УДК 633.34(55)

# OIL AND PROTEIN RESPONSEOF SOYBEAN (*GLYCINE MAX* L.) SEEDS TO WATER DEFICIT

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Поступила в редакцию 05.08.09 г.

**Влияние дефицита влаги на содержание масла и белка в семенах сои (*Glycine max* L.). – Бехтари Б., Хассеми Голезани К., Даббах Мохаммади Насаб А., Зехтабе Салмаси С., Тоорчи М. –** В 2004 г. в опытном хозяйстве сельскохозяйственного факультета Тебризского университета (Иран) на опытных и контрольных участках были проведены эксперименты по рандомизированному плану в трех повторностях. В полевых условиях исследовано влияние ограничений полива на накопление масла и белка в семенах двух сортов сои (Zane и Hack). Для экспериментальных и контрольных участков двух культурных сортов сои были установлены режимы полива $I_1$, $I_2$, $I_3$ и $I_4$, которые определяли на основании общего испарения из лотка 60±3, 80±3, 100±3 и 120±3 мм соответственно (класс А). Стадии созревания семян сравнивались как отдельные выборки. Результаты показали, что доли масла и белка в семенах разных урожаев значимо не зависели от режима полива. Однако выход как масла, так и белка на единицу площади значимо сокращался при увеличении дефицита воды. Кроме того, при увеличении среднего веса 100 семян содержание масла снижалось, а белка – увеличивалась. Предполагается, что выход масла и белка сои на единицу площади в условиях как достаточного, так и ограниченного полива можно улучшить повышением урожайности путём селекции семян высокоурожайных сортов.

*Ключевые слова*: *Glycine max*, накопление, масло, белок, урожай семян, дефицит воды.

**Oil and protein response of soybean (*Glycine max* L.) seeds to water deficit. – Behtari B., Ghassemi Golezani K., Dabbagh Mohammadi Nasab A., Zehtabe Salmasi S., and Toorchi M. –** A split-split-plot experiment with a randomized complete block design in three replications was conducted at the Research Farm of the Faculty of Agriculture, Tabriz University, Tabriz, Iran, in 2004. The limited-irrigation effect on the oil and protein accumulation in seeds for two soybean varieties (Zane and Hack) was investigated in field conditions. Several irrigation regimes $I_1$, $I_2$, $I_3$, and $I_4$ were assigned to the main plot, which were defined from the cumulative evaporation of 60±3, 80±3, 100±3, and 120±3 mm, from the pan (class A), respectively. The two soybean cultivars and few harvest stages were considered as subplots and subsubplots. The results indicated that the percentages of oil and protein in the seeds of various harvests were not significantly affected by water deficit. However, both oil and protein output per unit area was significantly reduced, as water deficit increased. Besides, when the mean weight of 100 seeds increases, the oil content decreases but the protein content rises. It is assumed that the 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.

*Key words*: *Glycine max*, accumulation, oil, protein, seed yield, water stress.

## INTRODUCTION

Oil and protein are the most important constituents of soybean seeds, and their synthesis and deposition in these seeds occur over a long period during pod-fill. Protein begins to accumulate in developing seeds in 10 – 12 days after flowering, with oil detected in 15 – 20 days after flowering (Hill, Breidenbach, 1974; Yazdi-Samadi et al., 1977). Rubel et al. (1972) have shown that developing soybean seeds contain 5% oil on the 25th day after flowering. The oil percentage increased slightly to ca. 20% in 40 days after flowering and essentially remained constant during the further seed development. Therefore, the period of drought stress can be expected to affect the deposition of these products under dryland conditions. While many studies have reported on the effect of moisture stress on seed weight (Thompson, 1978; Momen et al., 1979; Constable, Hearn, 1978; Snyder et al., 1982; Lawn, 1982), no effects on the oil and protein contents were usually reported. Using controlled irrigation experiments, Shaw and Laing (1966) found that the maximum protein percentage occurred when plants were stressed late in pod-fill and the minimum protein percentage occurred when they were stressed early in pod development. The same study revealed an inverse relationship for oil percentage which was high when stress occurred early and low when it 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, the leaf water potential as low as -23 bars occurring at any stage of soybean growth decreased the total oil and protein produced per plant, because the seed yield was reduced. Thomson (1978) showed that the protein percentage remained constant while the oil percentage increased when interval irrigations were increased throughout the whole growing season. Sweeney et al. (2003) noted that the yields from a single irrigation at $R_4$, $R_5$ or $R_6$ were similar and approx. 20% (on the average) more than the yield with no irrigation. The irrigation at $R_4$ increased the number of seeds per plant, whereas $R_5$ and $R_6$ irrigations increased the weight per seed. In addition, they found that the irrigation had the minimal effect on seed protein and a variable effect on oil content.

The response of soybean and other legumes to water deficit has been analyzed by various workers who often have documented reduced yields of these crops as a result of moisture stress (Runge, Odell, 1960; Shaw, Laing, 1966; Maurer et al., 1968, 1969; Miller et al., 1973; Sionit and Kramer, 1977; Muiehead, White, 1981; Karamanos, 1984; Villalobos-Rodriguez et al., 1984). The effects of moisture stress on oil and protein synthesis will also vary with the 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 and Thuriow (1974) and Specht et al. (1989) indicated that the crop response to irrigation was higher after flowering than at earlier growth stages. They found the pod-fill stage to be the most critical period for adequate moisture to obtain maximum yields. Latifi (1989) 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 soil moisture deficit (including prolonged one) at the specified developmental stages.

## MATERIALS AND METHODS

Our aim was to determine the effect of water deficit on the oil and protein percentage in soybean seeds. The study was carried out at the Research Farm of the Faculty of Agriculture, Tabriz University, Tabriz, Iran, which situated at an altitude of 1,360 m above sea level, latitude 38° 5' N and longitude 46° 17' E, in 2004. The normal May to September temperature (mean maximum, minimum, and average), evaporation and precipitation in this area are (28.2, 9.0, 20.1) °C, 14.4, and 68.9 mm, respectively. The soil is sandy loam, its bulk density, electrical conductivity; and average organic matter are 1.4 $g{\cdot}cm^{-3}$, 220 $\mu mos{\cdot}cm^{-1}$, 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, respectively.

Our treatment consisted of two soybean cultivars (Zan and Hack) examined under four irrigation regimes. The Zan variety is an indeterminate cultivar from group III soybean, and the Hack variety is an indeterminate and early-maturing cultivar from group II soybean. Irrigation regimes $I_1$, $I_2$, $I_3$, and $I_4$ were defined based on the cumulative pan evaporation of 60±3, 80±3, 100±3, and 120±3 mm (class A), respectively. Harvest stages to evaluate the constituents of soybean seeds commence in 38 days after flowering at 6-day intervals from each other. The soybean cultivars were grown on 2.5×5.0 m plots. Prior to planting, all plots had received monoammonium phosphate fertilizer broadcast at a rate of 100 kg/ha. Soybeans were seeded on rows of a 30 cm width with a row spacing of 5 cm, which resulted in plant populations of 600,000 per ha. The seeds were prepared with Captan. Each row received 5 g of granular inoculum of *Bradyrhizobium japonicum* Jordan bacteria dribbled into the furrow. The seeding dates were 4 and 5 June, 2004.

The experimental design was a split-split plot in randomized complete block arrangement with three replications. Irrigation regimes were assigned to main plot, and the two cultivars were allocated to its subplots. Harvest stages were considered as subsubplots.

The amount of irrigation was calculated by

$$V = (\Theta_{FC} - \Theta_{SM}).\rho b.A.d, \tag{1}$$

where

$$\Theta_{SM} = \frac{W_1 - W_2}{W_2} \times 100, \tag{2}$$

$V$ is the water amount consumed ($m^3$), $\Theta_{SM}$ the soil moisture content at a given time (days), $W_1$ and $W_2$ the wet and dry soil sample weights, $\Theta_{FC}$ the field capacity in the active root zone, $\rho b$ the bulk density, $A$ the plot area ($m^2$), and $d$ the active root zone (m). All plots were pre-irrigated (on 10 June, 2004) to ensure the soil profile to be at the field capacity at the planting time. At first, the irrigation amount was calculated on the basis of the 15 cm rooting depth. Consequently, after 15 July, the irrigation amount was computed based on our measurement of the rooting depth. A water counter with applied irrigation measured water. Evaporation was monitored daily. An accumulative pan (class A) for evaporation was installed at the center of the field for this purpose. Soil water content ($\Theta_{SM}$) was 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 the cumulative pan evaporation reached 60, 80, 100, and 120 mm duration of the growth season. At harvest, some plants in rows 2, 3, 4, and 5 of each plot were harvested and threshed. The seed yield and seed weight (100 seeds) were determined. The seed protein and oil contents were determined on a Seed Analyzer ZX-50 using laboratory regression by means of a Near Infrared Reflectance Spectroscopy instrument.

To show the regression relationship between the observed and predicted values, the following logistic regression equation (France, Thornley, 1984) was used:

$$Y = \frac{W0 \times Wf}{W0 + (Wf - W0)EXP(-aX)}.$$

Analysis of variance for all the data from our field experiment was conducted by MSTAT-C software (MSTAT-C, 1990). The means were considered significantly different when $P < 0.05$. Mean separation was by Duncan Multiple Ranges and LSD Test.

## RESULTS AND DISCUSSION

A summary of the statistical analyses of soybean seed weight, oil and protein contents is given in Table 1. Irrigation regimes had no significant effect on oil and protein percentages, and the harvest stages in both traits were highly significant ($P < 0.01$). The highest and lowest oil percentages were observed at $I_2$ (80 mm evaporation) and $I_4$ (120 mm evaporation), respectively. There were no interaction effects on oil and protein percentages for $I \times V$, $I \times H$, $V \times H$, and $I \times V \times H$.

**Table 1**

Analysis of variance for soybean seed weight (100 seeds), oil and protein percentages

| Source | Rep. | [a] $I$ | $E_a$ | [b] $V$ | $I \times V$ | $E_b$ | [c] $H$ | $I \times H$ | $V \times H$ | $I \times V \times H$ | $E_c$ | $CV$,% |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 probability levels, respectively. [a] $I$ are irrigation treatments: irrigation when the cumulative pan (class A) evaporation reached 60, 80, 100, and 120 mm, respectively; [b] $V$ are varieties (Zan and Hack); [c] H are the harvest stages: commence in 38 days after flowing at 6-day intervals from each other.

As shown in Fig. 1, the oil content is high at early stages of pod-fill, and then the oil content remained constant during the further seed development, especially after $H_5$ (62 days after flowering). The high oil percentage in the first harvest (38 days after flowering) shows that oil accumulation has been done before this stage. This result agrees with the observation of Yazdi-Samadi and Saadati (1978), and Rubel et al. (1972).

As expected, the protein content has revealed an inverse relationship with the oil content (an increased protein content corresponds to a decreased oil one). Given that the protein content in soybean seeds is usually nearly double the oil content, it is apparent that oil is relatively more sensitive to moisture stress (Rose, 1988). The highest protein content was in $H_5$ (62 days after flowering) and after this stage there were no significant differences between harvest stages in spite of the increased protein content (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 content differences were associated with moisture stress at specific crop stages.

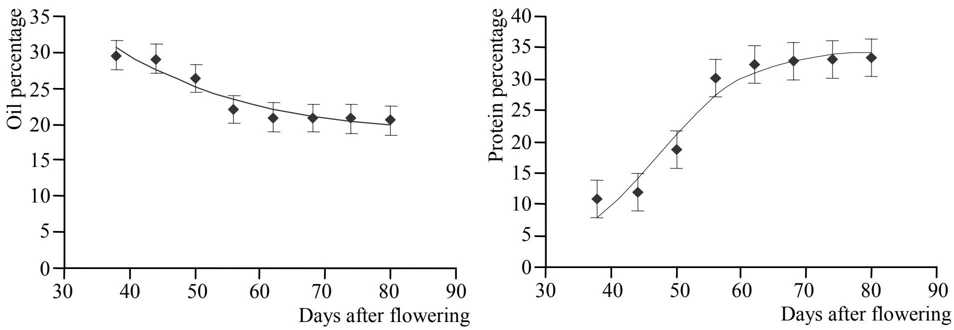


**Fig. 1.** Logistic regression relationship between observed and predicted values of oil (left) ($Y = \dfrac{-57.15\times 18.16}{-57.15+(18.16+57.15)EXP(-0.047x)}$, $R^2 = 0.91$) and protein (right) ($Y = \dfrac{0.046\times 34.6}{0.046+(34.6-0.046)EXP(-0.14x)}$, $R^2 = 0.95$) percentage

The effect of water stress on the grain, oil and protein yield was significant at a 0.05 probability level (Table 2). The soybean grain yield at $I_1$, $I_2$, $I_3$, and $I_4$ was 82.6, 47.4, 45.7, and 32.9 g/m$^2$, respectively. The grain yield at $I_1$ had a significant difference with other irrigation regimes, whereas the differences between $I_2$, $I_3$, and $I_4$ were not significant. Due to this cause, the oil and protein yield per unit area decreased (Table 3). This result is in general agreement with those 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 seeds (Yazdi-Samadi et al., 1977; Sale, Campbell, 1980). However, these contributions are relatively more important for oil. This agrees with the findings of Shaw and Laing (1966), who have concluded that low oil percentages occur with moisture stress later in the pod filling period.

Our comparison of the grain yield means between the cultivars has shown that the grain yield reduction in the Hack variety at $I_2$ and $I_3$ is less in comparison with the Zan one. This means that the Hack variety resistance to water deficit is stronger than that of the Zan variety (Table 3).

**Table 2**

Analysis 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$^{*}$ |
| *E*a | 6 | 426.608 | 18.809 | 56.162 |
| $^{b}V$ | 1 | 1095.745$^{**}$ | 46.171$^{**}$ | 89.226$^{**}$ |
| $I\times V$ | 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 probability levels, respectively; [a] *I* are the irrigation treatments: irrigation when the cumulative pan (class A) with evaporation reached 60, 80, 100, and 120 mm, respectively; [b] *V* the varieties (Zan and Hack).

**Table 3**

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

| | Grain yield, g/m$^2$ | | | | Oil yield, g/m$^2$ | | | | Protein yield, g/m$^2$ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $I_1$ | $I_2$ | $I_3$ | $I_4$ | $I_1$ | $I_2$ | $I_3$ | $I_4$ | $I_1$ | $I_2$ | $I_3$ | $I_4$ |
| Means | 82.6a[a] | 47.37b | 45.68b | 32.95b | 16.98a | 9.9b | 9.4b | 6.72b | 27.67a | 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 regimes on seed weight (100 seeds) at the 0.05 probability level was significant (Table 1). The highest and lowest seed weights were obtained at $I_2$ and $I_4$, respectively (Fig. 2). The plants at $I_1$ had excessive vegetative growth and seed numbers, which is caused by the decreased seed weight at this regime. Our comparison between the seed weight, oil and protein contents has indicated that seed weight is inversely related to oil content (an increased mean 100-seed weight corresponds to a decreased oil content percentage), but the protein content is higher.

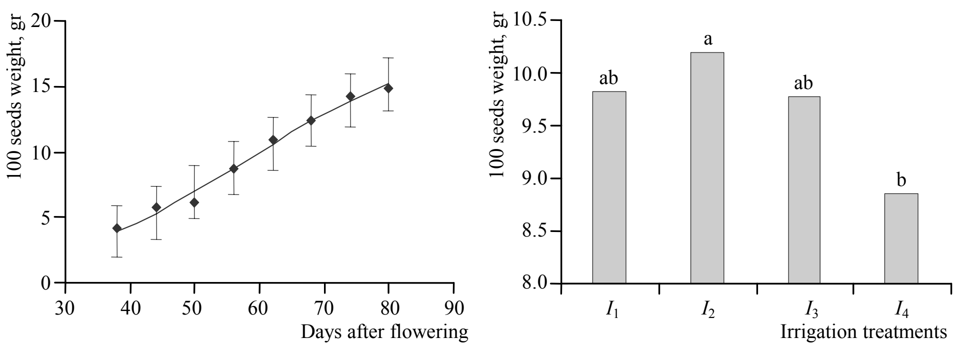


**Fig. 2.** Logistic regression relationship between observed and predicted values of 100 seed weights ($Y = \dfrac{0.39 \times 18.7}{0.39 + (18.71 - 0.39) EXP(-0.066x)}$, $R^2 = 0.99$) (left). 100 seed weights change in different irrigation treatments (right)

As shown in Fig. 2, the seed weight was low at the early stages of pod fill, and at the later stages of pod fill (about 70 days after flowering), the seed weight increased and remained constant during the further seed development. A relationship among seed weight, oil and protein contents exists, indicating that the oil and protein contents change in response to stress and are related to each other and to changes in seed weight. These results are consistent with those reported by Rose (1988).

Harvest stages and irrigation regimes interacted significantly on seed weight ($P < 0.01$). The seed weight was highest when the crop was irrigated at $I_1$ (60 mm pan evaporation) and harvested at $H_8$ (80 days after flowering).

As a result, the seed weight increase at the irrigation regimes until $H_4$ was similar. However, after this harvest stage, considerable differences were found between irrigation regimes and harvest stages, especially at $I_4$. There were interaction effects on seed weight for $V \times H$ (Table 1). The high and low seed weights were at $V_1H_8$ and $V_1H_1$, respectively.

The 3-way interaction between irrigation regimes, varieties, and harvest stages was significant for the 100-seed weight (Table 1). The 100-seed weight of both cultivars (Zan and Hack) at $I_1$ from 38 till 80 days after flowering was similar. Whereas the 100-seed weight at $I_2$, $I_3$ and $I_4$ (80, 100, and 120 mm of pan evaporation, respectively) at early harvest stages (i.e. 38, 44, and 50 days after flowering) for the Zan cultivar were low in comparison with the Hack one. At late harvest stages (i.e. 56, 62, 68, 74, and 80 days after flowering), the 100-seed weight for the Zan cultivar was often high in comparison with the Hack one. At last, the 100-seed weight at $I_2$, $I_3$, and $I_4$ for the Zan cultivar was high (Table 4).

**Table 4**

100-seed weight of soybean cultivars as affected by irrigation duration of different harvests

| Treatments | [a] $I_1$ | | $I_2$ | | $I_3$ | | $I_4$ | |
|---|---|---|---|---|---|---|---|---|
| | [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 |

[a] *I* are the irrigation treatments: irrigation when the cumulative pan (class A) evaporation reached 60, 80, 100, and 120 mm, respectively; [b] *V* the varieties: Zan and Hack; [c] *H* the harvest stages: commence at 38 days after flowering at 6-day intervals from each other; LSD at 0.05 and 0.01 equals to 1.973 and 2.542, respectively.

## CONCLUSIONS

Moisture stress during pod fill affects neither oil nor protein content in soybean seeds. The resulting seed composition is a balance of the reduction in seed weight and the reduction in the oil and protein contents per unit area. In this study, the amount and distribution of water were regular and distinctness, resulting in different effects on seed weight and different relative effects on the oil and protein components of seeds. Irrigation with short intervals and a low water volume is better than that with long intervals and much volume for soybean production. 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.

The authors gratefully acknowledge the assistance of Mrs. M. Sheikh Bagheri. They are also grateful to all those friends who participated.

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