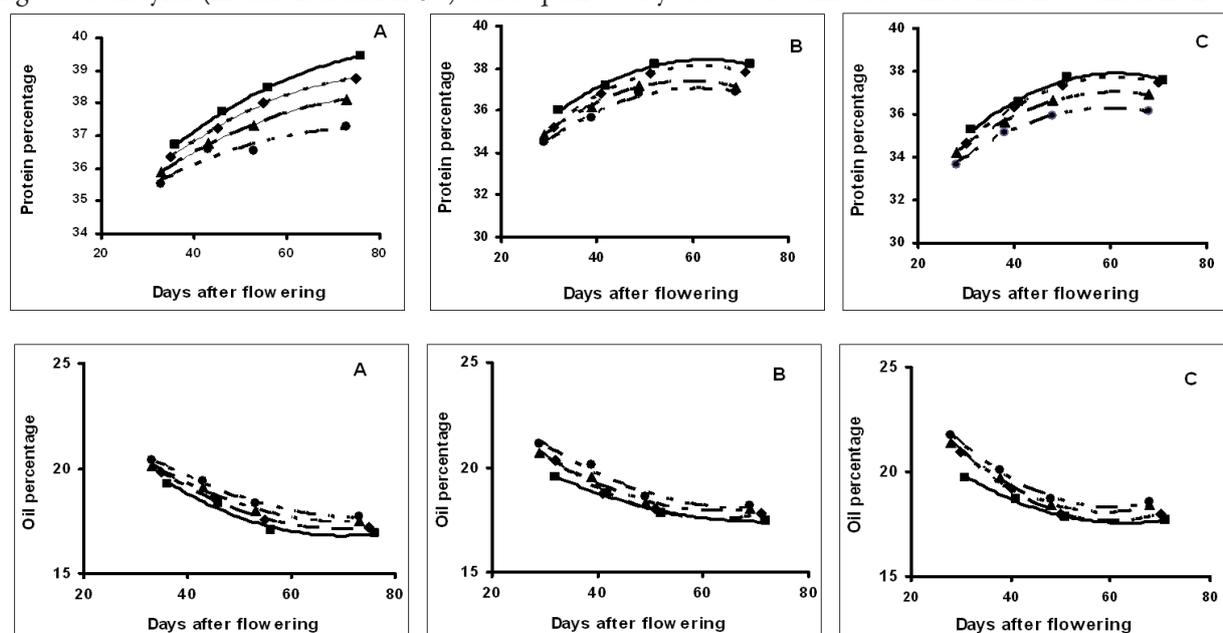
$$\text{Accumulation rate (mg d}^{-1}\text{)} = \frac{\text{Maximum weight (mg)}}{\text{Filling duration (day)}}$$

Analysis of variance of the data appropriate to the experimental design and comparison of means at $p \leq 0.05$ were carried out, using MSTATC software. SAS and Excel softwares were used for regression analysis and drawing figures, respectively.

Results

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 'L17' was attained about 10 days earlier than 'Williams' (Fig. 1). 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 'L17'. In contrast, oil percentage of all cultivars increased with increasing salinity. Oil percentage decreased as protein percentage increased with proceeding of grain filling (Fig. 1).

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 (Fig. 2). Maximum protein and oil contents per seed under salinity stress were achieved earlier than those under non-saline



A: Williams; B: Zan; C: L17; — 0 ds/m; - - 3 ds/m; ···· 6 ds/m; - · - · 9 ds/m

Fig. 1. Changes in seed protein and oil percentage of soybean cultivars under non-saline (control) and saline conditions

Tab. 1. Comparison of means of rate and duration of protein and oil accumulation of three cultivars of soybean under salinity stress

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 ⁻¹)	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
	'L17'	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 $p \leq 0.05$

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 (Fig. 2).

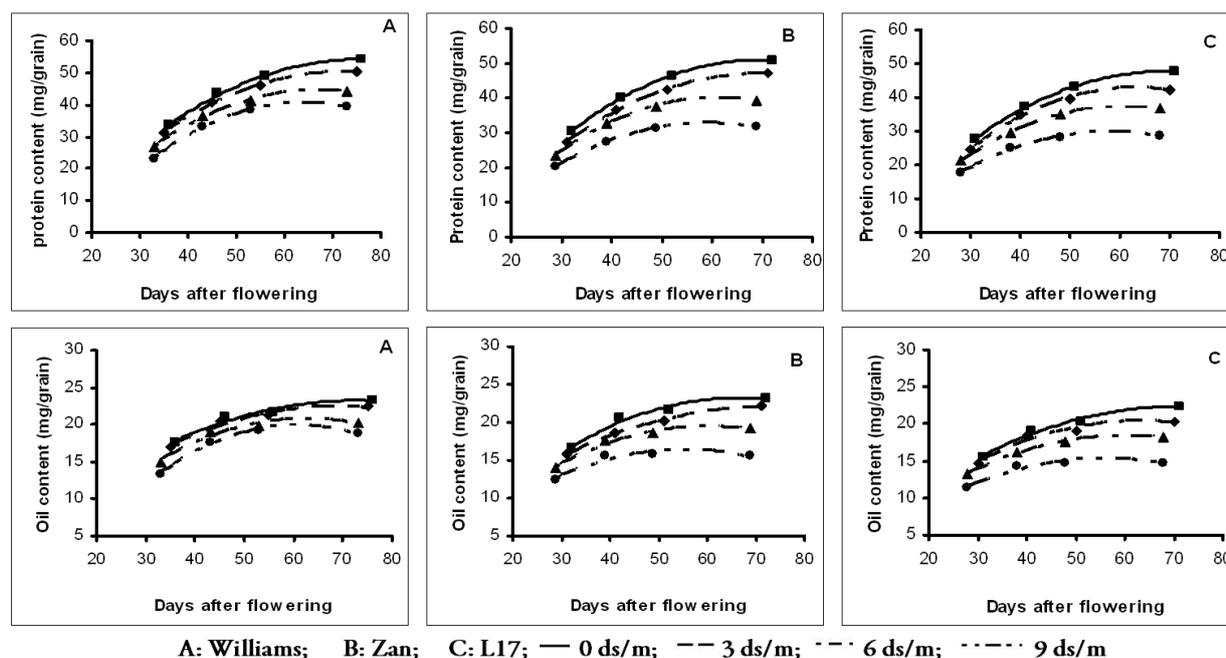
Effects of salinity on rate of protein accumulation, duration of protein and oil accumulation, grain yield per plant and protein and oil yields per plant were significant ($P \leq 0.01$), but its effect on rate of oil accumulation was not significant ($P \leq 0.05$). All these traits, except rate of protein accumulation and the duration of oil accumulation, were also significantly affected by cultivar (Tab. 1).

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 and the duration of oil accumulation for control and 3 dS/m salinity were not statistically similar (Tab. 1). 'Williams' had the highest rate and duration of protein accumulation and rate of oil accumulation, but 'L17' 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 'L17' were not significant (Tab. 2).

Discussion

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 (Figs. 1 and 2) 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's



A: Williams; B: Zan; C: L17; — 0 ds/m; - - 3 ds/m; ··· 6 ds/m; - · - · 9 ds/m

Fig. 2. Changes in seed protein and oil content of soybean cultivars under non-saline (control) and saline conditions

cells, 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 (Fig. 1), 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 (Fig. 2). These superiorities were associated with the production of larger grains under non-saline conditions (Ghassemi-Golezani *et al.*, 2009).

Salinity had little effect over the 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 (Tab. 1). Although, duration of protein accumulation and rate of oil accumulation for 'L17' 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 (Tab. 1). The greater grain, protein and oil yields per plant of 'L17' 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).

Conclusion

Oil percentage of soybean grains increases as protein percentage decreases under salinity stress. In contrast, 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.

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