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Phytohormonal Responses of Sunflower (*Helianthus annuus* L.) to Magnetized Water and Seed under Water Deficit Conditions

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Abstract: This experiment was conducted in 2009 in two separate fields in order to study the effects of magnetized seed and irrigation with magnetized water under water deficit conditions, on proline amino acid and some phytohormones in sunflower (Azargol hybrid). Experimental design was split plot in the form of randomized complete block design (RCBD) with four replications. Treatments included irrigation with normal and magnetized water, each in one of the separate fields, water deficit in main plots at 40% (control), 60% and 75% of available soil moisture depletion. Normal and magnetized seed were in sub plots. Results showed that types of irrigation and levels of water deficit affected proline at $p \leq 0.05$ and 0.01, respectively but types of irrigation affected abscisic acid and cytokinin at $p \leq 0.01$. Water deficiency had significant effect at $p \leq 0.05$ on abscisic acid. Irrigation and water deficit affected auxin and gibberellin significantly at $p \leq 0.01$. Water deficit treatments (at 60 and 75%) increased proline and abscisic acid content. Water deficit at 75% of available soil moisture depletion, increased proline by 25.18% and abscisic acid by 10.31% but reduced cytokinin by 6.37%, auxin by 24.32% and gibberellin by12.42% compared with control (40% of available soil moisture depletion). Magnetized water also increased all measured traits on average 17.45% but seed (normal and magnetized) could only affect auxin significantly at $p \leq 0.05$.

Key words: Abscisic acid · Auxin · Cytokinin · Gibberellin · Proline

INTRODUCTION

Sunflower is one of the most important oil crops under cultivation all over the world. This crop is susceptible to water deficiency at bud, flowering and grain filling stages and its yield will decrease in drought stress conditions [1, 2]. When plants face lack of water, they show different morphological and physiological reactions. Some plants accomplish their life cycle before dry period to avoid it but some others develop their root system, diminish their shoot growth, leaf area, number of stoma and increase the number of epidermal hair to tolerate the dry period [3]. Some molecular mechanisms are also responsible for drought tolerance such as synthesis of some polypeptides [4, 5].

Magnetic field is an unavoidable environmental factor that affects all living organisms on the earth. It's about hundreds of years that human has understood the essence of magnet and electricity and used this energy for different purposes. It is one of the factors that our today civilization is based on it and in recent decades researchers have tried to investigate its biological influences but it is not well understood yet. Most of these experiments have focused on bacteria and animal cells and have resulted that magnetic fields affect a wide range of cellular functions. Only few experiments have tested how magnetic fields affect plant cells [6, 7].

Direct magnetic fields are sometimes considered as stress to living organisms [8]. Magnetic fields can affect cell metabolisms in the same way as heat stress does and these fields can play the role of a protective mechanism to heat stress. Some studies have also tested the effects of interaction of magnetic fields and other environmental factors such as nutritional stresses, water deficiency, heavy metals etc. [9]. Generally, reports show that stimulation or inhibition of growth by magnetic fields, depends on species and physiological characteristics of the plants [10]. Changing pulses of magnetic fields can

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effectively stimulate cell development if applied correctly [11]. Other researchers represented that H_2O molecules can receive and absorb the energy of magnetic fields [12].

Osmotic adjustment is one of the most usual responses of plants to environmental stresses, specially osmotic variations of environment (as a result of drought or salinity stress). Osmotic adjustment refers to reduction of osmotic potential in cells due to accumulation of soluble substances under drought or salinity stress and is the main mechanism used by plants to avoid water stress in dry or saline conditions [13]. In this physiological mechanism, plant cells concentrate some ions in their vacuoles and some metabolites such as amino acids (mainly proline), monosaccharides, etc., in their cytozole. This will decrease osmotic potential and keep cell turgor pressure at high level to allow plants continue their physiological processes [13].

Proline accumulation is an important metabolic reaction that happens when cells dehydrate as a result of water deficiency or reduction of osmotic pressure. This process takes place not only in plants, but also in many other organisms. In plants encountered with conditions like drought, salinity, cold temperature and other factors that reduce cell water potential, proline content increase [14].

Abscisic acid (ABA) is a plant growth inhibitor that has different physiological roles such as induction of bud or seed dormancy, detachment of seeds and fruits, senescence and resistance to stresses in plants. Researches proved that resistance of tomato, potato, wheat, spinach and arabidopsis to drought stress is as a function of increase in their content of ABA [4, 5]. Alscher and Cumming, (1990) represented that concentration of ABA in some plants (alfalfa, tomato, cotton, rice, soybean and barley) would increase under drought stress and decrease when irrigated again [15].

Auxin in combination with cytokinin stimulates cell division [16]. Although reaction of auxin and cytokinin to drought stress is not well defined yet, but some researches represent that auxin [17] and gibberellin [18] levels in plants will decline under drought stress. Water stress can also make lower cytokinin level in xylem exudates and detach leaves [19]. Abscisic acid and cytokinin are known to act in apposite ways, as abscisic acid stimulates stoma to get closed and accelerates cells senescence, but cytokinin increases plants transpiration and delay senescence [20]. Finally, the objective of this study was to determine if magnetic energy has any effect on sunflowers hormones under water deficiency stress.

MATERIALS AND METHODS

This field experiment was conducted on 2009 at the research farm of Islamic Azad University, Karaj Branch, Iran, located in 51°6′ E, 35°45′ N, elev. 1313. The field was in arid to semiarid climate and soil type of the test site was sandy loam with pH of 7.8.

Two separate experiments were conducted in split plot arrangement in the form of randomized complete block design with four replications. In this experiment, three factors were studied:

Irrigation Water: Irrigation has been done at two levels, with normal water (W_1) and magnetized water (W_2). One of the two fields was irrigated with normal water and the other field was irrigated with magnetized water by hydrofix gated pipes, attached to a magnetic device with the power of at least 7000 gauss (1.5 Tesla).

Water Deficiency: As main factor in both fields, at 40% (control, T_1), 60% (T_2) and 75% (T_3) of available soil moisture depletion. In order to measure the percent of available soil moisture depletion, soil moisture blocks (chalk blocks) were installed in all plots, 30 cm below soil surface and connected to soil moisture meter by the means of fully isolated wires. Water deficit was started at sunflower R4 stage (the stage that inflorescence begins to open and if viewed from above, immature ray flowers are visible. This approximately happens at 1492 GDD units from planting [21]).

Seed: Normal (S_1) and magnetized (S_2), as sub factor in both fields. All seeds were first treated with Vitavax and then, those which needed to be magnetized were passed through a magnetic funnel.

On spring 2009, fields were prepared in conventional method (moldboard plow, 2 disks and leveler). According to the result of soil sample analysis, phosphorus (100 kgP/ha as triple-super phosphate, before planting), potassium (150 kgK/ha as potassium sulfate, before planting) and nitrogen (350 kgN/ha as urea, 2 parts before planting and 1 part at 8 leaves stage) were used.

In this experiment, planting density was 80000 plants per hectare. Each sub plot included six rows (6 m long and 60 cm spacing) and sunflower (*Helianthus annuus* L. Azargol hybrid) was planted with 20 cm of spacing on May. 13th, 2009, in both fields. Fields were also weeded by hand continuously until sunflower canopy dominated weeds.

In order to study sunflower phytohormones and proline amino acid, one plant was harvested together with root at R5 (beginning of flowering) and R6 (completion of flowering) growth stages. To determine auxin, gibberellin and cytokinin concentration, a Unicam model HPLC was used for extraction in Isocratic method. Auxin and cytokinin were extracted in a C18-HiqSil column (5 µm × 4.6 mm × 25 cm) and gibberellin was extracted in a Zarbax SB-S18 column (3.5 µm × 15 cm) and standard solutions were prepared for all hormones as 1g/Li in 20% methanol. Formic acid (1%) was added to these solutions and samples were kept at 4°C.

For extraction of gibberellin, 1 g of plant leaf was placed in a solution containing methanol - water - acetic acid (30-70-1) and homogenized by the means of a homogenizer. The solution was then centrifuged at 3000 round for 15 minutes. At last, upper solution was injected in C18-SPE column and eluted at 10 ml solution of ethanol - water - acetic acid (80-20-1). The extracted solution was dried in room temperature using a refrigerant and 1 ml of methanol was added again to make it the final solution for hormones extraction. For auxin and cytokinin extraction, 1.5 g of plant tissue was passed through 80% methanol. The extracted solution was dried at room temperature by refrigerant and 1 ml of 20% methanol, formic acid (1%) and 1 ml of 80% methanol were added. This final solution was used to measure hormones content.

Abscisic acid (ABA) was quantified according to Zhou *et al*., (2003) method [22]. To do this, LC/MS HPLC column (3.5 µm × 1.2 mm × 50 mm - Sunfire, Waters USA) was used.

To measure proline amino acid, Bates (1973) method was used. Samples unit is micromole in gram of fresh leaf. Standard curve was prepared to measure proline content and 1% pure proline was used to provide 1 - 160 µmol concentrations.

Finally, as each type of irrigation water treatment (normal and magnetized) was applied in one of the two fields, data were analyzed using combined analysis. Data were analyzed by SAS (ver. 9.1) [23] and means were compared by Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Proline: Analysis of variances showed that irrigation and water deficit levels have significantly affected proline amino acid at $p \leq 0.05$ and $p \leq 0.01$, respectively (Table 1). Means comparison (Table 2) also showed that irrigation with magnetic water (W_2) has produced 29.80% more proline than normal water (W_1). Magnetic water has probably improved osmotic adjustment by the production of more proline in order to lessen the effect of water deficiency on crop. Soltani *et al.* [12] concluded that magnetic field change ions concentration and consequently osmotic pressure to regulate water entrance to seeds.

Among three water deficit levels, T_2 and T_3 (moderate and sever deficits) produced 31.68% and 25.18%, respectively, more proline compared with T_1 (control). Garcia *et al.* [24] represented that although free proline content was raised in sunflower as the result of water deficit, but pure proline was not.

Table 1: Analysis of variances of measured traits

		Mean Squares (MS)				
SOV	df	Proline	Abscisic acid	Cytokinin	Auxin	Gibberellin
W	1	168.71 *	9130.08 **	2120.02 **	6888.02 **	14981.33 **
Error (W)	6	13.41	49.30	20.60	211.60	211.50
T	2	65.89 **	1110.14 *	276.81 ^{ns}	17787.64 **	18422.14 **
W×T	2	208.89 ^{ns}	5816.27 ^{ns}	1039.14 ^{ns}	53716.27 ^{ns}	77278.39 ^{ns}
Rep×T(W)	12	110.49	1518.93	1199.39	28458.79	40309.10
S	1	3.74 ^{ns}	36.75 ^{ns}	46.02 ^{ns}	2625.52 *	6210.75 ^{ns}
W×S	1	2.21 ^{ns}	270.75 ^{ns}	25.52 ^{ns}	50.02 ^{ns}	168.75 ^{ns}
T×S	2	16.05 ^{ns}	663.81 *	34.52 ^{ns}	346.64 ^{ns}	3380.68 ^{ns}
W×T×S	2	6.66 ^{ns}	33.93 ^{ns}	83.52 ^{ns}	636.02 ^{ns}	1621.18 ^{ns}
Error	18	6.63	188.16	100.82	319.03	1613.76
CV (%)	-	17.81	10.65	10.46	7.41	8.85

Abbreviations: SOV, sources of variation; DF, degree of freedom; REP, replication; W, irrigation water; T, water deficit; S, seed; NS, nonsignificant; **, significant at $p \leq 1\%$; *, significant at $p \leq 5\%$.

Table 2: Mean comparison of main effects of irrigation water (W_1, normal; W_2, magnetized), water deficit (T_1, 40%; T_2, 60; T_3, 75%) and seed (S_1, normal; S_2, magnetized) on measured traits

Treatments	Proline µmol/g.fw	Abscisic acid nmol/g.dw	Cytokinin nmol/g.dw	Auxin nmol/g.dw	Gibberellin nmol/g.dw
W_1	12.58 b	114.91 b	89.29 b	228.91 b	435.91 b
W_2	16.33 a	142.50 a	102.58 a	252.87 a	471.25 a
T_1	12.15 b	119.31 b	100.62 a	279.25 a	492.50 a
T_2	16.00 a	135.18 a	92.68 b	218.81 b	430.19 b
T_3	15.21 a	131.62 a	94.50 ab	224.62 b	438.06 b
S_1	14.73 a	129.58 a	96.91 a	248.29 a	464.96 a
S_2	14.17 a	127.83 a	94.95 a	233.50 b	442.21 a

Means in a column followed by the same letter are not significantly different at $P \leq 0.05$.

They also represented that proline content differ in different genotypes. Effects of proline content on plants resistance to drought stress was tested by Singh *et al.* [25]. They studied some barley genotypes under drought stress and found out that varieties with higher levels of proline can tolerate dry period better and when the period is over, grow faster than other varieties. So, proline content can be considered as screening test for resistance to drought in cereals breeding programs. Other experiments have also proved that increase in plants proline content help them survive under drought stress and recover better when dry period is finished [26, 27].

Magnetized seed had no significant effect on proline and was similar to normal seed.

Abscisic Acid (ABA): ABAs response to irrigation water and water deficit treatment was significant at $p \leq 0.05$ and $p \leq 0.01$ respectively. Means comparison showed that ABA accumulation in leaves has been 24.01% more in magnetized water than in normal water (Table 2). According to table 2 it can be concluded that sever water deficit has raised ABA content in leaves (T_2: 13.30% and T_3: 10.31% compared with T_1). It's well defined that lack of water in rhizosphere and reduction of turgor pressure in root cells will stimulate ABA synthesis in root and increase ABA transition to shoot. Alscher and Cumming, [15] expressed that chilling, salinity, heat and mechanical stresses will also enhance ABA synthesis in plants. In an experiment, ABA accumulation was measured soon after plant was faced drought stress. Results showed that water deficit will increase ABA synthesis and the hormone concentration is affected by duration and intensity of water deficit and also temperature [28].

Auxin (IAA): Analysis of variances showed significant effects of irrigation and water deficit at $p \leq 0.01$ on IAA but seed treatments (normal and magnetized) affected IAA only at $p \leq 0.05$ (Table 1). Mean comparison of irrigation showed that in magnetized water, plant has produced 10.46% more IAA than normal water (Table 2). In another experiment, stimulatory effect of magnetized water on growth indices was reported [29]. In this experiment, researchers represented that this can be as a result of increase in photosynthetic pigments, internal promoters of IAA or biosynthesis of proteins. They also represented that production of new proteins in plants treated with magnetized water, is linked with increase in IAA content. Again in this experiment, magnetized water boosted yield and yield components of chickpea (*Cicer arietinum* L.). These results are the reasons why magnetized water improves plants growth, IAA and photosynthetic pigments.

Mean comparison (Table 1) of water deficit levels shows that 60% and 75% deficit levels has produced lower auxin than control (T1, 40% of available soil moisture depletion). The maximum auxin is in T_1 and the minimum is in T_2 (27.62% lower than T_1). Yang *et al.* [30] also reported that auxin content will reduce in middle or late grain filling stages under water deficit condition. In seed treatments, maximum auxin was in normal (S_1) and minimum auxin, in magnetized (S_2) seed.

Gibberellin (GA): ANOVA showed significant effect of irrigation and water deficit on leaf gibberellin content at $p \leq 0.01$ (Table 1). Mean comparison also showed that in irrigation with magnetized water, plant has produced 8.10% more gibberellin than normal water (Table 2). Among three water deficit levels, the highest gibberellin content was observed in T_1 (control, 40% of available soil moisture depletion) and the lowest content was observed in T_2 (14.48% lower than T_1) and T_3 (12.42% lower than T_1) with no significant difference. Little is known about the effects of drought stress on gibberellins [31]. It is expected that during a period of slow growth, levels of

growth promoters, such as GA, decrease. Although this happened in wilted detached lettuce leaves [18], but did not happen in droughted intact sunflowers. Yang *et al.* [30] observed that although water stress treatments increase ABA, but they cause reduction of GA. Totally, it can be concluded that effects of drought stress on gibberellin is complicated [32].

Cytokinin: According to ANOVA, irrigation significantly affected cytokinin content at $p \leq 0.01$ (Table 1). Mean comparison (Table 2) shows that cytokinin level in irrigation with magnetized water is 14.88% more than normal water. Atak *et al.* [33] demonstrated that magnetic fields stimulate cytokinin synthesis and it increases production of all photosynthetic pigments. Moreover, it is reported that magnetic fields can differ some factors in water that affect water efficiency in plants cells. This process has some influences on plants growth at cell level. On the other hand, magnetized water can make some changes in phytohormones production that improves cells and plants growth [34].

Although analysis of variances showed no significant effect of water deficit levels on cytokinin, mean comparison put them in significantly different groups. Water deficit at 40% of available soil moisture depletion (T_1, control) produced the highest cytokinin level and water deficit at 60% (T_2), produced the least cytokinin content (8.56% lower than T_1). When sunflower encounters water deficit, transition of cytokinin to shoot would probably reduce because more cytokinin would be stored in roots [35]. Stress will also diminish cytokinin production in root and prevent it to be transited to shoot [36]. In another experiment, cytokinin level in stem was reduced 53% under water deficit condition [35].

CONCLUSION

Overall results of this experiment show that irrigation with magnetized water significantly increased all measured phytohormones in sunflower (on average, 17.45%). Increase in levels of gibberellin, auxin and zeatin (one of cytokinins) in sunflower affected by magnetic field is reported in other experiments [37]. Although water deficit levels increased proline and abscisic acid production, but they reduced content of cytokinin, auxin and gibberellin. Effect of water stress at 60% of available soil moisture depletion (T2) was higher than water deficit at 75% (T3) both in the traits that water deficit raised them or reduced them. Normal or magnetized water generally had no significant effect on measured traits.

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