Effects of withholding irrigation and Foliar Application of Zinc and Manganese on Fatty Acid Composition and Seed Oil Content in Winter Safflower

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Abstract:
Experiment was conducted in 2001 –2002 and 2002 –2003 crop years in Esfahan, Iran. The experimental design was the split factorial in a randomized complete block with three replications. Four drought stresses (S1= full irrigation, S2= withholding irrigation in vegetative growth stage, S3= withholding irrigation in flowering stage, and S4= withholding irrigation in seed filling stage) were randomized to the main plot units and 12 treatments from combination levels of three cultivars (C1= Zarghan 279, C2= Varamin 295 and C3= LRV 5151) and four foliar applications (F1= no foliar application, F2=foliar application of water, F3 = foliar application of Zinc Sulfate (3000 ppm) and F4=foliar application of manganese sulfate (3000 ppm)) were randomized to the subplot units. After seed harvest, oil yield and percent of seed oil were measured and fatty acid profiles of oil were determined by GC. Results presented that in S3 and C1 and C3 cultivars manganese foliar application significantly increased oil percent. In S4 oil percent and yield were increased in C1 and C3 cultivars with both zinc and manganese foliar applications. In some interaction levels zinc and manganese foliar applications significantly increased palmitic and oleic acids whereas these foliar applications decreased linoleic acid percent. Generally, drought stress during flowering stage imposed the most damage to oil percent and yield and also decreased linoleic acid content and increased stearic and oleic acid content, so flowering stage is more sensitive to drought than vegetative or grain filling stages and foliar application of zinc and manganese can compensate the negative effects of drought on safflower.

Key words: Fatty Acids - foliar application – manganese – safflower - zinc

Introduction

Oil and protein are two major components of seeds in oilseed crops and are affected significantly by environmental stresses. It was reported that 60 to 70 percent of total oils in safflower seeds are produced at a 22-days period after flowering (6). So any nutrient deficiencies or drought stresses that occur at this stage have the most effect on oil content and fatty acid profiles of the seed oil. Mekki *et a*l, (1999) reported that drought stress at seed filling stage significantly decreased oil yield and content in sunflower, while seed hull percent increased with drought. Although fatty acid content of oil was not affected by drought, palmitic acid content was increased and oleic acid content was decreased by drought stress. In that research foliar spray of potassium increased the oleic acid content of oil. Sawan *et al,* (2001) presented that application of zinc increased seed yield, seed protein content, protein and oil yield and total unsaturated fatty acids (oleic and linoleic) content in cotton. Increasing of seed oil and protein content in Indian mustard with application of zinc sulfate was reported (8). Application of manganese in flax also increased the oil percent and seed yield (5). In the present study, an attempt is made to investigate the effects of foliar application of zinc and manganese on oil content and fatty acid profiles in fall-sown safflower cultivars under drought conditions.

Materials and methods

Experiment was carried out at an experimental farm in Esfahan, I.R.Iran during two successive growing seasons, 2001-2002 and 2002-2003. Soil type was clay loam and from Aridisols series. The layout of the experiments was a split-factorial with organized treatments following a randomized complete block design, with three replications. Four stress treatments (S1=without stress; S2=withholding irrigation during vegetative growth stage; S3=withholding irrigation during flowering stage, and S4=withholding irrigation during grain filling stage) were randomized to the main plot units. Subplot units consisted of 12 treatments from combination of three cultivars (C1=Zarghan 279; C2= Varamin 295 and C3=L.R.V.5151) and four foliar application [F1=without foliar application; F2=foliar application of water; F3=foliar application of zinc sulfate (ZnSO4.7H2O) and F4=foliar application of manganese sulfate (MnSO4.H2O)]. C1 and C3 were spiny cultivars and relatively resistant to drought, but C2 was spinles cultivar and has less resistance to drought stress. Foliar application of Zn and Mn (3000 ppm) was applied twice. The first one was applied immediately after the end of rosette stage. The second foliar application was carried out two weeks after the first application (Lewis and McFarlane, 1986). We sprayed the plants with solution until the leaves were completely wet and the solution ran off the leaves (Sultana *et al*, 2001). All cultivars were sown on the final week of September, in 50 cm rows at a density of 40 seed m^{-2}. In order to determine the time of beginning of each drought stress treatment, safflower growth stages were determined according to the procedure of Tanaka *et al*., (1997). Seed harvest was carried out on 1.5 m^2 on two central rows of each plot**.** Seed oil was extracted with soxhlet and petroleum benzine was used as a solvent. Methylation of fatty acids was carried out according to the Metcalf *et al*., (1966). The fatty acid methyl esters were analyzed by a Unicam 4600 Gas Chromatograph. The chromatograph was fitted with a BPX 70, 30 m (length) x 0.25 µm (column i.d.) capillary column. Data were analyzed with SAS software and Duncan multiple range tests were used to determine the significance of differences between treatment means at 0.05 levels.

Results

Oil percent

At S1 level (Without drought stress) and in C1 cultivar (Zarghan 279) foliar application generally increased the oil percent compared to F1 (No foliar application) (table 1). At S3 level (Withholding irrigation at flowering stage) and in C1 cultivar F4 significantly increased oil percent (2.4%) compared to F1. At S4 level (Withholding irrigation at seed filling stage) and in C1 and C3 cultivars F3 and F4 significantly increased oil percent compared to F1 respectively. Generally foliar application increased seed oil percent relative to F1 (table 2). Withholding irrigation at the flowering stage (S3) significantly decreased seed oil percent compared to other stress levels (table 2). Results did not show any differences between C1 and C3 based on oil percent. However, C2 had minimum seed oil percent (table 2).

Oil yield

Results showed that at S3 and in C3, F4 significantly increased oil yield (27.6 %) compared to F1 (table2). At S4 level and in C1 cultivar, F3 and F4 levels and in C3, F4 significantly increased oil yield compared to F1. Generally minimum oil yield was seen at S3 level. C3 cultivar had the maximum seed oil yield relative to other cultivars and F3 and F4 significantly increased oil yield compared to F1 (table 2).

Fatty acid profiles

Maximum stearic and oleic acids percents were seen in withholding irrigation in flowering stage (S3), seed filling stage (S4) and vegetative stage (S2) respectively (table 2). However linoleic acid percent decreased with stress treatments, especially in S3 level. Generally minimum total unsaturated fatty acid percent (oleic + linoleic) was seen in S3 level. Maximum oleic acid percent and minimum linoleic acid percent were seen in C2 cultivar. C3 cultivar had the minimum oleic and the maximum linoleic acid percents so that total unsaturated fatty acid percent was the same between cultivars. Results showed that foliar application levels increased the total unsaturated fatty acid percent (i.e. palmitic, stearic and oleic acids) and decreased the linoleic acid percents.

Table 1. Mean comparisons of interaction effects of drought stress and foliar application on three safflower cultivars at two years of experiment. Within column, means followed by the same letter are not significantly different at p=0.05

Experimental treatments			Oil yield (Kg/ha)	Oil percent	Palmitic acid %	Total unsaturated fatty acids %
Without drought stress (S1)	Zarghan 279 (C1)	No foliar application (F1)	1497.3 b	31.8 b	8.0 ab	90.6 a
		Foliar application of water (F2)	1692.1 ab	35.1 a	8.6 a	89.6 bc
		Foliar application of Zn (F3)	1744.3 a	34.4 a	8.7 a	89.2 c
		Foliar application of Mn (F4)	1555.8 ab	35.7 a	7.6 b	90.3 ab
	Varamin 295 (C2)	No foliar application (F1)	1392.4 c	35.1 a	8.2 b	89.6 b
		Foliar application of water (F2)	1484.8 c	33.3 a	8.2 b	89.9 ab
		Foliar application of Zn (F3)	1885.1 a	36.1 a	7.6 b	90.7 a
		Foliar application of Mn (F4)	1651.7 b	33.4 a	9.7 a	88.4 c
	LRV 5151 (C3)	No foliar application (F1)	1573.1 b	33.1 a	8.1 a	89.9 a
		Foliar application of water (F2)	1701.9 ab	35.3 a	9.3 a	88.6 b
		Foliar application of Zn (F3)	1693.6 ab	34.6 a	8.3 a	89.5 ab
		Foliar application of Mn (F4)	1824.9 a	33.9 a	8.7 a	89.3 ab
Withholding irrigation at vegetative growth stage (S2)	Zarghan 279 (C1)	No foliar application (F1)	1302.6 a	34.6 a	7.6 b	90.7 a
		Foliar application of water (F2)	1208.3 a	34.5 a	8.2 b	89.9 a
		Foliar application of Zn (F3)	1421.6 a	34.4 a	8.3 b	89.8 ab
		Foliar application of Mn (F4)	1412.2 a	35.0 a	9.1 a	88.9 b
	Varamin 295 (C2)	No foliar application (F1)	1046.4 c	31.8 a	8.5 a	89.5 a
		Foliar application of water (F2)	1340.1 ab	33.4 a	8.1 a	89.8 a
		Foliar application of Zn (F3)	1377.4 a	33.9 a	7.8 a	89.6 a
		Foliar application of Mn (F4)	1135.7 b	32.9 a	8.2 a	89.7 a
	LRV 5151 (C3)	No foliar application (F1)	1270.9 b	34.3 a	8.2 a	90.2 a
		Foliar application of water (F2)	1368.8 b	32.8 a	8.5 a	89.4 a
		Foliar application of Zn (F3)	1693.3 a	35.1 a	8.1 a	89.7 a
		Foliar application of Mn (F4)	1676.6 a	33.3 a	7.9 a	89.9 a
Withholding irrigation at flowering stage (S3)	Zarghan 279 (C1)	No foliar application (F1)	748.7 a	27.2 b	7.4 b	90.3 a
		Foliar application of water (F2)	742.9 a	28.2 ab	9.2 a	88.7 a
		Foliar application of Zn (F3)	718.6 a	27.5 ab	8.9 a	88.9 a
		Foliar application of Mn (F4)	780.4 a	29.6 a	7.9 b	89.9 a
	Varamin 295 (C2)	No foliar application (F1)	615.9 a	25.9 a	8.2 b	89.7 a
		Foliar application of water (F2)	741.5 a	28.8 a	8.5 ab	89.6 a
		Foliar application of Zn (F3)	751.3 a	26.9 a	9.1 a	88.8 a
		Foliar application of Mn (F4)	799.7 a	26.2 a	8.2 b	89.5 a
	LRV 5151 (C3)	No foliar application (F1)	805.0 b	26.9 b	8.6 a	89.2 a
		Foliar application of water (F2)	925.4 ab	32.0 a	8.6 a	89.5 a
		Foliar application of Zn (F3)	896.4 ab	28.9 a	7.9 a	90.1 a
		Foliar application of Mn (F4)	1112.4 a	32.3 a	8.2 a	89.7 a
Withholding irrigation at	Zarghan 279 (C1)	No foliar application (F1)	1192.9 b	32.3 c	8.7 a	89.3 a
		Foliar application of water (F2)	1093.6 b	31.7 c	8.3 a	89.7 a

	Foliar application of Zn (F3)	1646.6 a	36.8 a	7.9 a	89.8 a
	Foliar application of Mn (F4)	1428.9 a	33.9 b	8.4 a	89.9 a
Varamin 295 (C2)	No foliar application (F1)	1341.4 a	34.1 a	8.8 a	89.4 a
	Foliar application of water (F2)	1262.9 a	34.1 a	8.4 a	89.6 a
	Foliar application of Zn (F3)	1297.8 a	34.0 a	8.1 a	89.8 a
	Foliar application of Mn (F4)	1527.8 a	35.6 a	8.5 a	89.6 a
LRV 5151 (C3)	No foliar application (F1)	1457.4 b	32.9 b	7.9 a	89.8 a
	Foliar application of water (F2)	1421.5 b	34.6 a b	8.4 a	89.4 a
	Foliar application of Zn (F3)	1515.8 ab	34.9 ab	8.2 a	89.7 a
	Foliar application of Mn (F4)	1703.1 a	35.3 a	7.9 a	89.9 a

Table 2. Mean comparisons of main effects on oil properties at two years of experiment. Within column, means followed by the same letter are not significantly different at p=0.05.

Treatments	Oil percent	Palmitic %	Stearic %	Oleic %	Linoleic %	Total unsaturated fatty acids %	Oil yield (Kg/ha)
Drought Stresses							
S1	34.2 a	8.4 a	1.9 c	16.1 d	73.3 a	89.6 ab	1641.4 a
S2	33.8 a	8.2 a	2.0 bc	17.0 c	72.4 b	89.8 a	1354.5 b
S3	28.4 b	8.4 a	2.1 a	18.8 a	70.5 c	89.5 b	810.7 c
S4	34.4 a	8.3 a	2.0 b	17.4 b	71.7 b	89.7 a	1409.7 b
Foliar Applications							
F1	31.8 b	8.2 b	1.9 b	17.2 b	72.3 a	89.8 a	1187.0 b
F2	32.9 a	8.5 a	2.0 ab	16.9 b	72.4 a	89.5 b	1248.6 b
F3	33.1 a	8.2 b	2.1 a	17.6 a	71.7 b	89.6 ab	1386.8 a
F4	33.0 a	8.4 ab	2.0 ab	17.6 a	71.5 b	89.6 ab	1393.9 a
Cultivars							
C1	32.8 a	8.3 a	2.0 a	17.5 b	72.0 b	89.7 a	1267.3 b
C2	32.2 b	8.4 a	2.0 a	18.1 a	71.1 c	89.6 a	1228.2 b
C3	32.2 a	8.3 a	2.1 a	16.4 c	72.8 a	89.6 a	1416.7 a

Discussion

Flowering stage in fall-sown safflower took from the last week of May to the end of June (about one month). In this period high air temperature coincided with the drought stress in S3 level, and seed oil content was significantly decreased by drought stress at this period, because of reduction of seed capacity for accumulation of oil. Mekki *et al*., (1999) reported that drought stress at seed filling period decreased the seed oil content in sunflower. However, in safflower drought stress at seed filling period had no significant effect on seed oil content.

Zinc and manganese are two essential micronutrients that regulate the enzyme activity for cell metabolism and our results showed that foliar spraying of these elements, so increasing of their concentration in shoots, had changed the oil quantity and quality and even in drought condition at flowering and seed filling stages their efficacy were highlighted. Oil yield and percent increment in two cultivars, C1 and C3, at flowering drought condition revealed that we can modify the drought impact by foliar spraying of these elements. C1 and C3 cultivars were spiny and C2 was a non-spiny cultivar and there was positive correlation between this character and oil yield and percent.

Analysis of fatty acid profiles showed that 99.5 % of safflower oil was made of four fatty acids including stearic (2%), palmitic (8.3 %), oleic (17.3 %) and linoleic (71.9 %), and these results were in agreement with Ngaraj and Anjani (1997). However, we could not see any linolenic acid in fatty acid profiles. Drought stress at flowering stage resulted in decreasing the un-saturated fatty acids and increasing the saturated fatty acids. Younis *et al*., (2001) also reported the same results in soybean. Finally flowering stage of safflower plant is more sensitive to drought than vegetative or grain filling stages and foliar application of zinc and manganese can compensate the negative effects of drought on oil yield and percent in safflower plant.

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