Quality and quantity responses of soybean (*Glycine max* L.) seeds to water deficit

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Abstract

A split-split-plot experiment with randomized complete block design in three replications was conducted in 2004 at the Research Farm of the Faculty of Agriculture, Tabriz University, Tabriz, Iran. The effects of limited irrigations on oil and protein accumulation of seeds in two soybean varieties (Zane and Huck) in the field were investigated. Irrigation treatments were assigned to main plot, and two soybean cultivars were allocated to the subplots. Harvest stages were considered as the sub subplots. Irrigation treatments I1, I2, I3 and I4 were defined based on the cumulative evaporation of 60±3, 80±3, 100±3 and 120±3 mm, from pan (class A) respectively. The results indicated that percentages of oil and protein in the seeds were not significantly affected by water deficit at different harvest stages. However, both oil and protein out put per unit area were significantly reduced, as water deficit increased. By increasing means 100 seeds weight, percentage oil content decreased, but percentage protein content was increased. In general, it was concluded that soybean oil and protein production per unit area under full and limited irrigation conditions could be improved by increasing seed yield via selection of high-yielding varieties.

Keywords: Accumulation, Oil, Protein, Seed yield, Soybean, Water stress

Introduction

Oil and protein are the most important constituents of soybean seed, and their synthesis and deposition in the seed occurs over long period during pod-fill. Protein begins to accumulate in developing seeds 10-12 days after flowing, with oil being detected 15-20 days after flowering (Hill and Breidenbach 1974; Yazdi-Samadi *et al.* 1977). Rubel *et al.* (1972) have shown that developing soybean seeds contained 5% oil at 25 days after flowering. The oil percentage increased slightly to around 20% by 40 days after flowering and essentially remained constant during the remainder of seed development. Therefore, it is expected that under dryland conditions period of drought stress will the deposition of these products. While many studies have reported on the effect of moisture stress on seed weight (Thompson 1978; Momen *et al.* 1979; Constable and Hearn 1978; Snyder *et al.* 1982; Lawn 1982b), effects on oil and protein contents were usually not reported. Using controlled irrigation experiments, Shaw and Laing (1966) found that maximum protein percentage occurred when plants were stressed late in pod-fill and minimum protein percentage occurred when plants were stressed early in pod development. The same study revealed the inverse relationship for oil percentage, which was high when stress occurred early and low when stress was late in pod-fill. Sionit and Kramer (1977) concluded that moisture stress applied at various growth stages did not appreciably affect oil and protein percentage. However, a leaf water potential as low as
- 23 bars occurring any stage of growth of soybean decreased the total oil and protein produced per plant, because seed yield was decreased. Thomson (1978) showed that, when the interval irrigations was increased throughout the whole growing season, protein percentage remained constant while oil percentage increased. Sweeney *et al.* (2003) noted that yields from a single irrigation at R4, R5 or R6 were similar, and averaged approximately 20% more than yield with no irrigation. Irrigation at R4 increased seeds plant -1, whereas R5 and R6 irrigations increased weight per seed. In addition, they found that the irrigation had minimal effect on seed protein and variable effect on oil content.
The response of soybean and other legumes to water deficits has been analyzed by various workers who often have documented reduced yields of these crop as a result of moisture stress (Runge and Odell, 1960; Shaw and Laing, 1966; Maurer *et al.*, 1968, 1969; Miller *et al.*, 1973; Sionit and Kramer, 1977; Muirhead and White, 1981; Karamanos, 1984; Villalobos-Rodriguez *et al.*, 1984). Effects of moisture stress on the oil and protein synthesis will also vary with environment, but should be related to the effects on yield and seed weight. However, this relationship has not been examined in those fields where stress develops, perhaps erratically, over time. Doss *et al.* (1974) and Specht *et al.* (1989) indicated that the crop response to irrigation was higher after flowering than at earlier growth stages. They found that the pod-fill stage was the most critical period for adequate moisture to obtain maximum yields. Latifi (1980) also determined that increased protein and decreased oil of soybean were associated with irrigation at early pod set and seed filling in Nebraska.
This study was undertaken to obtain information on the soybean oil and protein yield, and protein and oil contents in response to several of soil moisture deficits and to prolonged soil moisture deficits at specified developmental stages.

Materials and Methods

The strategy was to determine the effect of water deficits on oil and protein percentage of soybean seeds. The study was carried out in 2004 at Research Farm of the Faculty of Agriculture, Tabriz University, Tabriz, Iran, which situated at an altitude of 1360 m above sea level, latitude 38° 5′ N and longitude 46° 17′ E. The normal May to September temperature (mean maximum, minimum and average), evaporation and precipitation of this area are 28.2, 9, 20.11 C°, and 14.4 and 68.9 mm, respectively. The soil is a sand loam, the bulk density, electrical conductivity; average organic matter, were 1.4gr.cm-3, 220µmos.cm-1 and 0.8%, respectively. The laboratory measured field capacity (-10 kPa) and plant wilting point (-946 kPa) of this soil in the active root zone (30 cm) averaged 15.1% and 10.4% by volume.
Treatment consisted of two soybean cultivars (Zan and Hack) evaluated at four irrigation regimes. Zan variety is an indeterminate cultivar from group III soybean and Hack variety is an indeterminate and early-maturing cultivar from group II soybean. Irrigation treatments I1, I2, I3 and I4 were defined based on the cumulative evaporation of 60±3, 80±3, 100±3 and 120±3 mm, from pan (class A) respectively. Harvest stages to evaluated constituents of soybean seed commence 38 days after flowering at intervals 6 days each other. Soybean cultivars were grown in 2.5×5m plots. Prior to planting, all plots received monammonium phosphate fertilizer broadcast at a rate 100 kg/ha. Soybeans were seeded on row widths of 30 cm, and at within-row spacing of 5 cm, which resulted in plant populations of 600,000 plant/ha. Seeds were prepared with Captan. Each row received 5gr of granular inoculums of *Bradyrhizobium Japonicum* Jordan bacteria dribbled into the open furrow. Furrow were then covered and tamped down. Seeding date were four and 5 June 2004.
The experimental design was a split-split plot in randomized complete block arrangement with four replications. Irrigation treatments were assigned to main plot, and two cultivars were allocated to the subplots. Harvest stages were considered as the sub subplots.
The amount of irrigation was calculated by:

$V=(\theta FC-\theta SM).pb.A.d$ (1)

Where

$\theta_{SM} = \frac{w1 - w2}{w2}$ (2)

V is the amount water consumed (m3), θ_{SM} is the soil moisture content in a given time (day), *W1* and *W2* are the wet and dry soil sample weight, θ_{FC} is the field capacity in the active root zone, *pb* is the bulk density, *A* is the plot area (m2) and *d* active root zone (m). Irrigation amount was calculated on the basis of 30cm rooting depth. Consequently, after 15 July, irrigation amount was computed based on the full rooting depth of 120cm. irrigation was applied by a water counter with measured of water. All plots were pre-irrigated (on 10 June 2004) to ensure that the soil profile was at field capacity at planting time. Evaporation was monitored daily. Accumulative pan (class A) evaporation was installed at the center of field for this purpose. Soil water contents (θ_{SM}) were measured on soil cores collected with a hand sampler at soil depths of 15, 30, 45, 60 and 75 cm near the center of randomly selected plots properly after cumulative pan evaporation reached 60, 80,100 and 120mm. At harvest, some plants in rows 2, 3, 4 and 5 of each plot were harvested and threshed. Seed yield and seed weight (100 seeds) were determined. Seed protein and oil contents were determined using a Seed Analyzer ZX-50 using laboratory regression for this Near Infrared Reflectance Spectroscopy instrument.
In order to show of the regression relationship between observed and predicted values using a followed logistic regression equation (France and Thornley, 1984):

$$Y = \frac{W'0 \times Wf}{W'0 + (Wf - W'0)EXP(-\mu X)}$$

An analysis of variance for all data from field experimental was conducted by MSTAT-C software (MSTAT-C, 1990). Treatments means were considered significantly different at P<0.05. Mean separation was by Duncan Multiple Ranges Test.

Results and Discussion

Summery of the statistical analyses of soybean seed weight, percent oil and protein contents are given in Table 1. Irrigation treatments had not significant effect on oil and protein percentages and the harvest stages in both traits were highly significant (P<0.01). Highest and lowest oil percentage were I2 (80 mm evaporation) and I4 (120mm evaporation), respectively. There were no interaction effects on oil and protein percentages for I×V, I×H, V×H and I×V×H.

Table 1. Analyses of variance for soybean seed weight (100 seed), oil and protein percentages.

Source	Rep.	[a] I	E_a	[b] V	I×V	E_b	[c] H	I×H	V×H	I×V×H	E_c	CV%
d.f.	2	3	6	1	3	8	7	21	7	21	112	
Seed weight	12.58	15.85*	2.29	0.29	2.99	2.7	401.7**	3.1**	14.2**	3.25**	1.49	12.67
Oil%	0.66	2.253	1.6	0.28	0.63	0.99	369.27**	0.49	1.41	1.03	1.19	4.58
Protein%	0.27	13.79	12.89	3.41	2.06	6.73	234.21**	6.03	12.52	7.51	7.81	10.96

*, ** significant at 0.05 and 0.01 levels, respectively.

[a] I= Irrigation treatments: irrigation when cumulative pan (class A) evaporation reached 60, 80, 100 and 120 mm, respectively.

[b] V= variety: Zan and Hack

[c] H= Harvest stages: commence at 38 days after flowing at intervals 6 days each other.

As shown in Fig.1 early stages of pod-fill oil content is high, then amount of oil content was remained constant during the remainder of seed development essentially after H5 (62 after flowering). High oil percentage in first harvest (38 days after flowering) show that oil content accumulation accomplished before this stage. This result agrees with observation of Yazdi-Samadi and Saadati (1978) and Rubel *et al.* (1972).

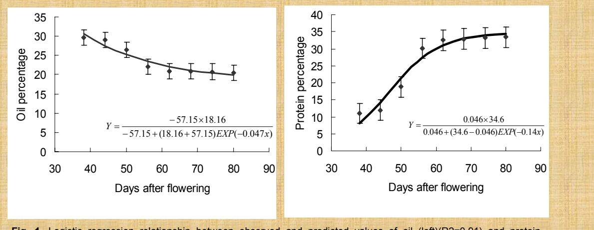

Fig. 1. Logistic regression relationship between observed and predicted values of oil (left)(R2=0.91) and protein (right)(R2=0.95) percentage.

So that expected, protein content revealed the inverse relationship with oil content and by increased the percentage protein content decreased percentage oil content. Given that protein content of soybean seed is usually nearly double the oil content, it is apparent that oil is relatively more sensitive to moisture stress (Rose, 1988). Highest protein content was in H5 (62 days after flowering) and after this stage in spite increased protein content were no significant different between harvest stages (Fig. 1). Thompson (1978) found little influence of irrigation on protein and oil in Australia. On the other hand, Sionit and Kramer (1977) determined that slight protein and oil differences were associated with moisture stress at specific crop stages.

The effect of water stress on grain, oil and protein yield were significant at 0.05 probability level (Table 2). The soybean grain yield at I1, I2, I3 and I4 were 82.6, 47.4, 45.7 and 32.9 gr/m2, respectively. The grain yield at I-1 treatment had significant difference with other irrigation regimes, whereas, the difference between I2, I3 and I4 were not significant. To this cause oil and protein yield per unit, area decreased (Table 3). This result is in general agreement with results obtained by Korte *et al.* (1983) and Kadhem *et al.* (1985). During later stages of pod fill both oil and protein are still being deposited in the seed (Yazdi-Samadi *et al.*, 1977; Sale and Campell, 1980). However, these contributions are relatively more important for oil. This agree with the findings of Shaw and Laing (1966), who concluded that low oil percentages occurred with moisture stress later in the pod filling period.
Compared of the grain yield means between cultivars shown that the grain yield reduction in Hack variety at I2 and I3 as compared with the Zan variety were less. So, result that Hack variety resistance to water deficit more than Zan variety (Table 3).

Table 2. Analyses of variance for soybean grain, oil and protein yield.

Source	d.f	Grain yield	Oil yield	Protein yield
Rep.	2	402.6	22.082	31.66
[a]I	3	2724.568*	114.802*	305.97*
Ea	6	426.608	18.809	56.162
[b]V	1	1095.745**	46.171**	89.226**
I×V	3	106.614	4.88	9.95
Eb	8	41.25	1.891	5.79
%CV		12.31	12.77	13.87

*, ** significant at 0.05 and 0.01 levels, respectively

[a] I= Irrigation treatments: irrigation when cumulative pan (class A) evaporation reached 60, 80, 100 and 120 mm, respectively.

[b] V= variety: Zan and Hack

Table 3. Means of grain, oil and protein yields for irrigation and variety treatments

	Grain yield (gr/m²)				Oil yield (gr/m²)				Protein yield (gr/m²)			
	I_1	I_2	I_3	I_4	I_1	I_2	I_3	I_4	I_1	I_2	I_3	I_4
Means	82.6a[a]	47.37b	45.68b	32.95b	16.9a	9.9b	9.4b	6.72b	27.7a	15.32b	15.25b	11.19b
		V_1	V_2			V_1	V_2			V_1	V_2	
Means		45.5b	58.8a			9.38b	12.2a			15.4b	19.3a	

[a]Means followed by the same letter are not significantly different at the *P*=0.05 level (Duncan's test).

The effect of irrigation treatments on seed weight (100 seed) at the 0.05 probability level was significant (Table 1). Highest and lowest seed weight amounts obtained at I2 and I4 irrigation treatments, respectively (Fig. 2). Plants in the I1 were excessive vegetative growth and seed number that cause of decreased seed weight in this treatment. Compared to the seed weight , oil and protein content indicated that, seed weight was inversely related to oil content by increasing means 100 seed weight, percentage oil content decreased, but percentage protein content was increased.
As shown in Fig. 2 early stages of pod filling seed weight is low, and later stages of pod fill about 70 days after flowering seed weight increased and remained constant during the remainder of seed development. Relationship among seed weight, oil and protein content, indicating that changes oil and protein content in response to stress were related to each other and to changes in seed weight. These results are consistent with reported by Rose (1988).

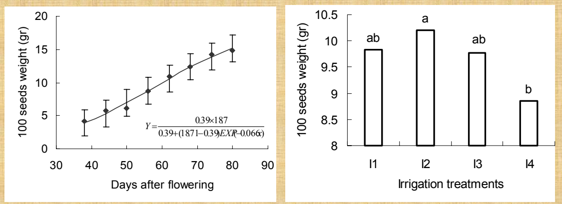

Fig. 2. Logistic regression relationship between observed and predicted values of 100 seed weights (, R2=0.99) (left). 100 seed weights change in different irrigation treatments (right).

Harvest stages and irrigation treatments interacted significantly on seed weight (P<0.01).Seed weight was highest when the crop was irrigated with I1 (60mm evaporation from pan) and harvested at H8 (80 days after flowering).
As a result, increasing of seed weight at irrigation treatments until H4 was similar. However, after this harvest stage considerable differences found between irrigation treatments and harvest stages especially at I4. There were interaction effects on seed weight for V×H (Table 1).high and low amounts of seed weight were V1H8 and V1H1, respectively. after flowering was similar. Whereas, the 100 seeds weight at I2, I3 and I4 treatments (80, 100, and 120 mm evaporation from pan, respectively) at early harvests (i.e. 38, 44 and 50 days after flowering) for Zan cultivar were low compared to Hack cultivar. At least harvests (i.e. 56, 62, 68, 74, and 80 days after flowering) 100 seeds weight for Zan cultivars were often high compared to Hack cultivar. At last, 100 seeds weight at I2, I3, and I4 for Zan cultivar were high amounts (Table4).

Table 4. 100 seed weight of soybean cultivars as affected by irrigation treatments duration of difference harvests.

	[a] I_1		I_2		I_3		I_4	
Treatments	[b] V_1	V_2	V_1	V_2	V_1	V_2	V_1	V_2
[c] H_1	5.01	4.45	2.68	5.67	1.917	5.93	2.22	5.46
H_2	5.15	6.06	3.85	7.35	6.09	5.47	4.46	7.91
H_3	6.69	6.22	5.94	7.33	5.7	5.74	4.45	6.66
H_4	8.66	8.47	8.85	8.97	8.73	8.23	8	9.23
H_5	10.18	9.79	12.23	12.01	12.36	10.56	9.7	10.48
H_6	13.07	12.49	13.26	13.27	13.86	12.48	11.18	10.68
H_7	14.67	13.93	15.99	14.79	16.47	13.25	13.51	11.37
H_8	16.39	16.01	17.41	13.6	14.53	14.93	14.69	11.6

LSD 0.05 and 0.01 equals 1.973 and 2.542, respectively

[a] I= Irrigation treatments: irrigation when cumulative pan (class A) evaporation reached 60, 80, 100 and 120 mm, respectively.

[b] V= varieties: Zan and Hack

[c] H= Harvest stages: commence at 38 days after flowing at intervals 6 days each other.

Conclusions

Moisture stresses during pod fill will not affect both the oil and protein content of soybean seeds. The resulting seed composition is a balance of the reduction is seed weight and the reduction in quantities of oil and protein content per unit area. In this study, the amount and distribution of water were regular and distinctness, resulting in differing effects on seed weight and differing relative effects on oil and protein components of the seed. Irrigation with short time interval and low water volume is better than irrigation with long time interval and much volume in soybean production. In general, it was concluded that soybean oil and protein production per unit area under full and limited irrigation conditions could be improve by increasing seed yield via selection of high-yielding varieties.

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