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Effects of foliar application of methanol on growth and root characteristics of chickpea (*Cicer arietinum* L.) under drought stress

Saeed Reza Hossinzadeh[1*], Ali Ganjeali [2]Azam Salami[3] and Rahele ahmadpour[4]

[1]Young Researchers Club, Tarbiat Moalem university, Tehran, Iran
[2] Biology department, Faculty of Science, Ferdowsi University of Mashhad, Iran
[3]Biology department, Faculty of Science, Tarbiat Moalem university, Tehran, Iran
[4]Young Researchers Club, Faculty of Science, Ferdowsi University of Mashhad, Iran

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ABSTRACT

Available water is an important factor for plant growth in arid environments. Foliar application of methanol are believed to be more important in drought tolerance. In order to evaluate the effects of foliar application of methanol on some morphological characteristics of chickpea under drought stress, an factorial experiment was conducted factorial based on completely randomized design with three replications in 2011 at the Recearch Center for Sciences of Ferdowsi University of Mashhad. The first factor was different levels of methanol including, 0 (control), 20, 25, 30, 35 volumetric percentage (v/v), which were used as foliar applications at three times during growth season of chickpea, with 10 days intervals. Second factor was moisture regimes in two levels, 25 and 100 percent of field capacity. Results showed that there was significant difference ($P \leq 0.01$) between methanol levels concentrations regarding to plant height, root dry weight, tap root length, root area, root area to leaf area ratio, total root length, leaf area, root to shoot ratio, number of lateral root, root volume and root fresh weight. Spraying with 25% volume level significantly increased in plant height, number of lateral root, root dry weight, tap root length, root area, root area to leaf area ratio, total root length, root to shoot ratio and leaf area compared with control. Results indicated that interactions between drought and methanol was significant differences ($P \leq 0.05$) in traits such as, root to shoot ratio, leaf area and total root length.

Key word: Foliar application, Methanol, Root characteristics, Drought stress

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INTRODUCTION

Generally, drought is one of the limiting crop production factors in arid environments [2,17].The amount of water needed for plant growth and development in chickpea is an important factor and can have significant influence on growth and morphological characteristics [3].Production of biomass by plants depends to great extent on environmental factors such as water supply, air temperature and carbon dioxide concentration in the canopy[13].Taking this point, many researchers tend to use growth regulators to improve crop growth and production. Increasing the yield in the unit of surface is one of the most important issues that have attracted many researchers' attention. Photosynthesis is the substantial process for the production of organic matter in plants [2].Usually, the amount of the produced dry matter has a direct relationship with the photosynthesis efficiency of the plant and also the way in which CO_2 fixation occurs in crops. Therefore, Methanol spray is a method which increases crop CO_2 fixation in unit area [11].Today, in order to achieve this goal, compounds such as methanol, ethanol, propanol, butanol and amino acids like glycine, glutamate and asparate are used. Recent investigation showed that C_3 crops yield and growth increased via methanol spray and methanol may act as C source for these crops [15].Abundant dioxide carbon supply from methanol causes the photo respiration to be shifted from catabolism to anabolism[14]. Photorespiration can be minimized with methanol spray, since 25% of carbon wastes during photorespiration [3]. That is because methanol is absorbed in plant and rapidly metabolized to CO_2 in plant tissue due to smaller size of methanol rather than CO_2[7]. The major source of methanol production in plant is cellular pectin demethylation. Such volatile organic compound i.e., methanol exist leaves via stomata and it is obvious that plant tissues metabolize methanol [22]. A small proportion of this endogenous methanol reaches leaf surfaces, where it is volatilized or consumed by methylotrophic bacteria. These bacteria are capable to grow on methanol and generate plant growth regulators such as auxin and cytokinin [10].Also these bacteria are associated with nitrogen metabolism in plants through production of bacterial urea [21]. Nonomura and Benson (1992) foliar application of methanol increase the growth and yield of c_3 species and methanol is considered as a source of carbon for plants [2].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) [22]. Several studies have been shown that foliar application of methanol can prevent of biomass reduction [9-18].Li et al. (1995) revealed that Grain yield, 1000-seed weight and number ofpods per plant of soybean treated by in Methanol significantly increased compared to control [11].Foliar application with 5-10% methanol increase plant growth and yield [7]. In order to better absorption of methanol by the leaves, after foliar application, hours of darkness is necessary [13]. Hemming and Criddle (1995) reported that foliar application of methanol cause to rise in Carbon conversion efficiency [15]. Experiments have shown that foliar application of 20% methanol to peanut could increase leaf area index, crop growth rate, pod and grain yield, number of ripened pod and grainprotein of peanut [16]. Nadali et al. (2010) stated that 21% (v/v) methanol spray poses the greatest impact on yield, and other physiological traits[12]. Positive effects of foliar application of methanol on growth and yield of other Plant have been confirmed in previous studies. Thus, the objectives of this study were to investigate the effects foliar application of methanol and drought stress on morpphological characteristics of chicpea (*Cicer arietinum* L.).

MATERIALS AND METHODS

In order to study the effect of drought stress and foliar application of methanol on root characteristics of chickpea (*Cicer arietinum* L.) during 2011 summer, an experiment was conducted at the Research Center for Sciences of Ferdowsi University of Mashhad. This study was done as a factorial based on completely randomized design with three replications. The first factor was the foliar application of methanol in five levels [0 (control), 20, 25, 30, 35 volumetric percentage (v/v)] that to prevent of methanol poisoning at light presence, 2 gr lit-1 of glycine was added to prepared solution [2,10].The second factor was drought stress in two levels, 25 and 100 percent of field capacity. Soil test results revealed that the soil texture is of the sandy-loam type (8.88% clay, 55.12% sand and 36% silt) with pH and EC values being 7.9 and 1.2 dS/m, respectively. In addition to the before mentioned, the organic matters of the region's soil are potassium, phosphorus and nitrogen which are given in Table 1. The foliar application of methanol was done at three times during growth season of chickpea, with 10 days intervals. The first foliar application of methanol was performed 4 week after planting on September 12 and other spraying was done during early of bloom and early of pod formation respectively. Spray application was continued until solution drops flow from plant surface. Plants were harvested via distructive of pot at the end of growth and then plant shoot part from root were separated. Finally, the morphological traits such as; plant height, , leaf number, leaf area, root dry weight, tap root length, total root length, root area, root area/ leaf area and root /shoot were measured.

Table 1 - Soil characteristics used in the experiment

pH	EC ds/m	N %	K (ppm)	Na (ppm)	P (ppm)	Clay %	Silt %	Sand %
7.9	1.2	0.56	6.138	0.59	35	8.88	36	55.12

RESULTS AND DISCUSSION

The results of analysis variance demonstrated that the effects of drought stress and methanol foliar application on plant height was significant($P \leq 0.01$). Interactions between drought and methanol was not significant (Table 2). Based on result of mean comparison, the highest plant height was related to the level of 25% methanol, which there was no significant differences with all levels of methanol and the lowest was observed in control treatment(Table 3).In the study on cotton, the highest plant height was observed in the treatment of 30 volumetric percentage of methanol[15].They expressed that methanol increasedCO_2 assimilation [2]. Methanol to formaldehyde is converted by the enzyme methanol oxidase then be converted to format (Methanoeic acid). In the next step, format converted to CO_2 by format dehydrogenase, Therefore increased CO_2interacelular[2]. In the study reported on cotton, spraying methanol, leads to the stimulation of growth and plant height, by increasing cytokinin[28]. Methylothrophyc bacteria live on the leaves of most crop plants, these bacteria, with receiving methanol provide the necessary substrate for auxin and cytokinin hormones [26].Analysis of variance showed that effect of methanol was significant on root dry weight at P ≤ 0.01 probability level but effect of drought stress and also interaction between methanol and drought stress was not significant (Table 2). The highest root dry weight was in 25% methanol concentration that there was no significant differences with 30% level and the lowest of it was related to control (Table 3). Nadeali (2010) reported that the maximum root dry weight compare with to control was in 20% and 30% (v/v). In another study on soybean, applying 21% (v/v) methanol caused root yield to be increased by 38%, compared to 0 (control) [11]. It seems that methanol with increasing in CO_2 fixation, caused increasing root yield. Also according to Ehyaee (2010), the most root dry weight was observed in 30% methanol concentration. The effect of the drought stress and concentration of methanol on tap root length was significant (P≤0.01).Interactions between drought and methanol was not significant (Table 2).Result showed, all levels of methanol, were placed in a statistical group and control level, was in another group(Table 3).The root system due to its proximity to water, is considered as the first sensor, therefore plays an important role in resistance to drought[24]. In a study on tomato was observed that foliar application of methanol, leading to an increase in root and shoot dry weight at all levels[25].In this study, was observed that methanol has a positive effect on tap root length. The results of analysis variance demonstrated that the effects of drought stress and methanol foliar application on root area was significant (P≤0.01) but interactions between drought and methanol was not significant (Table 2). result of mean comparison (Table 3) showed that the most root area related to 25% [v/v] methanol which had significant difference with other treatments. The lowest root area was observed control treatment. Root area increase, due to increased entrance water points, leading to increased absorption level and water uptake efficiency[1].It seems that the root area increase, with increased root dry weight is related, On the other hand, increase in dry matter due to increased net photosynthesis[14]. Zebic et al (1992) reported that increase in net photosynthesis due to rapid oxidation of methanol to carbon dioxide and reduces the plants photorespiration [14].The effect of the drought stress and methanol spraying on root area to leaf area ratio was significant at probability levels of 5 and 1%, respectively. Interactions between drought and methanol was not significant (Table 2). The maximum and the minimum root area to leaf area ratio were observed at 25% [v/v] and control respectively (Table 3). Mirakhori et al. (2011) concluded that methanol spraying had a positive effect on root area to leaf area ratio of soybean.

The results of analysis variance demonstrated that the effects of drought stress and methanol foliar application on total root length was significant (P≤0.01). Also, interactions between drought and methanol was significant at P ≤ 0.05 probability level (Table 2). Based on result of mean comparison, the highest total root length was related to the level of 25% methanol, which there was significant differences with all levels and the least for this trait was observed in control (Table 3). In interaction of methanol and drought stress, the maximum total root length was obtained in 25 volumetric percentage (v/v) in non-stress conditions and the minimum for this trait was observed control in stress conditions (Figure 1). Researches done on sugar beet showed that methanol foliar application leads to increased the length and volume root in drought condition [14]. In this study, also observed that methanol has a positive effect on total root length.

The results of analysis variance demonstrated that the effects of drought stress and methanol foliar application on leaf area was significant (P≤0.01). Also, interactions between drought and methanol was significant at P ≤ 0.05 probability level (Table 2). All applied methanol levels increased number of lateral roots compared to control, except the highest concentration (35%) that may be has imposed a toxic effect (Table 2).The maximum leaf area was observed at 25% (v/v), which there was no significant differences with 20% and 30% methanol. The lowest leaf area was observed in 35% methanol, which there was no significant differences with control (Table 3). It seems that methanol with increasing leaf area caused increasing photosynthesis in the plants and protects leaves and probability it was due to increases root yield. In interaction of methanol and drought stress, the highest leaf area, was obtained in 25 volumetric percentage (v/v) in non-stress conditions and the lowest was observed control in stress conditions (Figure 2).There are some reasons for increase leaf yield. The leaves of many plants have covered by methylobacterium. These bacteria are capable to grow on C compounds such as methanol and generate plant growth regulators such as auxinand cytokinin [26].Also, according to view of Makhdum et al. (2002), methanol treated cotton showed increased leaf area index and turgidy. The results of analysis variance demonstrated that the effects of drought stress and methanol foliar application on root to shoot ratio was significant (P≤0.01). Also, interactions between drought and methanol was significant at P ≤ 0.05 probability level (Table 2).The highestroot to shoot ratio was obtained when methanol was used at 25%. The lowest on root to shoot ratio was observed control (Table 2).In interaction of methanol and drought stress, the highest root to shoot ratio, was obtained in 25 volumetric percentage (v/v) and the lowest was observed control (Figure 3).More ratio of root to shoot (the water absorption organs to water consumer organs) plant resistance to enhance drought tolerance improves [1]. in this study was observed that the methanol with root increase will lead to plant resistance to drought stress. These results are in consistent with results of Makhdum et al (2002) who reported that methanol spray increased root to shoot ratio.

Table 2. Analysis of variance of growth and root characteristics of chickpea in different levels of foliar aplication of methanol under drought stress

S.O.V	Degree of freedom	Plant height (cm)	Root dry Weight (mg)	Tap root length (cm)	Root area (cm^2)	Root area/ leaf area	total root length (cm)	Leaf area (mm^2)	Root/Shoot	Number of lateral root	Root volume (cm^3)	Root fresh weight (mg)
						Mean Square						
Methanol	4	**39.449	**0.887	**33.137	**969747682.34	0.387**	992308.1 **	68453200.383**	0.263 **	3.746 **	0.001 ^{ns}	57.271 **
Stress	1	**748.701	^{ns}0.069	**99.008	**1090055367.1	0.256 *	247198.8 **	**1685055885.6	0.195 **	2.319 ^{ns}	0.009 **	85.197 **
M×S	4	^{ns}5.519	^{ns}0.159	^{ns}7.196	^{ns}80986561.802	0.106 ^{ns}	93964.6 *	*13058787.883	0.046 *	0.482 ^{ns}	0.001 ^{ns}	2.269 ^{ns}
Error	20	2.965	0.132	4.767	62093285.496	0.049	175232.6	3905537.233	0.015	0.790	0.002	4.097
C.V	-	5.12	19.08	8.07	14.01	16.06	8.16	4.79	10.12	27.14	6.63	12.78

*ns: Non-significant, * and **: significant at $P \leq 0.05$ & $P \leq 0.01$*

Table 3. Comparison of growth and root characteristics of chickpea under different levels of foliar aplication of methanol under drought stress.

Methanol levels	Plant height (cm)	Root dry weight (mg/plant)	Tap root length (cm)	Root area (mm^2)	Root area/ leaf area	total root length (cm)	Leaf area (mm^2)	Root/Shoot	Number of lateral root	Root fresh weight (mg)
Control	b30.62	c1.469	b23.00	e41600	1.077 c	4825.1 b	41600 e	0.922 d	13.23 b	12.59 c
20% (v/v)	a35.68	bc1.880	a27.58	d50480	1.193 bc	4934.2 b	50480 d	1.091 cd	17.27 a	14.38 bc
25% (v/v)	a35.73	a2.422	29.08 a	a76000	1.726 a	5844.0 a	76000 a	1.456 a	20.00 a	20.77 a
30% (v/v)	a35.35	ab2.140	a28.08	b58590	1.486 ab	5050.3 b	58590 b	1.342 ab	20.80 a	16.51 b
35% (v/v)	33.47 ab	c1.626	a27.50	c54490	1.375 abc	5011.2 b	54490 c	1.178 bc	18.92 a	14.95 bc

The columns that have letters in common are not significantly different at $P \leq 0.01$ according to duncan test.

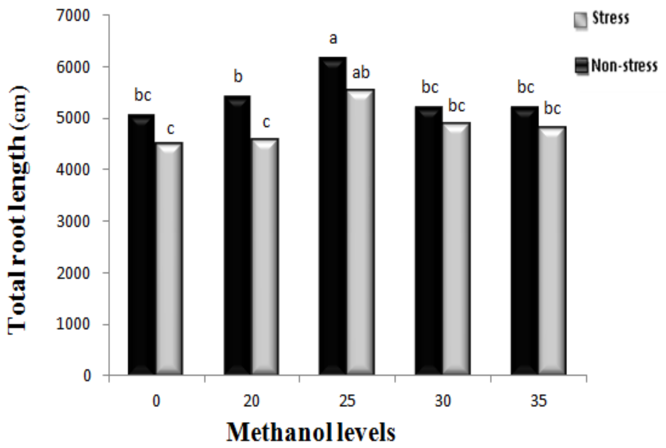

Fig. 1- Interaction effect of methanol and drought stress on total root length

The columns that have letters in common are not significantly different at P $\leq$ 0.05 according to duncan test.

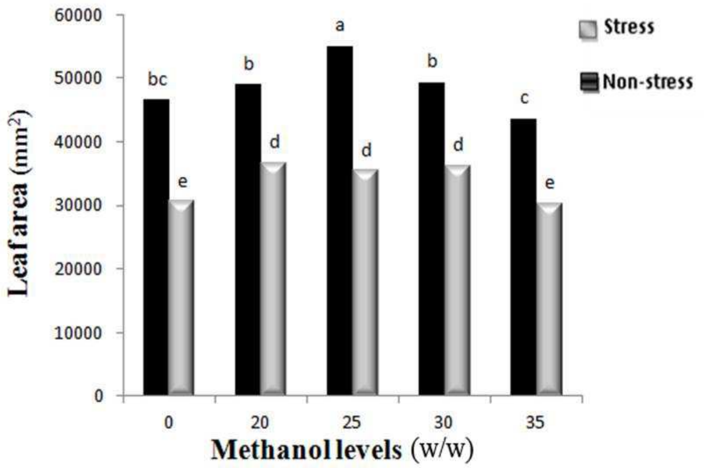

Fig. 2- Intraction effect of methanol and drought stress on total root length

The columns that have letters in common are not significantly different at P $\leq$ 0.05 according to duncan test.

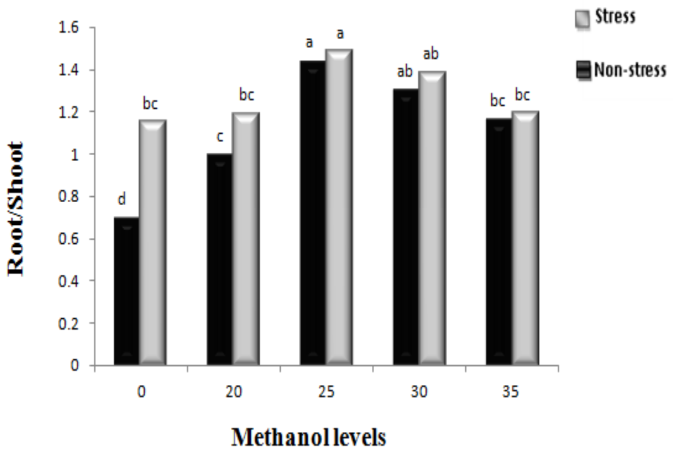

Fig. 3- Intraction effect of methanol and drought stress on total root length

The columns that have letters in common are not significantly different at P $\leq$ 0.05 according to duncan test.

Foliar application of methanol had a significant effect ($P \leq 0.01$) on number of lateral roots but effect of the drought stress and interaction between drought and methanol was not significant (Table 2).All applied methanol levels increased number of lateral roots compared to control. The highest number of lateral roots was obtained using 30% methanol, which there was no significant differences with all levels of methanol (Table 3).In the present research, the effect of methanol spraying and interactions between drought and methanol was not significant on root volume but drought stress was had a significant ($P \leq 0.01$) effect on root volume(Table 2). Ganjeali et al (2004) reported that drought conditions decreased the root volume in different genotypes of chickpea. Drought stress treatment and foliar application of methanol had a significant effect($P \leq 0.01$) on root fresh weight but interactions between drought and methanol was not significant (Table 2). Based on result of mean comparison, the highest root fresh weight was related to the level of 25% methanol, which there was significant differences with all levels. The lowest root fresh weight was observed in control (Table 3). Zbieć et al. (2003) observed the fresh weight of root increased by using 20% or 30 % methanol solutions.

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