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**THE RELATIONSHIP BETWEEN LAI AT EFFECTIVE SEED FILLING PERIOD  
STAGE WITH OIL, PROTEIN, POD AND GRAIN DRY WEIGHT OF SOYBEAN  
UNDER DROUGHT STRESS AND MICRONUTRIENTS FERTILIZATION**

**KOBRAEE S<sup>1\*</sup> SHAMSI K<sup>1</sup> AND VAGHAR MS<sup>2</sup>**

**1:** Department of Agronomy and Plant Breeding, College of Agriculture, Kermanshah  
Branch, Islamic Azad University, Kermanshah, Iran

**2:** Department of Agronomy and Plant Breeding, College of Agriculture, Ghasre Shirin,  
Branch, Islamic Azad University, Ghasre Shirin, Iran

\*Corresponding Author: Tel.: 00988317243181, E Mail: [Kobraee@yahoo.com](mailto:Kobraee@yahoo.com)

**ABSTRACT**

In order to investigation of the relationships between leaf area index and pod dry weight with oil and protein contents in soybean seed, a field experiment was conducted in Kermanshah, Iran during 2010 growing season. The experimental design was randomized complete blocks in split plots arrangement with three replications. Treatments included four different regimes of irrigation in main plot and eight foliar treatments were allocated as subplots. At effective seed filling period stage, five plants were selected from each plot, randomly, and leaf area index was measured. Also, at harvesting time, pod and grains dry weight, oil and protein content were determined. Based on results obtained, the highest pod dry weight (PDW), grain dry weight (GDW), and oil content were belonged to I1 treatment. In addition, withholding irrigation at flowering stage (I2) had the greatest impact on reducing oil content. In different irrigation regimes, oil content and GDW were positively, and protein concentration was negatively related to LAI. In different irrigation regimes, unlike protein content, oil concentration showed a significant increase as GDW from low up to high. Results of this study showed that relationship between oil and protein contents was slightly affected by irrigation and micronutrient fertilization. Furthermore, zinc

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application had the most impact on oil and protein percent and increased these traits up to 19.51% and 38.78%, respectively.

**Keywords:** LAI, Micronutrients, Seed Filling Period, Soybean, Water Stress

## INTRODUCTION

Soybean is a legume that grows in tropical, sub tropical and temperate climates. In addition, Soybean is a major source of vegetable protein and oil for human and animal consumption and also for industrial use (Biodiesel). Mineral nutrition in plants is essential for plant growth, and plays an important role in seed quality. Therefore, Deficiencies in minerals due to drought stress at any plant growth stage result in poor seed quality [1]. Soybean plants need to be irrigated frequently in order to avoid yield and quality losses. Some of morphological traits such as LAI increase with regular irrigation particularly at critical growth stages of plant. Furthermore, water stress is recognized as a reduction factor in soybean oil and protein contents [2, 3, and 4]. Protein and oil content, chemical composition of oil and protein content, and grain appearance are three important factors determining the quality of soybeans [5, 6, and 7]. In the other side, researchers suspect that, there exists an inverse relationship between seed protein and oil concentration, hence, making it difficult to improve both traits

simultaneously [8, 9]. However, [10] reported there is significantly and positively correlated between quality traits such as oil content with seed dry weight as a quantity trait. The objective of present study, therefore, is to estimate the relationships between leaf area index at effective seed filling period with selected quality parameters related to seed such as oil and protein content and quantity trait grain dry weight, when that affected by combination of micronutrients foliar application and drought stress, Simultaneously.

## MATERIALS AND METHODS

The experiment was conducted in an experimental field area of the Islamic Azad University, Kermanshah Branch in Iran during 2010 growing season. The site of study was situated at 34<sup>0</sup>23' N, 47<sup>0</sup> 8' E and 1351 m above sea level. Before the beginning of the experiment, soil samples were taken to determine the physical and chemical properties. A composite soil sample was collected at a depth of 0 - 30 cm. It was air dried, crushed, and tested for physical and chemical properties. The

texture of the soil based on silty clay with (silt 49.1%, clay 42.4%, and sand 8.5%), pH 7.3, organic matter 2.6%, total nitrogen 0.11%, phosphorus 8.2 ppm, potassium 531 ppm, zinc, iron and manganese 0.81, 2.76, and 4.49 mg/kg, respectively. The experimental design was randomized complete blocks in split plots arrangement with three replications. Main plots included four different regimes of irrigation: (I1) Irrigation at all of growth stages, (I2) Irrigation Withholding at flowering stage, (I3) Irrigation Withholding at pod set stage and (I4) Irrigation Withholding at seed filling period. Eight foliar treatments: (1) spray with distilled water, (2) zinc spray, (3) manganese spray, (4) iron spray, (5) zinc and manganese spray, (6) zinc and iron spray, (7) manganese and iron spray, and (8) zinc, manganese and iron spray, were allocated as subplots. Before sowing, soybean seeds (cv. Williams) were inoculated with *Bradyrhizobium japonicum* and sown at a high-planting rate the field. When the unifoliate leaves were expanded, the plots were hand-thinned to obtain a uniform plant population of 33 plants per m<sup>2</sup>. The quantity of irrigation water in each plot was calculated according to [11], controlled by counter and exercise irrigation treatments at different growth

stages according to [12]. At the V4 growth stage, the plots were sprayed twice (with one week interval) with 0.5% (w/v) or distilled water until the leaves were wet. At effective seed filling period stage (one week after pod beginning), five plants were selected from each plot, randomly, and LAI by leaf area meter L13100-LI-cor model, was measured. At the end of growing season, 1m<sup>2</sup> middle rows of each plot were completely harvested by taking margins into account. After deducting 13% moisture, grains dry weight was calculated. Also, oil and protein percent in soybean seed were measured according to [13, 14].

## RESULTS AND DISCUSSION

Adequate information about relationships between leaf area index and grain dry weight with oil and protein concentrations will help researchers to improve the effectiveness of breeding methods. Based on **Table 1**, the leaf area indexes of soybean in different withholding irrigation (I1 to I4) are varied by 3.63 in regular irrigation to 2.88 in withholding irrigation at seed filling period stage (20.7% lower). [15] Stated that leaf area index is reduced by water deficit due to reduction in canopy assimilation. Also, the highest pod dry weight (PDW), grain dry weight (GDW), and oil content were belonged to I1

treatment. However, withholding irrigation at flowering stage had the greatest impact on reducing oil content (11.9% lower compared with regular irrigation). In contrast, withholding irrigation at seed filling period stage increased seed protein content by 38.54%. [16] Emphasized that composition of soybean seed can be affected by environmental factors. Also, [17] reported that fertilizers application are the most important factors affecting the seed composition of soybean. The differences between values of LAI and GDW among the eight micronutrient foliar categories were quite small. Indeed, micronutrient fertilization had little effect on these traits. PDW, oil and protein contents were more influenced by micronutrient application. LAI increased with iron application, while, the highest values of PDW and GDW were recorded in ZnMn foliar application. Zinc application had the most impact on oil and protein percent and increased these trials up to 19.51% and 38.78%, respectively. [18] Reported that the effect of zinc fertilizer on oil and protein percent in safflower seeds was higher than the other fertilizers application. In addition a similar result was obtained by [19]. Overall, Fe, ZnMn and Zn had the greatest effects on LAI, PDW

and GDW, oil% and protein%, respectively. Similar results at regular irrigation were observed in **Table 2**. In regular irrigation, Fe led to increase LAI up to 3.82, while, when drought stress occurred at flowering, pod set and seed filling period stages, iron foliar application had less impact on LAI. In the other hands, Fe use efficiency is higher when sufficient water is availability. The relationships between evaluated traits were shown in following regression equations. The relationship between LAI and oil, protein, PDW, and GDW under different irrigation regimes were shown in regression equations [Eq. 1, 2, 3, and 4]:

$$\text{Oil: } Y=106.7+57.34X+9.23X^2 R^2=0.944 \quad [1]$$

$$\text{Protein: } Y=65.55+16.08X+2.32X^2 R^2=0.914 \quad [2]$$

$$\text{PDW: } Y=128.7+77.89X+11.95X^2 R^2=0.976 \quad [3]$$

$$\text{GDW: } Y=23.93+14.49X+2.44X^2 R^2=0.932 \quad [4]$$

The relationship between LAI and oil, protein, PDW, and GDW at micronutrients foliar treatments were shown in regression equations [Eq. 5, 6, 7, and 8]:

$$\text{Oil: } Y=408.0+255.6X-38.26X^2 R^2=0.770 \quad [5]$$

$$\text{Protein: } Y=-699.4+456.4X-70.59X^2 R^2=0.442 \quad [6]$$

$$\text{PDW: } Y=-58.07+36.10X-5.34X^2 R^2=0.046 \quad [7]$$

$$\text{GDW: } Y=-52.18+32.79X-4.88X^2 R^2=0.198 \quad [8]$$

Whereas Y is oil, protein, pod dry weight, and grain dry weight, and X is LAI.

The relationship between GDW and oil, and protein under different irrigation

regimes were shown in regression equations [Eq. 9, and 10]:

$$\text{Oil: } Y=35.03-13.47X+2.65X^2 \quad R^2=0.997 \quad [9]$$

$$\text{Protein: } Y=62.69-16.68X+2.73X^2 \quad R^2=0.998 \quad [10]$$

The relationship between GDW and oil, and protein at micronutrients foliar treatments were shown in regression equations [Eq. 11, and 12]:

$$\text{Oil: } Y=39.15-15.78X+3.07X^2 \quad R^2=0.061 \quad [11]$$

$$\text{Protein: } Y=1.96+30.01X-6.104X^2 \quad R^2=0.678 \quad [12]$$

In Eq. 9, 10, 11, and 12; Y is oil and protein, and X is grain dry weight. These equations are shown that in different irrigation regimes, oil content and GDW were positively, and protein concentration was negatively related to LAI (Fig a, b, and d). Also, PDW with increases in LAI up to 3.1 was decreased and when LAI increased by over 3.3, pod dry weight was increased (**Figure 1c**). Concerning micronutrient foliar treatments, although regression analysis showed that oil concentration, PDW, and GDW were positively, and protein content was negatively related to LAI, but, do not support by very high positive  $R^2$  (**Figure 2 a, b, c, and d**). In our experiment, the relationship between GDW as an independent variable and oil and protein content as dependent variables at different irrigation regimes (**Figure 3 a and b**), and micronutrients foliar treatments (**Figure 3 a to d**) were studied.

In different irrigation regimes, unlike protein content, oil concentration showed a significant increase as GDW from low up to high (**Figure 3 a and b**). The increase was 12.60% for oil content when the GDW changed from 2.35 to 3.48 g/plant. Regardless of irrigation and fertilizer treatments, a similar observation was made by [20]. In contrast, changes in protein content in the range of GDW were -2.34%. Also, a similar trend in oil and protein content were observed at micronutrients foliar treatments, but, do not support by very high positive  $R^2$  (Fig 3 c and d). These results were agreed with [21] in soybean and inconformity with [22] in maize. These researchers reported that there is a positive relationship between seed weight and oil content, but seed size had no significant effect on protein content. Indeed, according to **Figures 1, 2, and 3**, there are the positive and negative relationships between LAI and GDW with oil and protein concentration, respectively. Previous studies were emphasized on the importance investigation of relationships between grain weight, oil and protein content in soybean [21], mungbean [23], and Cotton [20]. Furthermore, the results obtained revealed that irrigation had the more impact on the relationships between LAI, GDW with oil

and protein content compared micronutrient application treatments. As shown in **Figure (4)**, under different irrigation regimes a negative and at micronutrient foliar treatments a positive relationships were found between oil and protein content in soybean seed, but not with high positive  $R^2$ . Overall, results of this study showed that relationship between oil and protein contents was slightly affected by irrigation and micronutrient fertilization.

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**Table1: The Means of LAI, Pod and Grain Dry Weight at Effective Seed Filling Period Stage (ESFP), oil and Protein Content in Soybean at Harvesting Time in Different Irrigation Regimes and Micronutrients Leaves Sprayed**

Treatments	LAI	Pod dry weight (g/plant)	grain dry weight (g/plant)	Oil (%)	Protein(%)
<b>Irrigation regimes</b>					
I1	<u>3.63</u>	<u>3.61</u>	<u>3.48</u>	<u>20.28</u>	<u>37.66</u>
I2	3.34	2.09	2.53	17.86	<u>37.96</u>
I3	3.14	1.85	2.64	18.01	37.64
I4	2.88	3.46	2.35	18.05	38.54
<b>Micronutrient foliar application</b>					
M0	3.18	2.01	2.52	18.08	36.97
Zn	3.32	2.36	2.61	<u>19.51</u>	<u>38.78</u>
Mn	3.31	3.01	2.84	18.61	37.08
Fe	<u>3.37</u>	2.92	2.90	18.67	38.08
ZnMn	3.21	<u>3.30</u>	<u>2.93</u>	18.69	37.75
ZnFe	3.19	2.71	2.75	18.15	38.75
MnFe	3.18	2.91	2.72	18.20	37.75
ZnMnFe	3.21	2.82	2.72	18.51	38.73

NOTE: I1: Irrigation at all of growth stages, I2: Irrigation Withholding at flowering stage, I3: Irrigation Withholding at pod set stage, I4: Irrigation Withholding at seed filling period, and M0: distilled water spray. LAI: leaf area index, ESFP,: effective seed filling period stage

Table 2: The Means of LAI, Pod and Grain Dry Weight at Effective Seed Filling Period Stage (ESFP<sub>s</sub>), Oil and Protein Content in Soybean at Harvesting Time in Different Irrigation Regimes and Micronutrients Leaves Sprayed Interaction Effects

Treatments	Means				
	LAI	Pod dry weight (g/plant)	Grain dry weight (g/plant)	Oil (%)	Protein (%)
M0I1	3.68	2.64	3.17	19.60	38.32
ZnI1	3.71	3.28	3.25	21.80	39.60
MnI1	3.73	4.26	3.48	20.97	37.85
FeI1	3.82	3.57	3.71	20.70	38.31
ZnMnI1	3.59	4.59	3.80	19.95	38.93
ZnFeI1	3.48	3.21	3.52	19.50	39.00
MnFeI1	3.52	3.69	3.48	19.70	37.21
ZnMnFeI1	3.48	3.51	3.45	20.16	38.82
M0I2	3.27	1.42	2.32	17.30	36.20
ZnI2	3.38	1.76	2.47	19.00	39.20
MnI2	3.52	1.96	2.71	17.77	37.68
FeI2	3.48	2.42	2.75	17.90	37.73
ZnMnI2	3.27	2.41	2.55	17.87	37.65
ZnFeI2	3.35	2.21	2.48	18.07	38.63
MnFeI2	3.21	2.42	2.51	17.20	38.07
ZnMnFeI2	3.21	2.17	2.47	17.83	38.77
M0I3	2.91	1.48	2.37	17.50	36.76
ZnI3	3.27	1.29	2.48	18.70	37.85
MnI3	3.25	2.09	2.75	17.54	36.52
FeI3	3.18	2.08	2.80	18.30	38.22
ZnMnI3	3.15	2.12	2.80	18.50	37.31
ZnFeI3	3.21	1.91	2.59	17.61	38.52
MnFeI3	3.00	1.65	2.61	17.82	37.69
ZnMnFeI3	3.17	2.15	2.70	18.22	38.50
M0I4	2.85	2.52	2.22	17.93	36.74
ZnI4	2.91	3.11	2.27	18.51	38.50
MnI4	3.00	3.79	2.43	18.27	36.44
FeI4	2.76	3.60	2.48	17.85	38.12
ZnMnI4	2.85	4.09	2.45	18.54	37.26
ZnFeI4	2.71	3.52	2.41	17.56	38.57
MnFeI4	3.00	3.89	2.27	18.11	37.06
ZnMnFeI4	3.00	3.41	2.28	17.93	38.82

NOTE: Similar letters in each column shows non-significant difference according to LSD test in %5 level. I1: Irrigation at all of growth stages, I2: Irrigation Withholding at flowering stage, I3: Irrigation Withholding at pod set stage, I4: Irrigation Withholding at seed filling period, and M0: distilled water spray. LAI: leaf area index, ESFP<sub>s</sub>: effective seed filling period stage

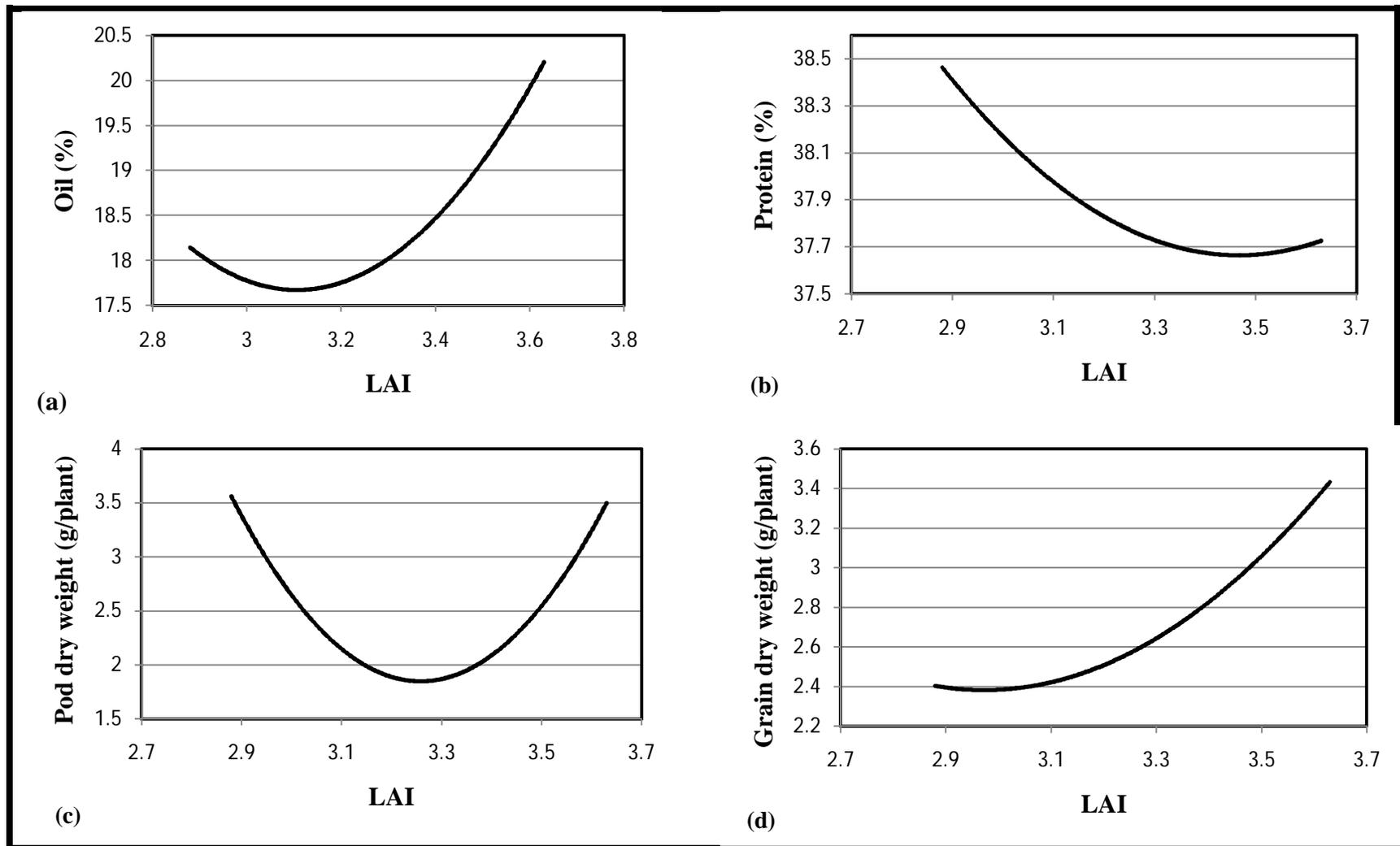


Figure 1: The Relationship Between LAI with (a) oil, (b) Protein, (C) Pod Dry Weight and (d) Grain Dry Weight in Soybean Under Different Irrigation Regimes

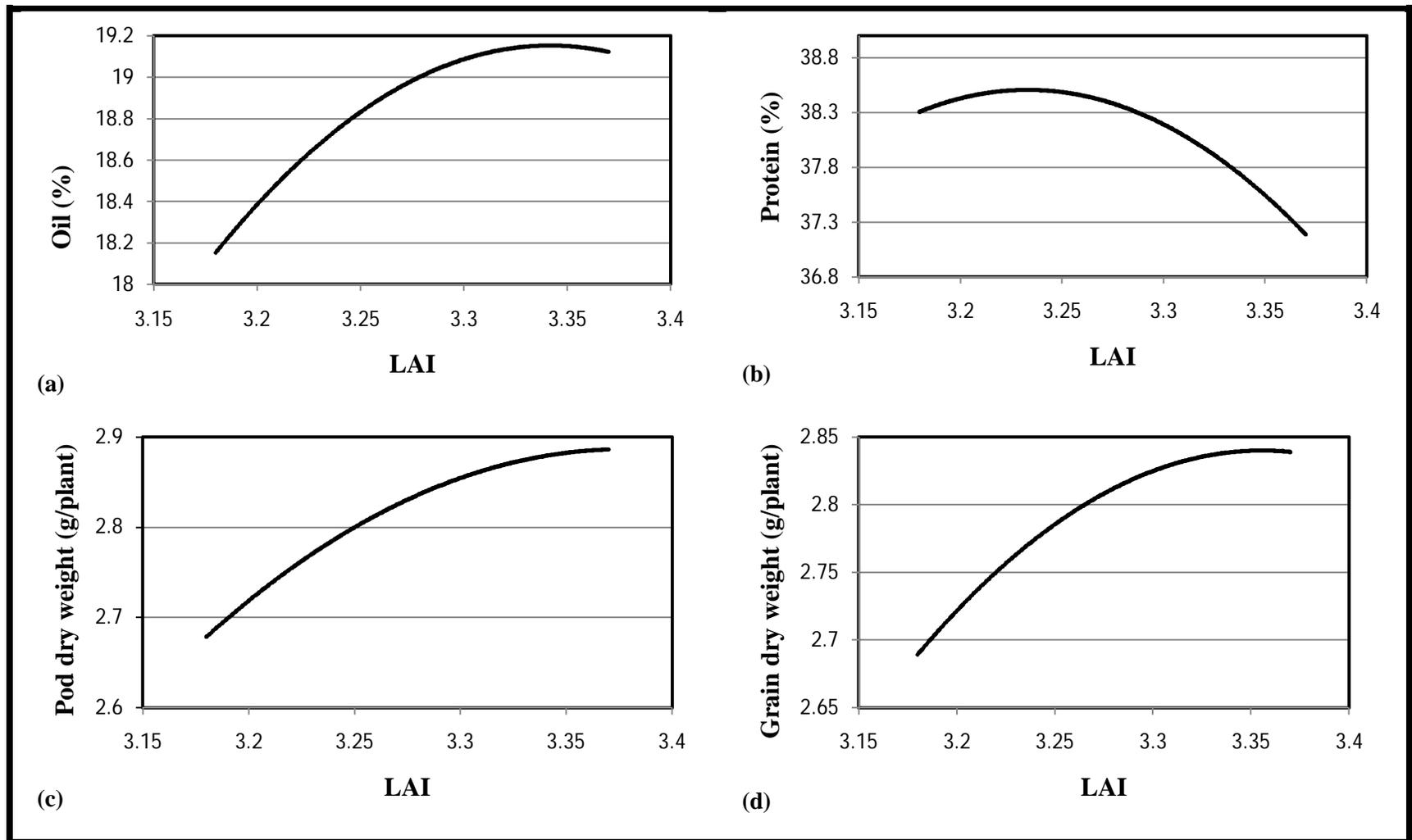


Figure 2: The Relationship Between LAI with (a) oil, (b) Protein, (c) Pod Dry Weight and (d) Grain Dry Weight in Soybean at Micronutrient Foliar Applications

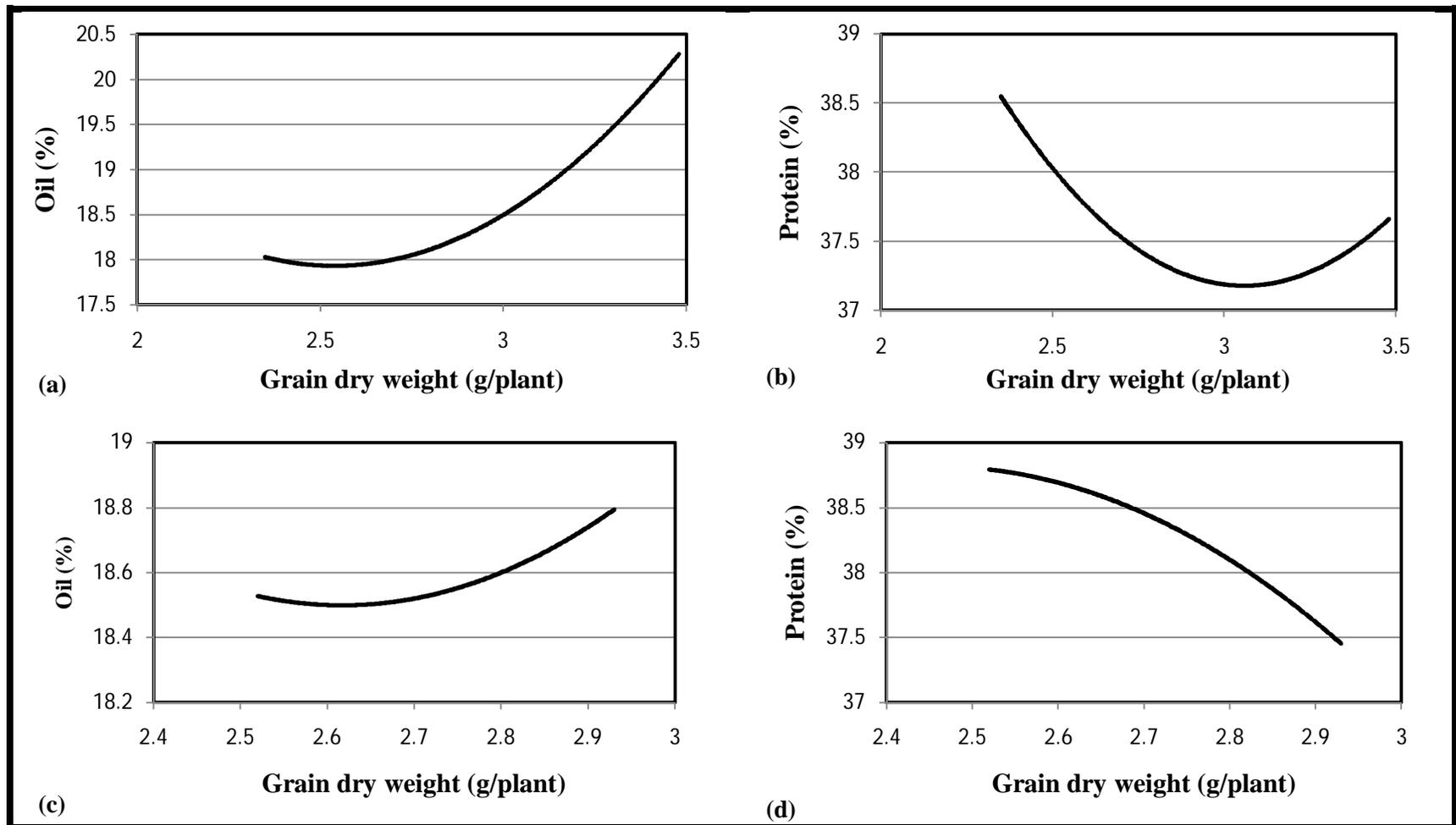


Figure 3: The Relationship Between Grain Dry Weight with (a) Oil, (b) Protein at Different Irrigation Regimes, and (c) Oil and (d) Protein at Micronutrient Foliar Applications in Soybean Seeds

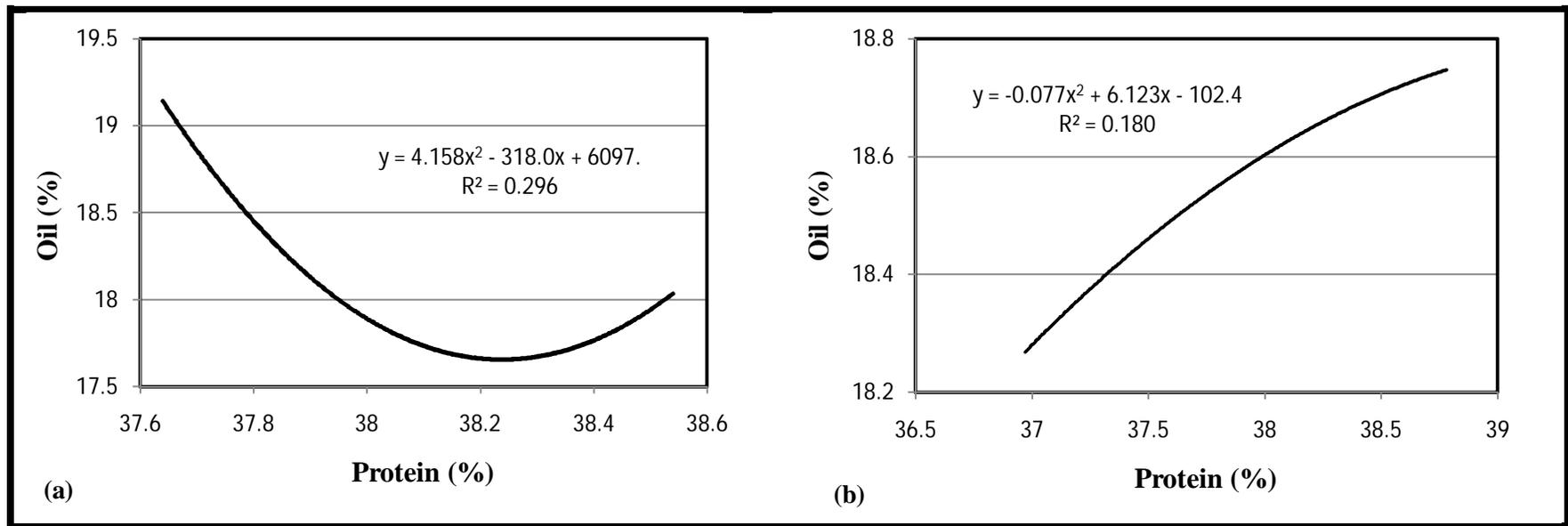


Figure 4: The Relationship Between Oil and Protein Contents in Soybean Seed (a) Under Different Irrigation Regimes (b) at Micronutrient Foliar Application