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Effect of Methanol Spraying on Physiological Characteristics, Oil and Protein Yields of Soybean (cv. Williams) under Deficit Irrigation

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ABSTRACT

*Most agricultural areas are located in arid zones and drought is one of the most critical limiting factors of photosynthesis and crop yields, hence, treatments like methanol spraying can partly reduce damage from water stress. To investigate the effects of times and number of methanol spraying on some physiological characteristics of soybean under deficit irrigation, a factorial split-plot experiment based on a randomized complete block design with four replications was done at Karaj, Iran, in 2010. The first factor was drought stress in two levels (based on depletion of a_1=40% and a_2=70% of available soil moisture). The second factor was spraying times of methanol in two levels (in the morning at b_1=8-10 AM and in the evening at b_2=19-21 PM). Third factor was foliar application number of methanol with three levels (each c_1=7, c_2=14 and c_3=21 days, Methanol spray was applied 5, 3 and 2 times during growth season of soybean, respectively). All treatments were sprayed with 21% (v/v) methanol concentration. 2 g lit^{-1} glycine was added to prepared solutions. In this study, grain yield, oil and protein percentage and yields, relative water content (RWC), leave chlorophyll content were determined. Results indicated a significant different (at 0.01probability level) between different levels of water stress on all evaluated traits and normal irrigation showed more favorable effects on measured parameters. Soybean grain yield under normal and deficit irrigation were 3187 and 1526 kg ha^{-1}, respectively. However, no significant difference ($P>0.05$) was shown between different times of methanol spraying over studied characteristics. In contrast, measured traits were significantly affected by the number of methanol application. Moreover, results demonstrated that significant differences exists ($p>0.05$) between interaction effects a×b, a×c, b×c and a×b×c in all traits evaluated. In normal and deficit irrigation maximum grain yield was produced by methanol spraying every other week in the evening and every 7 days in the morning, respectively. Grain yield were positively correlated with relative water content (RWC) (r=+0.89**) and chlorophyll content (r=+0.94**). It seems by measuring these traits can predict yield at a certain period.*

Keywords: methanol, soybean, water relative content, chlorophyll content, oil, protein.

INTRODUCTION

Water amount needed for plant is a critical factor on soybean growth and development and can play a good role in yield and production of crop [3]. Drought stress considers as a vital limiting factor of soybean growth which dwindles growth during plant vegetative stage [4]. Short maturation varieties compared to long duration varieties of soybean less respond to water stress, then in arid zones or areas where water is limited, short maturation varieties should be planted [9]. Daneshian *et al*. (2002) reported that drought stress at pod formation stage enhanced flower and pod shedding and diminish the soybean seeds number [14]. Korte *et al*. (1983) conducted that drought stress during early reproductive stages increases the flowers and pods reduction [20]. Water shortage, thus, is the main serious factor affecting flower and pod shedding, because adequate water supply prevents destructive changes in the area of pedicles falling. Water stress during grain filling significantly declined soybean yield by 32-42% [32]. They found that drought stress during seed development decreased yield, shortens the grain-filling stage and lowers final seed size and in severe stress seeds will be wrinkle and ill-formed.

Leave relative water content is one of the significant changes caused by drought stress. Relative water content regards as an index of plant drought resistance. According to Kaiser *et al*. (1985), reduction of relative water content by 70-100% photosynthesis capacity can reduce due to stomata closing which is quickly reversible [18]. As leaf RWC decrease by 35-70%, photosynthesis will reduce and improve only with water supplying. The main reason may be light inhibition, as carboxilation, Calvin cycle and light respiration will reduce, apparently electron translocation is a more limitative factor and when leaf RWC decrease by 30%, reduction of photosynthesis capacity is the result of cytoplasmic membrane injury which causes plant death. RWC represents a useful indicator of the state of water balance of a plant tissue and is a suitable and accurate method for tracking of water within the tissue which follows changes of tissues dry matter [13].

According to reports of Haghparast (1997), drought stress can destroy cell wall, leading to leakage of cell and vacuole fluid into the environment, high concentration and electrical conductivity (EC) of solution [30]. Highly viscous fluid, thus, can be a sign that more cells are destroyed [11]. Nonomura and Benson (1992) found that foliar application of methanol increase the growth and yield of c3 species and methanol is considered as a source of carbon for plants [2]. Zbiec et al. (2003) found that loss of light respiration in plants treated with methanol related to rapid oxidation of methanol to carbon dioxide and ribulose-l, 5-bisphosphate and less competitive of O_2 [21]. Methanol molecules are smaller than the carbon dioxide and absorbed sooner by plant, moreover, foliar application of methanol delayed senescence of leaves through ethylene production in plant, this increases photosynthetic active period and leaf area duration (LAD) [31]. Li et al. (1995) found that grain yield, seeds weight, and number of pods per soybeans plant compared to control significantly increase by methanol spraying and applying a 25% volumetric solution of methanol prepare the greatest effect on soybean growth and yield [36]. In order to better leave absorption, darkness is necessary for a few hours after methanol spraying [6].

Andres *et al*. (1990) found that foliar application of methanol can enhance activity of FBPase, an important enzyme controlling photosynthesis [29]. Hemming and Criddle, (1995) found that methanol application cause rise in Carbon conversion efficiency [5]. Castrillo and Trujillo (1994) observed a strong correlation between RWC and chlorophyll, protein and RUBP [22]. A positive correlation was found between the soybean yield and maximum florescence, photochemical capacity, chlorophyll content and RWC [8]. Shamsi (2010) showed that while drought stress decreased RWC and chlorophyll content, proline content also increased [19]. Nevertheless, further studies provided a strong positive correlation between wheat yield and RWC.

Azizi et al. (2008) found a similar effective correlation between yield and RWC, a negative strong correlation between yield and cell membrane stability, and a strong positive correlation between yield days to ripening in lentil (*lens esculentum* L.) [35]. The reports by Araus *et al*. (1998) illustrated that a strong correlation exist between stomata conductance, RWC, and co2 assimilation [15]. RWC may indicate relation between physiological traits and drought tolerance levels, as RWC is a stable characteristic which causes complete the plant tissue of sorghum water within a few days or a week [34]. Burquez (1987) also indicated a positive correlation between RWC and leaf thickness of napus [1]. In one experiment, reduction of RWC resulted from reduction on soil water and photosynthesis reduction during water loss arose from stomata closing can also negate carbon assimilation rate [28]. Tahara *et al*. (1990) [25] and Paknejad *et al*. (2009) [8] suggested a positive relation at grain filling stage and wheat cultivars produced higher yield, had a greater RWC as well. Allan *et al*. (2008) [17] and Mirakhori *et al*. (2009) reported that 60.9-81.5% reduction in leave RWC of soybean may occur due to 0-6 day's irrigation period [24]. Mirakhori *et al*. (2009) [24] and Nadali *et al*. (2010) demonstrated that 21% (v/v) methanol spray poses the greatest impact on yield, and other physiological traits [12]. Jafari-Paskiabi *et al*. (2011) indicated that concentration and time spraying methanol affected on pod and seed yield of cowpea also among methanol concentration treatments, maximum pod and seed were recorded for the 20% and 30% methanol treatments, respectively [23]. Thus, the objectives of this study were to determine the effects of time and number of methanol application on soybean quality yield under deficit irrigation.

MATERIALS AND METHODS

The study was performed in 2010 at Karaj, Iran (35° 41' 15"N, 50° 56' 51" E, 1190 m). Soil type was clay loam with pH of 7.6 and 2.7 ds m^{-1} salinity at the depth of 0-30 cm. A factorial split-plot experimental was conducted using a randomized complete block design with four replications. In these experiments, the first factor was two levels of water stress (based on 40 and 70% depletion of available soil moisture a_1 and a_2, respectively), second factor was the time of methanol spraying in two levels (8-10 AM=b_1 and 19-21 PM=b_2) and the third factor was in three levels (foliar application of methanol weekly=c1, biweekly=c2 and three weekly=c3 which was performed spraying 5, 3 and 2 times, respectively).

To avoid the toxicity in the presence of direct sunlight and chlorophyll degradation, 2 g lit^{-1} of glycine was added to prepared solution [2, 10]. Moreover, when spraying the plots of other treatments were sprayed by water and glycine at time of spray solution. The first spraying was performed on July 16 and 60 days after planting according to weather for 20 years with increasing temperature over 25 $^{\circ}$C. Spraying was continued until the solution drops on plant surface. Irrigation time was determined using gypsum blocks in term of the Soil Moisture Depletion (SMD). Gypsum blocks already calibrated and applied available moisture depletion curve provided by Paknejad *et al*. (2007) in Research Field of Islamic Azad University-Karaj Branch [7]. Irrigation was done when the moisture meter showed 20 and 80 numbers. Plots were furrow irrigated soon after planting all treatments irrigated until the fifth stage. To avoid interference among watering treatments, 250 cm distance between drought treatments was considered.

The soybean was planted at an average density of 40 plant m^{-2}, 0.5 m row spacing and 5 cm distance between seeds within rows. Each plot involved six 5m rows. Soybean seeds were disinfected then inoculated by soybean inoculums and was manually sown in 4 May 2010. Soybean were seeded at high density and then thinned to the target densities (40 plant m^{-2}) after their establishment. Relative water content (RWC) was measured within 24 hours after spraying.

From each plot 3 leaves (including top, middle, and bottom) was selected and transferred immediately to the laboratory as well as weighed (wet weight) with the 0.001 g accuracy. These leaves, then, were placed in distilled water for 24 hours and re-weighed (saturated weight). The average of three leaves measured was put in the formula. Leaves Relative Water Content (RWC) was calculated with according to the following equation [16]:

$$\%RWC = \frac{Fw - Dw}{Sw - Dw} \times 100$$

Where, F_w, D_w and S_w describe fresh, dry and saturated leaf weights, respectively. Leaf chlorophyll concentration was determined by "SPAD *CL-01*" chlorophyll-meter system in all plots. The obtained numbers were fitted the following model which was originated for soybean [24]:

$$y_2 = 7/479\ (x) + 51/6\ (mg/m^2)$$

Y and x are chlorophyll concentration (mg m−2) and SPAD readings, respectively. Harvesting was conducted at 125 days after planting and grain yield were measured in an area of 4 m^{-2}. Kejeldal method was applied for protein content determination and for oil extraction socsole device was used. All data were subjected to ANOVA using the GLM procedure of SAS. Treatment means were separated using Duncan test at $P<0.05$. The graphs were fitted using Excel (2003).

RESULTS AND DISCUSSION

The results of ANOVA (Table 1) demonstrated that the effects of drought stress level on grain yield, protein and oil percentage and yields were significant, but time of spraying had no significant differences on all measured traits at $P<0.05$ probability level. So this finding contradicts those obtained by Jafari-Paskiabi *et al*. (2011) [23]. Moreover, there was a significant difference between numbers of methanol application on all evaluated features. Grain yield, besides, was further affected by the interactions of water stress and number of spraying, water stress and time of spraying, time and number of spraying. Based on the results, there was significant ($P<0.05$) differences between interaction effects of drought, time and number of methanol spraying was detected on all traits. The highest (3187 kg/ha) and lowest (1526 kg/ha) grain yield was obtained in under normal and deficit irrigation, respectively. Here, yield reduction equals to 52.1% was detected in deficit irrigation compared to normal irrigation (Table 2). Yield loss was anticipatable due to water stress was applied from primary growth stages. This result is supported by previously published works [8, 24] in which the maximum soybean yield was produced under normal irrigation condition. Pandey et al. (1984) found that soybean grain yield was linear affected by water. Based on result, in normal irrigation condition the highest grain yield (3403 kg/ha) related to methanol spraying per 14 days in the evening [33]. However, foliar application of methanol per 14 days in the evening did not differ significantly in comparison with foliar application of methanol per 7 days in the morning and evening. In other hand, the lowest grain yield (2928 kg/ha) was obtained in methanol spraying per 14 days in the morning and had no statistically significant with methanol spraying per 21 days in the morning. Maybe, methanol spraying per 21 days produced a less carbon assimilation.

Under normal irrigation condition, there was no difference between spraying in the morning and evening and methanol spraying per 14 days was superior due to usage reduction of methanol and higher yield. For deficit irrigation, the highest (2025 kg/ha) and lowest (1090 kg/ha) grain yield were produced in methanol spraying per 7 days in the morning and methanol spraying per 21 days in the evening, respectively (Figure 1). Under deficit irrigation, spraying in the morning is better. This is probably due to closing of stomata in the evening induced by decreasing turgor and then more foliar application of methanol compensated to some extent damage from deficit irrigation and the changes trend of yield under deficit irrigation conditions affected by the number of methanol application which these result is in correspond to that of Khashaman (2010) [26].

Drought stress reduces oil percentage as the most (27.34%) and least (22.80%) oil percentage were generated in a_1 and a_2, respectively. A_2 showed a reduction as much as 2% related to normal conditions (a_1) (Table 2). Daneshian *et al*. (2002) reported that drought stress reduced soybean yield due to reduction of grain number per plant and 1000 grain weight [14]. They also found that as water stress increased, further seed oil and less protein rate is induced, however, eventually drought stress a marked negative effect on the oil and protein yield because of yield loss. Increasing effects of methanol spraying on the oil and protein percent have been confirmed in soybean [29]. In normal irrigation, spraying of methanol every other week in the morning produced the greatest oil percent (28.27%) which had no significant different with spraying in the evening. on the contrary, spraying of methanol every 21 days in the morning induced the smallest oil percent (22.30%) which placed statistically in a same group with spraying weekly in the evening (Figure 2). Thus, it may be concluded that, under normal irrigation more time methanol application can create destruction of chlorophyll and less applications of methanol can decrease plant assimilation.

In deficit irrigation foliar application of methanol every other week in the evening made the utmost oil percent (24.21%) and spraying of methanol every 7 days in the morning generated the minimum oil percent (20.78%), however, it had no significant difference with methanol application in the evening every 21 days. Finally, methanol spraying suggested in the evening every other week. The highest (890.93 kg/ha) and lowest (429.90 kg/ha) oil yield were observed in a_1 and a_2, respectively (Table 2). In normal irrigation spraying of methanol every 7 days in the evening generated the peak oil yield (952 kg/ha), besides, methanol application every 21 days in the evening breed the lowest yield oil (554 kg/ha). As oil yield affected by grain yield, more application time of methanol has favorable effects on oil yield. Under deficit irrigation methanol spray every 7 days in the morning and foliar application of methanol every 21 days presented the uppermost (573 kg/ha) and least (364 kg/ha) oil yield, respectively. In deficit irrigation because of more application time of methanol, greatly reduce damage from deficit irrigation, and changes trend of yield affected by more application time of methanol (Figure 3). Thus, it can be deductible more application times of methanol impose positive influences on oil yield, because oil yield and grain yield have direct relation and consequence for both normal and drought stress recommended methanol spray every 7 days in the morning.

Negative correlation between grain oil and protein percent has been reported [37]. The most (36.60%) and least (32.38%) protein percent was reported in a_1 and a_2, respectively. Under normal irrigation conditions, the maximum (39.23%) content of grain protein was discovered with methanol spray fortnight in the evening, however, showed no statistically significant ($P<0.05$) difference relative to methanol application weekly in the evening and put in a top group, on the other hands, the minimum (34.28) content of grain protein was observed by methanol spray every 21 days in the evening and did not significant different compared to foliar application of methanol in the morning every 21 days. In deficit irrigation, the highest (34.89%) and lowest (32.29%) grain protein rate were found in treatments which received methanol every other week in the morning and every 21 days in the evening, respectively. Nonetheless, foliar application of methanol every other week and every 21 days in the morning and evening were statistically similar. Accordingly, in normal and deficit irrigation foliar application of methanol fortnight in the evening and morning were appropriate (Figure 5).

The maximum protein yield (1243.93 kg/ha) was observed in $a_1$and minimum protein yield (698 kg/ha) was produced in a_2 which showed a yield loss as much as 44% compared to a_1 (Table 2). Under normal irrigation, methanol spray weekly, in the morning gave the greatest protein yield (1529.56 kg/ha) and was statistically similar to foliar application of methanol in the evening. Conversely, foliar application of methanol every other week in the morning presented the lowest protein yield (1019.37 kg/ha), although, methanol spray in the morning and evening were not significantly different and put in a same group. In water stress conditions, methanol spray weekly in the morning and foliar application of methanol fortnight in the morning led to the utmost (1077.29 kg/ha) and least (938.63 kg/ha) protein yield (Figure 5).

Overall, foliar application every 7 days in the morning and evening in both normal and water stress is better. More time spraying of methanol can reduce damage from water stress and the changes trend of yield affected by time of methanol application which this finding is in agreement with Khashaman (2010) observations [26]. Result also showed that RWC, leave chlorophyll content were affected by deficit irrigation considerably (0.05), while, the time of methanol spraying had any significant effect on studied traits. There was a significant difference between different levels the frequency of methanol spraying in all traits. Moreover, results demonstrated that significant differences exists ($P>0.05$) between interaction effects a×b, a×c, b×c on soybean yield and a×b×c in all soybean evaluated traits. Soybean, leave RWC, leaf chlorophyll index (SPAD), were significantly (at 0.05 probability level) affected by interaction of a×b×c. The relationship between RWC and grain yield is well known, therefore, the yield can be predicted by measuring the RWC.

Result from the correlation among the traits (Table 3) showed the highest correlation between grain yield and RWC (r=0.89**) was occurred 60 days after planting (Figure 6). Then, RWC measurement at this time can predict yield better. Figure 7 shows changes for RWC in $a_1b_2c_2$ and $a_1b_1c_2$ which had the best and worst grain yield under normal conditions, respectively. Indeed, grain yield was less severely reduced in treatments showed the maximum grain yield than treatments with minimum grain yield since methanol spraying began (53 days after planting). Apparently, at final stages of soybean growth, RWC tend to decline because plant become woodier and then RWC reduce both in normal and deficit irrigation. The relations evaluation showed that the most grain yield is combined with the most RWC and the least grain yield comes to the least RWC (Figure 8). In deficit irrigation, methanol spraying in more times could reduce damage from drought stress and RWC was less decreased compared to its application in less times. Makhdum *et al*. (2002) reported that methanol spraying could increase from 20 to 50% cotton (*Gossypium hirsutum* L.) leaves thickness [27]. On the other hand, as leaf thickness increase, RWC will improve [1]. Tahara *et al*. (1990) found that the highest grain yield obtained had a significant RWC than the lowest grain yield [25]. A strong positive relation between leave chlorophyll content and grain yield (Table 3), therefore, grain yield can estimate through chlorophyll content measurement.

Trend in chlorophyll content changes shows that as plant close to final stages of growth, leave chlorophyll content decrease due to decomposition of leave chlorophyll content and translocation of photosynthetic materials to grain. Result showed that the uppermost correlation between grain yield and chlorophyll content (r=0.94**) was came about 67 days after planting (Figure 9).

Figure 6 shows changes for leave chlorophyll content in $a_1b_2c_2$ and $a_1b_1c_2$ which created the greatest and smallest grain yield under normal conditions, respectively.

Table1: Analysis of variation for effects of timing and number of methanol foliar application and its period under deficit irrigation conditions.

SOV	df	Mean Squares (MS)				
		Grain Yield	Oil%	Oil yield	Protein%	Protein yield
Replication	3	0.00^{ns}	2.07^{ns}	154.26^{ns}	0.44^{ns}	614.02^{ns}
Soil moisture (A)	1	33.1**	101.50**	728464.35**	166.35**	786388.48**
Time of foliar application (B)	1	0.03^{ns}	0.20^{ns}	12.01^{ns}	2.97^{ns}	2.38^{ns}
A*B	2	0.01**	0.47^{ns}	12506.24**	12.42*	10.40^{ns}
Error (AB)	9	0.10	1.61	21.33	1.28	19.76
Number of foliar application (C)	2	0.65**	61.74**	274340.24**	48.79**	400224.74**
A*C	2	0.21**	11.51*	46870.43**	9.35**	137407.97**
B*C	2	0.22**	1.84^{ns}	159.24^{ns}	1.25^{ns}	1701.29*
A*B*C	2	0.06*	11.17*	19627.36**	1.36^{ns}	1515.72*
Error	24	0.01	1.23	398.29	2.45	571.93
CV (%)	-	4.62	6.91	3.50	3.75	1.75

***, Significant at 0.01 level *, Significant at 0.05 level n.s, non significant.*

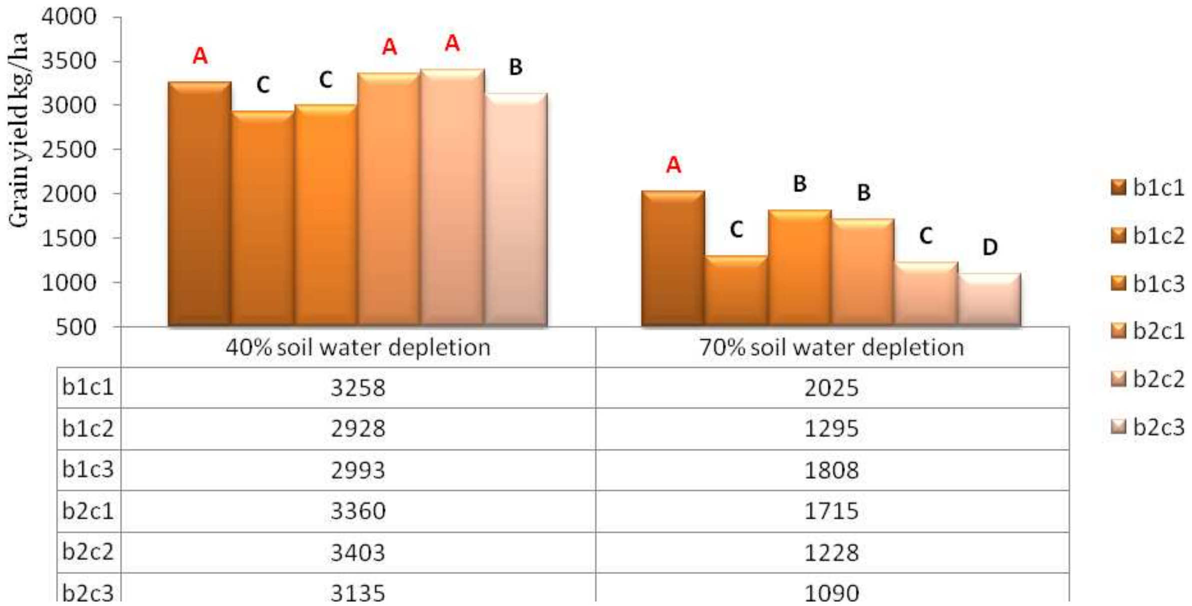

Fig 1: Effect timing (b_1=8-10 AM and b_2=19-21 PM) and number (c_1=each 7 days, c_2=each 14 days, c_3=each 21 days) Foliar application of methanol on grain yield under water deficit conditions.

Definitely, during the first steps of spraying $a_1b_1c_2$ showed higher chlorophyll content compared to $a_1b_2c_2$ (Figure 10). On the other hand, between first to second steps $a_1b_2c_2$ had the better performance than $a_1b_1c_2$. The reason for this may be due to less assimilation as a consequence the lack of full stomata opening, or toxicity of methanol consumption. Figure 11 shows changes for leave chlorophyll content in $a_2b_1c_1$ and $a_2b_2c_3$ which had the greatest and least grain yield under deficit irrigation. As observed, the highest grain yield has the most chlorophyll content and the lowest grain yield has the least. Probably, at final stages of soybean growth, translocation of photosynthetic materials to grain increase and membrane leakage increase as well. Since when plant close to its growth final stages, it will be woody and its membrane destroy.

Table 2: Means comparison time and number of methanol foliar application under water deficit conditions.

Treatment	Levels	Grain yield ($kg.ha^{-1}$)	Oil (%)	Oil yield ($kg.ha^{-1}$)	Protein (%)	Protein yield($kg.ha^{-1}$)
Soil moisture(A)	a1=40%	3187 a	24.83 a	890.93 a	36.60 a	1243.93 a
	a2=70%	1526 b	21.92 b	429.90 b	32.38 b	698 b
Time of foliar application (B)	b1=8-10AM	2384 a	23.31 a	608 a	34.11 a	1127 a
	b2=19-21PM	2330 a	23.44 a	607 a	34.61 a	1128 b
Number of foliar application (C)	c1=each 7 days	2589 a	22.13 b	757 a	34.19 b	1293 a
	c2=each 14 days	2213 b	25.64 a	549 b	36.19 a	978 c
	c3=each 21 days	2268 b	22.36 b	516 c	32.71 c	1110 b

Mean with the same letters in each column does not have significant difference at the 5% level of probability.

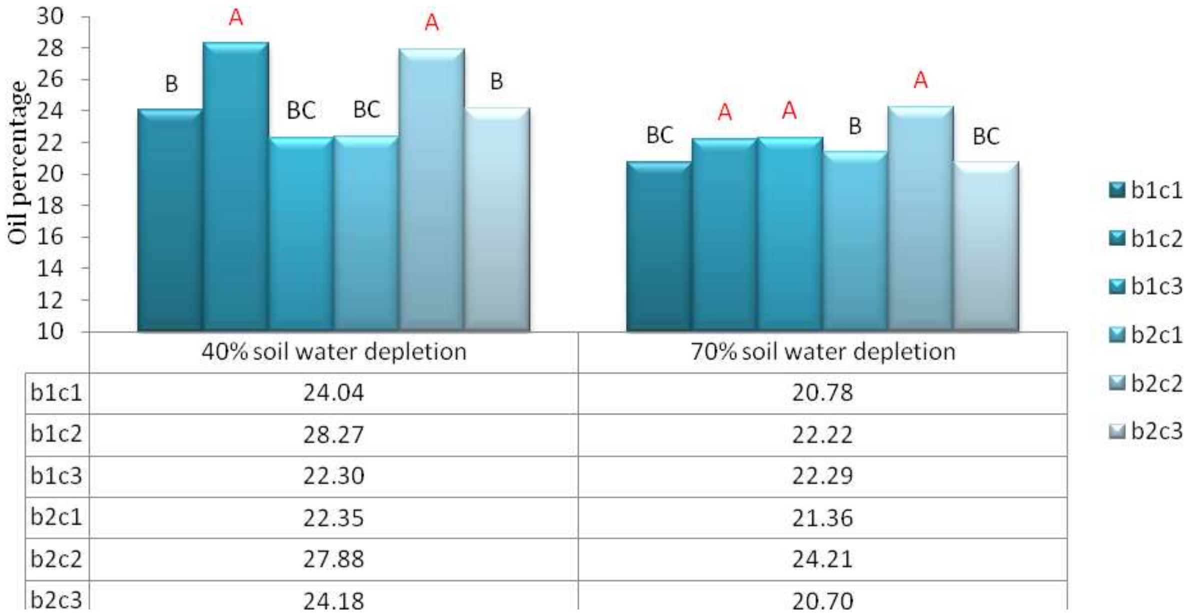

Fig 2: Effect timing (b1=8-10 AM and b2=19-21 PM) and number (c1=each 7 days, c2=each 14 days, c3=each 21 days) Foliar application of methanol on oil percentage under water deficit conditions.

Table 3: Correlation Coefficient of the Studied Traits

CHL5	CHL4	CHL3	CHL2	CHL1	RWC5	RWC4	RWC3	RWC2	RWC1	YIELD	
											YIELD
										0.87**	RWC1
									0.92**	0.95**	RWC2
								0.74**	0.64*	0.80**	RWC3
							0.89**	0.78**	0.70*	0.85**	RWC4
						0.96**	-0.88**	0.79**	0.71**	0.86**	RWC5
					0.85**	0.81**	0.78**	0.97**	0.91**	0.93**	CHL1
				0.89**	0.91**	0.92**	0.90**	0.89**	0.79**	0.93**	CHL2
			0.93**	0.89**	0.86**	0.85**	0.87**	0.90**	0.80**	0.97**	CHL3
		0.88**	0.94**	0.88**	0.94**	0.95**	0.90**	0.87**	0.72**	0.89**	CHL4
	0.95**	0.85**	0.88**	0.82**	0.89**	0.90**	0.88**	0.82**	0.67*	0.83**	CHL5

*ns, * and **: Non-significant, significant at 5 and 1 % probability levels, respectively*

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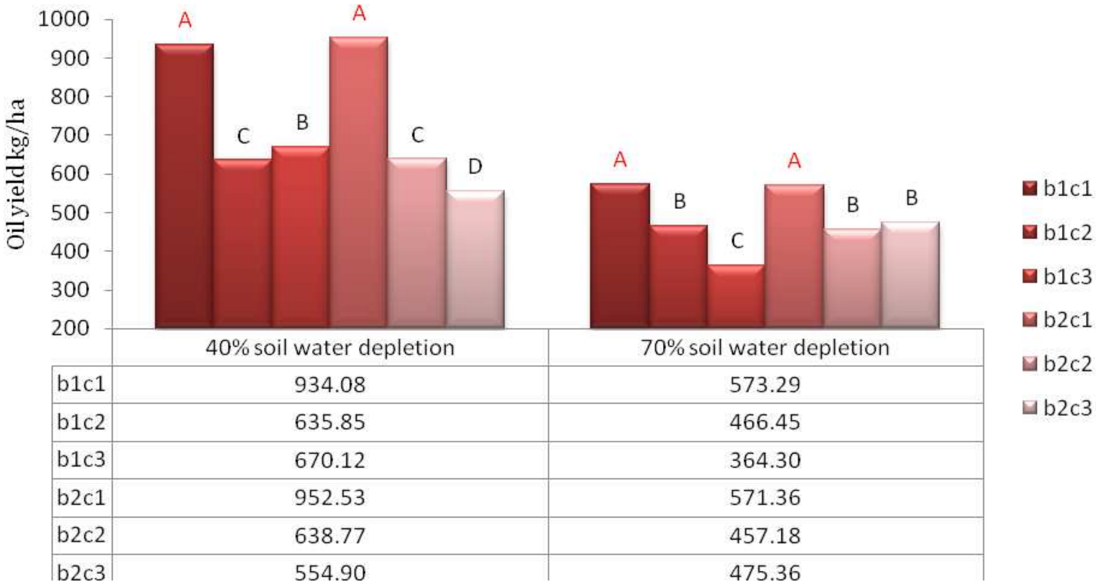
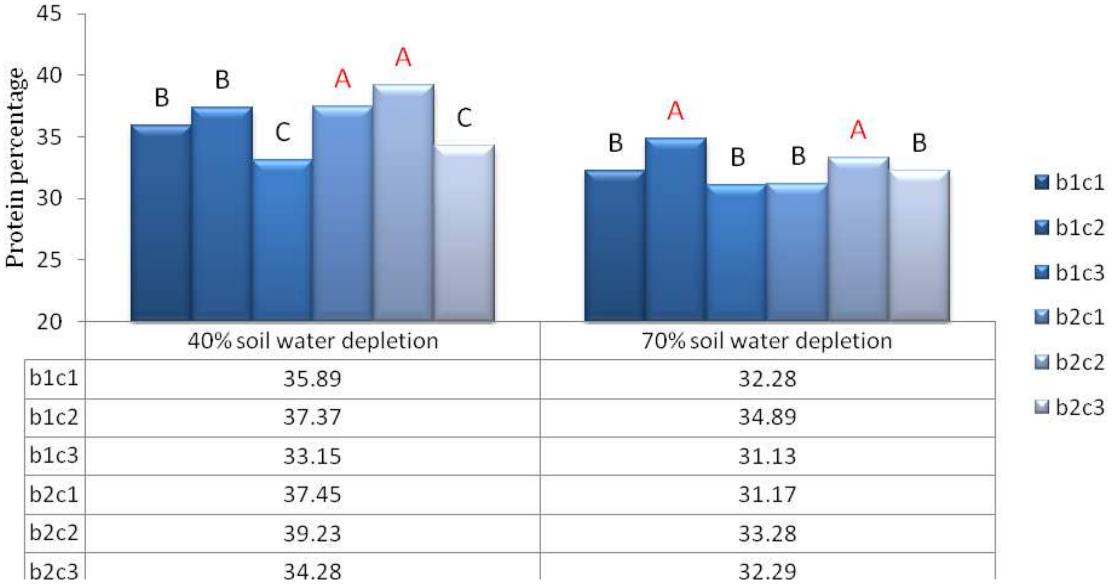


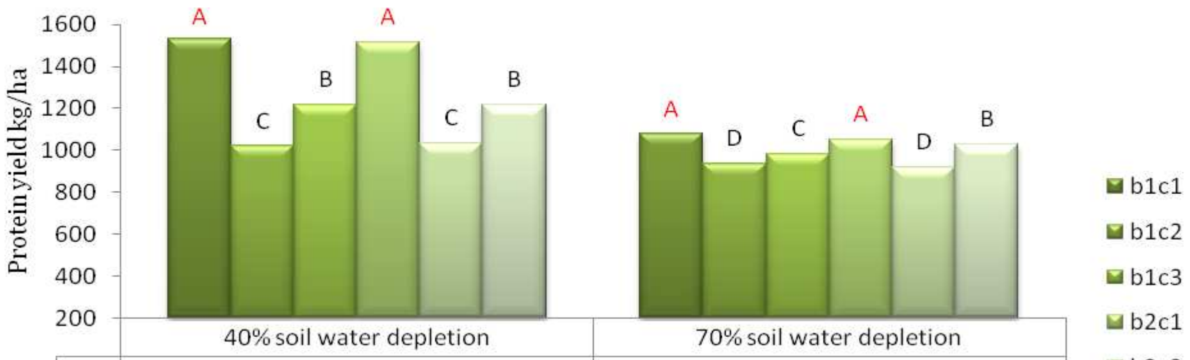
	40% soil water depletion	70% soil water depletion
b1c1	934.08	573.29
b1c2	635.85	466.45
b1c3	670.12	364.30
b2c1	952.53	571.36
b2c2	638.77	457.18
b2c3	554.90	475.36

Fig 3: Effect timing (b1=8-10 AM and b2=19-21 PM) and number (c1=each 7 days, c2=each 14 days, c3=each 21 days) Foliar application of methanol on oil yield under water deficit conditions.



	40% soil water depletion	70% soil water depletion
b1c1	35.89	32.28
b1c2	37.37	34.89
b1c3	33.15	31.13
b2c1	37.45	31.17
b2c2	39.23	33.28
b2c3	34.28	32.29

Fig 4: Effect timing (b1=8-10 AM and b2=19-21 PM) and number (c1=each 7 days, c2=each 14 days, c3=each 21 days) Foliar application of methanol on Protein percentage under water deficit conditions.



	40% soil water depletion	70% soil water depletion
b1c1	1529.56	1077.29
b1c2	1019.37	938.63
b1c3	1216.22	984.03
b2c1	1517.31	1050.36
b2c2	1035.06	921.56
b2c3	1216.90	1026.58

Fig 5: Effect timing (b1=8-10 AM and b2=19-21 PM) and number (c1=each 7 days, c2=each 14 days, c3=each 21 days) Foliar application of methanol on Protein yield under water deficit conditions.

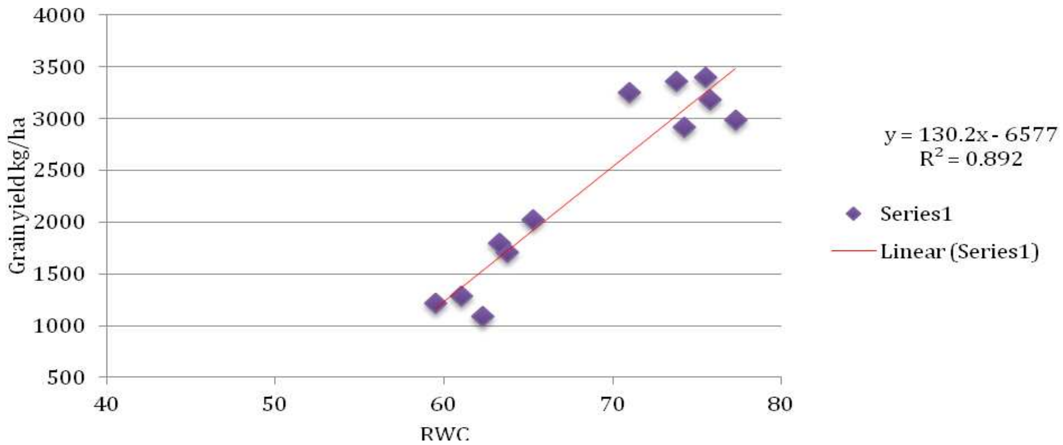


Fig 6: Correlation between Grain yield and RWC

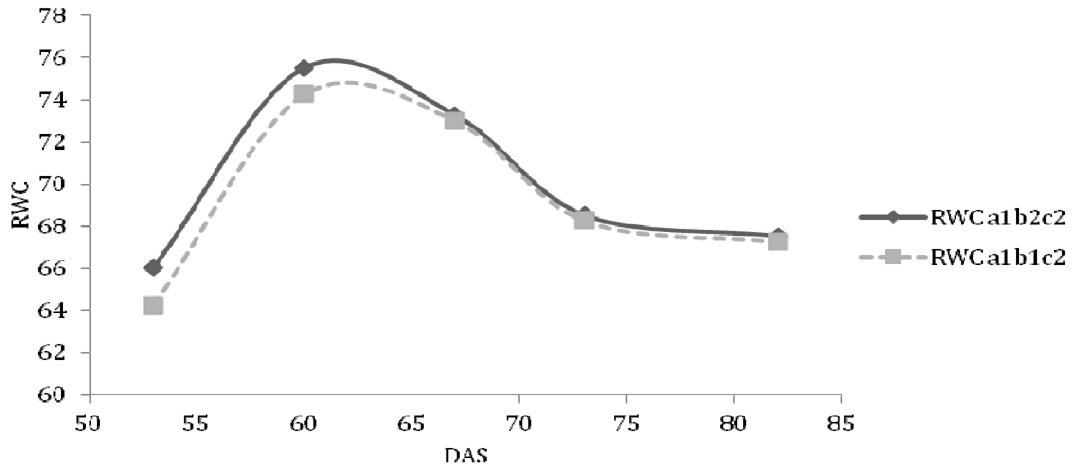


Fig 7: Changes for RWC in $a_1b_2c_2$ and $a_1b_1c_2$ which had the best and worst grain yield under normal conditions, respectively.

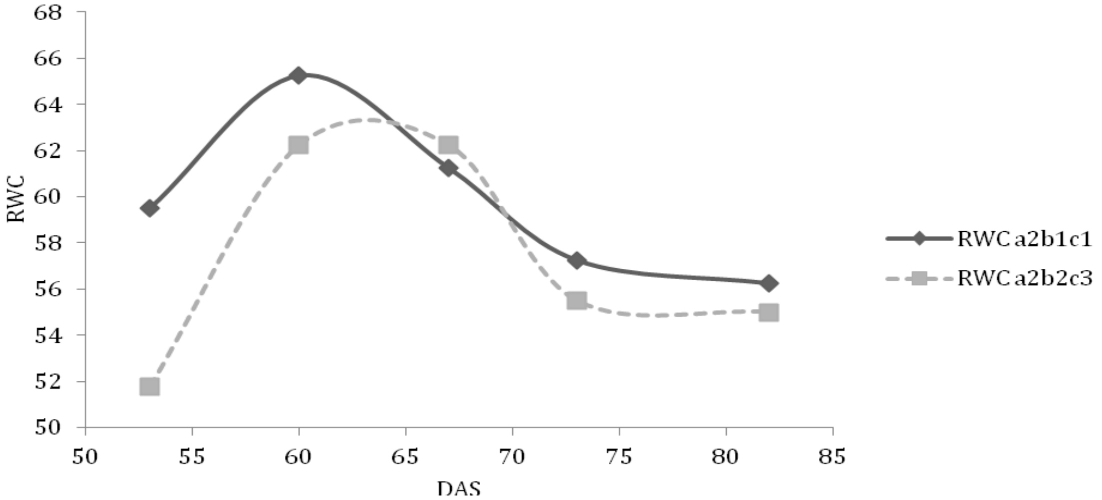


Fig 8: Changes for RWC in $a_2b_1c_1$ and $a_2b_2c_3$ which had the best and worst grain yield under deficit conditions, respectively.

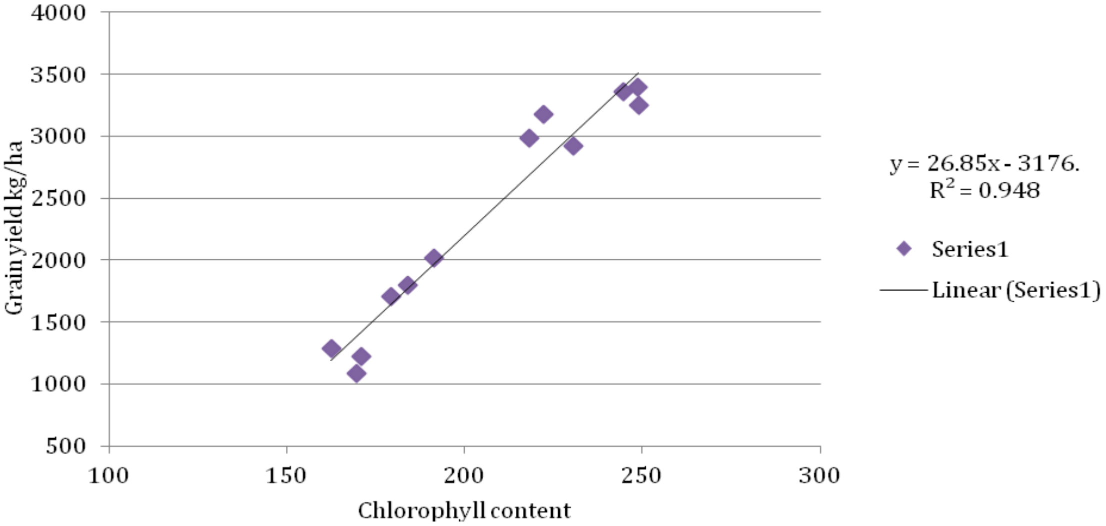


Fig 9: Correlation between Grain yield and chlorophyll content

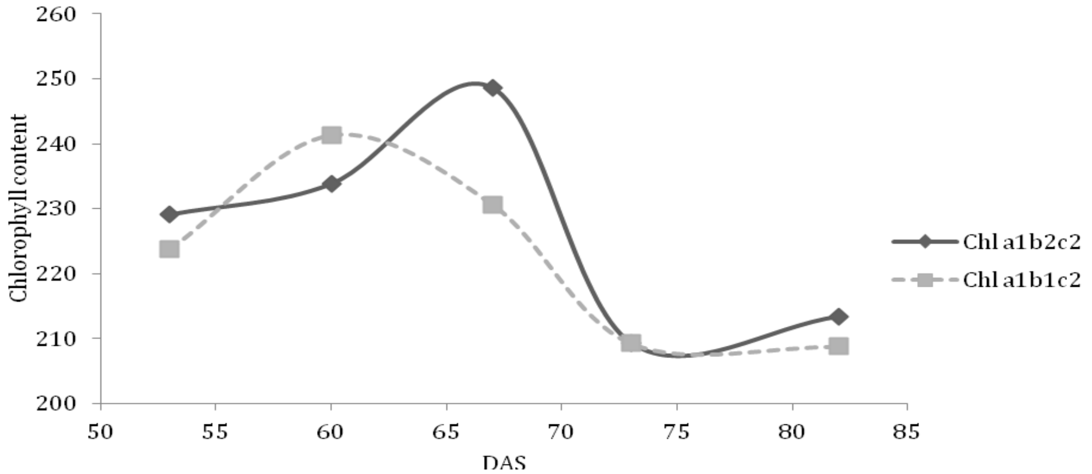


Fig10: Changes for Chlorophyll content in $a_1b_2c_2$ and $a_1b_1c_2$ which had the best and worst grain yield under normal conditions, respectively.

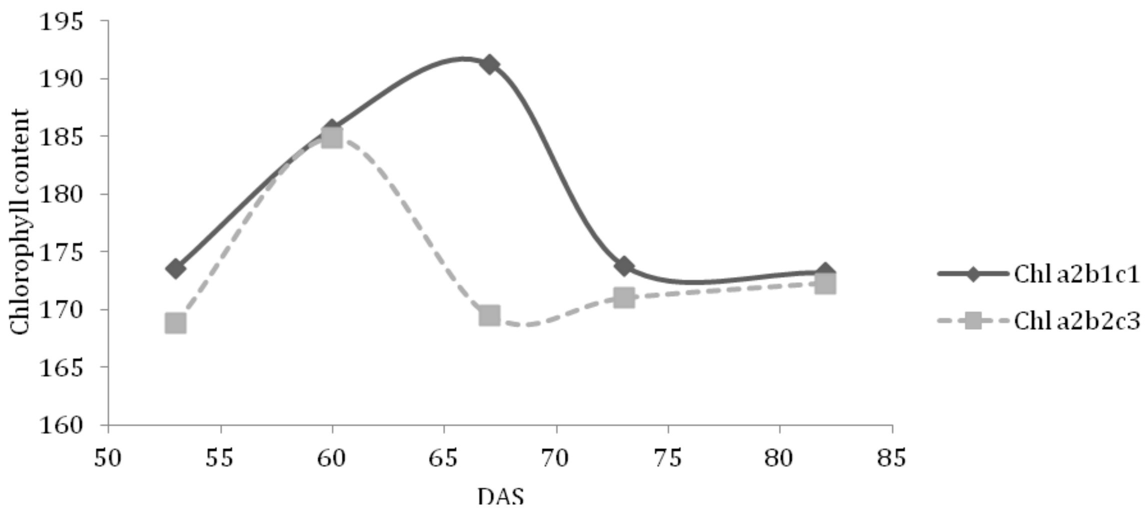


Fig11: Changes for Chlorophyll content in $a_2b_1c_1$ and $a_2b_2c_3$ which had the best and worst grain yield under deficit conditions, respectively.

CONCLUSION

Soybean yield is affected by methanol spraying and under water stress higher frequency of spraying somewhat can reduce destructive effects of drought and prevent of yield loss. Based on the results, the highest impacts under normal irrigation and drought conditions were found by methanol spraying every 14 and 7 days, respectively. Under normal irrigation condition foliar application of methanol in the morning and evening provided the maximum yield but in deficit irrigation condition the maximum yield obtained when methanol applied in the morning. Apparently, by increasing applications, methanol can have better impacts on RWC, and leave chlorophyll, and prevents light respiration such an anti- stress substance and can be used by plant as a carbon source.

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