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**Annals of Biological Research, 2012, 3 (9):4282-4286**
*(http://scholarsresearchlibrary.com/archive.html)*

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**ISSN 0976-1233**
**CODEN (USA): ABRNBW**

# The effects of Time Spraying Amino Acid on water deficit Stress on yield, yield component and some physiological characteristics of grain corn (TWC647).

**P. Kasraie[1] , M. Nasri[1] ,M. Khalatbari[2*]**

*[1]Department of Agronomy, Varamin- Pishva Branch, Islamic Azad University, Varamin, Iran.*
*[2]Department of Agronomy, Share-Ghods Branch, Islamic Azad University, Share-Ghods, Iran.*

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**ABSTRACT**

*This study investigated the effects of time spraying Amino Acid on the yield and yield components and some physiological traits in grain corn (Zea mays L. var. TWC647) under water deficit. Research was conducted with complete randomized block experimental design with split-plot arrangement with three replications. In this experiment, the main plots consisted of water deficit with 3 levels :( Cut Irrigation in Vegetative (A1), Cut Irrigation in flowering (A2), Cut Irrigation in grain filling (A3). Subplots were time spraying Amino Acid in 3 levels: (Control )Non Amino Acid (B1), Amino Acid spraying before water deficit stress (B2), and Amino Acid spraying after water deficit stress (B3). Results of analysis of variance showed that the interaction effects of water deficit and time spraying Amino Acid on the characteristics of Number of rows per spike, Number of grains per row, 1000grains weight, grain yield, biological yield, harvest index, protein yield, protein percentage and proline were significant at the 5% level, However, there was not significant the plants per square meter and number of spikes per plant of water stressed plants treated with time of Amino Acid foliar Application. In this study, the maximum grain yield (7406.1kg/ha$^{-1}$) and the plants per square meter, number of spikes per plant, number of rows per Spike, number of grains per row, 1000grains weigh, Proline , Harvest Index were obtained from Cut Irrigation in vegetative with Amino Acid foliar before water deficit stress. Seed protein percentage increased as the amount of water deficiency. lowest grain yield was assigned of Cut Irrigation in flowering with Control (none Amino Acid) with (2258.6 kg/ha$^{-1}$) had no significant difference with Cut Irrigation in grain filling with control treatment and the lowest 1000grains weight (183.4 gr) allocated to the same treatment.*

**Key words**: Corn, water deficit Stress, Time Spraying Amino Acid, yield, yield component.

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## INTRODUCTION

Water availability is a major limiting factor for plant growth and it is the main factor responsible for reductions in corn (*Zea mays L.*) production in the Iran. Drought affects nearly all the plant growth processes; however, the stress response depends upon the intensity, rate, and duration of exposure and the stage of crop growth [20]. Inhibition of leaf growth by water stress can be considered to be an adaptive response. Thus it limits leaf area production, eventually plants rate of transpiration [9]. Water stress in particular stages of corn phonology affects seed qualitative Properties such as oil and protein's percentage [18]. Determining crop yield response to irrigation is important for crop selection, economic analysis, and for practicing effective irrigation management strategies. If water is limited, it is important to know how to time irrigations to optimize yields, water use efficiency and, ultimately, profits [5]. Proline accumulation in plants exposed to water deficit is a well-known stress response [16]. The response results from a stimulation of proline biosynthesis as well as an inhibition of its utilization. Proline accumulation is usually accompanied by inhibition of growth [14]. Irigoyen *et al* in 2006 who reported that water stress reduced soluble protein content in both tissues; however, the decline in soluble protein content was detected at greater $\Psi w$ in nodules than in leaves. Proline and TSS increased in leaves and nodules [7]. Udomprasert *et al*, (2010) indicated that Leaf samples were collected at wilting and at recovery for proline and ABA analyses using spectrophotometric technique and gas chromatography, respectively, compared to control plants. It was found that water stress at both tassel initiation and anthesis caused an increase in proline and ABA levels in both corn varieties [21]. Water stress can affect growth, development, and physiological processes of corn plants, which can reduce biomass and, ultimately, grain yield due to a reduction in the number of kernel per ear or the kernel weight [19]. The findings are in consonance with dlaasseg *etal* in 2007 who reported that a significant grain yield reduction (10 to 17%) was observed after stress during the vegetative period at early ear shoot and ovule development in 2005. A 58% grain yield reduction was associated with stress at 75% silking in 2006[4]. In the 3-week period after silking, water deficits consistently reduced yields approximately 30% in both years. Significant reductions in kernel numbers were associated with yield reductions from stress before or during silking and pollination. Kernel weights were significantly reduced by stress during or after silking. Trends in the percentage of developed kernels in each of three ear sections indicated that the ability of kernels to compete for products of photosynthesis correlated with the comparative age of the ovule-or kernel at the time of water deficit. Several studies have shown significant effect of stress timing on corn yield [8, 10].Payero *et al* in 2009 by evaluating the morphological and physiological responses to water stress showed that Irrigation timing affected the DM of the plant, grain, and cob, but not that of the Stover. It also affected the percent of DM partitioned to the grain (harvest index), which increased linearly with ETC and averaged 56.2% over the two seasons, but did not affect the percent allocated to the cob or Stover [13].Fattahi neisiani *et al* in 2009 which reported that protein content decreased but proline and malondialdehyde content increased under water stress [6]. In this investigation, available strategies for an improved tolerance to water deficit are discussed.

## MATERIALS AND METHODS

Field experiments were conducted in 2011 at the University Varamin- Pishva, NE (35.19N 51.39E, 898 m above sea level). This study investigated the effects of spraying time at Amino Acid on the yield and yield components and some physiological traits in grain Corn (TWC647) under water deficit stress.Research was conducted with complete randomized block experimental design with split-plot arrangement with three replications. In this experiment, the main plots consisted of water deficit with 4 levels :( Cut Irrigation in Vegetative (A1), Cut Irrigation in flowering (A2), and Cut Irrigation in grain filling (A3). Subplots were spraying proline time in 3 levels: - (Control ) Non Amino Acid (B1), Amino Acid spraying before water deficit stress(B2) , Amino Acid spraying after water deficit stress(B3). The climate at Varamin is arid and semi arid, with average annual precipitation and reference evapotranspiration of approximately 170 and 200 mm, respectively. On average, about 56% of the annual precipitation occurs during the growing season, which extends from October to April. The experimental soil consisted of 22% clay, 31% silt and 46% sand.

Initially, Plant nutrient feed of phosphorus was added by applying 110 Kg/ha triple super phosphate after cultivation. Nitrogen fertilizer was added in three periods; application of 33% N at cultivation time, application of 33% N fertilizer at stem elongation stage and application of 33% N fertilizer in beginning of flowering stage. A subplot size of 4.5 × 5 m, having six rows five meter long each was used. Uniformity of sowing depth was achieved by using a hand dibbler to make holes of 3-5 cm deep. The space between rows was 75 cm wide. All the experimental units were irrigated after planting. Before harvesting, yield components such as the Plants per square meter, Number of spikes per plant, Number of rows per spike and Number of grains per row, of 10 spikes were selected randomly from each plot at maturity, and then recorded. Grain yield was calculated in each split-plot after grain moisture reached 14% and the weight of each grain was determined after counting and finally the harvest index was calculated by ratio of grain yield to total above ground biomass. Within each plot, an area of 6 m2 was hand harvested to determine grain yield and total above ground biomass.Seed protein percentage was determined by Bradford method in 1976, using bovine serum albumin (BSA) as a standard [2]. Protein yields were calculated by multiplying grain yield to protein percentage of seed.

**Proline assay:** Leaf Samples (0.2 g) were homogenized in a mortar and pestle with 3 ml sulphosalicylic acid (3% w/v), and then the homogenate was centrifuged at 18,000 g for 15 min. Two milliliters of the supernatant were then put into a test tube into which 2 ml of glacial acetic acid and 2 ml of freshly prepared acid ninhydrin solution (1.25 g ninhydrin dissolved in 30 ml glacial acetic acid and 20 ml 6 M orthophosphoric acid) were added. Tubes were incubated in a water bath for 1 h at 100°C, and then allowed to cool to room temperature. Four milliliters of toluene were added and mixed on a vortex mixer for 20 seconds. The test tubes were allowed to stand for at least 10 min to allow the separation of the toluene and aqueous phases. The toluene phase was carefully pipetted out into a glass test tube, and its absorbance was measured at 520 nm by spectrophotometer [GBC, Cintra 6, and Australia]. The concentration of proline was calculated from a proline standard curve and was expressed as mmol per gram of fresh weight [1].

The data were subjected to analysis of variance using MSTAT-C computer software. Duncan's multiple range tests ($p < 0.05$) was applied for mean separation when F values were.

## RESULTS

Results of analysis of variance indicated that the interaction effects of water deficit and time of Amino Acid foliar Application on the characteristics of Number of rows per spike, Number of grains per row, 1000grains weight, grain yield, biological yield, harvest index, protein yield, protein percentage and proline were significant at the 5% level, However, there was not significant the plants per square meter and number of spikes per plant of water stressed plants treated with time of Amino Acid foliar Application (Table 1).

**Table 1: Mean squares of some agronomic traits**

| | | M.S | | | | |
|---|---|---|---|---|---|---|
| S.O.V | df | Plants per square meter | Number of spikes per plant | Number of rows per spike | Number of grains per row | 1000 grains weight |
| Block | 2 | 0.085 ns | 0.029 ns | 5.01 ns | 17.42 ns | 13.42ns |
| Irrigation (A) | 2 | 0.388 ns | 0.118 ns | 23.09 * | 110.09* | 204.45 ** |
| Error(a) | 4 | 0.124 | 0.045 | 4.85 | 13.87 | 9.25 |
| Amino Acid(B) | 2 | 0.21 ns | 0.030 ns | 5.92 ns | 73.4* | 439.92** |
| Ir* Amino Acid (A×B) | 4 | 2.14 ns | 0.025 ns | 16.25 * | 385.92** | 508.08** |
| Error(b) | 12 | 0.66 | 0.011 | 2.05 | 10.99 | 11.42 |
| C.V | | 6.8 | 7.21 | 14.85 | 10.11 | 6.25 |

**, ** means significant in 0.05 and 0.01 level of probability respectively and NS: non-significant.*

Data of interactive effect between that water deficit and time of Amino Acid foliar Application has been demonstrated in table 3, 4.

**Table 2. Mean squares of some agronomic traits and seed qualitative parameters.**

| | | *M.S* | | | | | |
|---|---|---|---|---|---|---|---|
| S.O.V | df | Harvest Index | Grain yield | Total above ground biomass | Proline | Seed protein percentage | Protein yield |
| Block | 2 | 28.33ns | 32452.1 ns | 125432.3 ns | 0.00025ns | 0.0654ns | 18452.01ns |
| Irrigation (A) | 2 | 209.4* | 499807.6 ** | 5104002.21** | 0.00090ns | 0.2041* | 62800.2* |
| Error(a) | 4 | 35.42 | 28453.21 | 699255.1 | 0.00038 | 0.0124 | 1053.2 |
| Amino Acid (B) | 2 | 249.35* | 899453.01 ** | 2025483.9** | 0.00242* | 0.1021ns | 290882.6** |
| Ir*Amino Acid (A×B) | 4 | 988.25** | 1025472.11** | 3222874.3** | 0.01999** | 0.21102* | 425201.3** |
| Error(b) | 12 | 22.85 | 19966.8 | 470259.1 | 0.00024 | 0.0333 | 9990.6 |
| C.V | | 12.21 | 16.18 | 17.45 | 4.45 | 3.32 | 12.1 |

**, ** means significant in 0.05 and 0.01 level of probability respectively and NS: non-significant.*

**Table 3. Means of some agronomic traits**

| Treatment | Plants per square meter | Number of spikes per plant | Number of rows per spike | Number of grains per row | 1000grains Weight (gr) |
|---|---|---|---|---|---|
| Cut Ir in Vegetative* Control (A1B1) | 6.1 a | 1.2 a | 13.7 bc | 15.7 b | 260.1b |
| Cut Ir in Vegetative* Amino Acid foliar before water deficit stress (A1B2) | 6.2 a | 1.6 a | 14.8 a | 19.4 a | 297.8 a |
| Cut Ir in Vegetative* Amino Acid foliar after water deficit stress (A1B3) | 6.1 a | 1.3 a | 14.1 abc | 16.9 b | 263.6 b |
| Cut Ir in flowering*Control (A2B1) | 5.9 a | 1.1 a | 12.6 c | 11.8 d | 197.3 d |
| Cut Ir in flowering* Amino Acid foliar before water deficit stress (A2B2) | 6.3 a | 1.5 a | 13.1 bc | 16.9 b | 258.9 b |
| Cut Ir in flowering* Amino Acid foliar after water deficit stress(A2B3) | 6 a | 1.2 a | 12.7 c | 13.3 cd | 211.8 cd |
| Cut Ir in grain filling*Control (A3B1) | 6.1 a | 1.4 a | 13.9 abc | 10.4 d | 183.4 d |
| Cut Ir in grain filling * Amino Acid foliar before water deficit stress (A3B2) | 6 a | 1.8 a | 14.1 abc | 16.2 b | 223.1 c |
| Cut Ir in grain filling * Amino Acid foliar after water deficit stress (A3B3) | 6.1 a | 1.5 a | 13.1 bc | 13.8 c | 208.6 cd |

*Means with the same letter in each column have not statistically significant difference.*

**Table 4. Means of some agronomic traits**

| Treatment | Seed protein Percentage (%) | Protein Yield (kg ha-1) | Proline (µgr/gr) | Total above ground biomass (kg ha-1) | Grain yield (kg ha-1) | Harvest Index (%) |
|---|---|---|---|---|---|---|
| Cut Ir in Vegetative* Control (A1B1) | 8.68 b | 459.3 d | 0.817c | 9947.2 c | 3986.5 c | 40 ab |
| Cut Ir in Vegetative* Amino Acid foliar before water deficit stress (A1B2) | 8.47 b | 874.4 a | 0.986 a | 15565.6 a | 7406.1 a | 47.5 a |
| Cut Ir in Vegetative* Amino Acid foliar after water deficit stress (A1B3) | 8.56 b | 640.1 b | 0.911 b | 13857.6 b | 5486.7 b | 39.6 b |
| Cut Ir in flowering*Control (A2B1) | 10.18 a | 221.9 f | 0.868 bc | 10385.7 c | 2258.6 d | 21.7 d |
| Cut Ir in flowering* Amino Acid foliar before water deficit stress (A2B2) | 9.94 a | 532.13 c | 1.013 a | 16985.6 a | 5289.4 b | 31.1 c |
| Cut Ir in flowering* Amino Acid foliar after water deficit stress(A2B3) | 9.99 a | 298.3 e | 0.978 a | 14285.2 b | 2979.8 d | 20.8 d |
| Cut Ir in grain filling*Control (A3B1) | 10.21 a | 227.7 f | 0.874 bc | 109485.2 b | 2325.2 d | 21.2 d |
| Cut Ir in grain filling * Amino Acid foliar before water deficit stress (A3B2) | 9.85 a | 547.8 c | 1.012 a | 16568.6 a | 5396.2 b | 32.6 c |
| Cut Ir in grain filling * Amino Acid foliar after water deficit stress (A3B3) | 10.09 a | 322.8 e | 0.981 a | 14347.4 b | 3257.4 cd | 22.7 d |

*Means with the same letter in each column have not statistically significant difference.*

## DISCUSSION

The result of Interactions table showed ,the highest Number of rows per spike, Number of grains per row, 1000grains weight, grain yield, and harvest index, were obtained under Cut Irrigation in Vegetative with Amino Acid foliar before water deficit stress (A1B2)with average14.8(N.o), 19.17(N.o), 297.8 (gr),7406.1(kg/ha$^{-1}$), and 47.5(%) respectively. On the other hand, lowest grain yield was assigned of Cut Irrigation in flowering with Control (none Amino Acid) with (2258.6 kg/ha$^{-1}$) had no significant difference with Cut Irrigation in grain filling with control treatment and the lowest 1000grains weight (183.4 gr) allocated to the same treatment.The highest and lowest biological yield were achieved at Cut Irrigation in flowering with Amino Acid foliar before water deficit stress (16985.6 kg/ha$^{-1}$) and Cut Irrigation in Vegetative with Control treatments (9947.2 kg/ha$^{-1}$), respectively.Water stress at vegetative stage reduced protein content of seed. The result of table 4 showed the highest and lowest seed protein percentage achieved from Cut Irrigation in grain filling with Control (10.21%) stage and Cut Irrigation in Vegetative with Amino Acid foliar before water deficit stress (8.47%) treatment, respectively.In this study, the most Protein Yield was observed on water deficit stress at Vegetative stage with Amino Acid foliar before water deficit stress treatment (874.4 kg/ ha$^{-1}$).The proline was measured in leaf corn at Amino Acid foliar before and after water deficit stress. The proline was observed in Cut Ir in flowering with Amino Acid foliar before water deficit stress (1.013 µgr/gr) was highest that the Cut Ir in Vegetative with Control treatments (0.817 µgr/gr).In this investigation, available strategies for an improved tolerance to water deficit are discussed. These results indicate that irrigation at flowering and grain filling stages of maize are sensitive under water deficit stress. The Grain yield timing can have a considerable effect on physiological characteristic corn. Oktem in 2008 who reported that the relationships between fresh spike yields and the irrigation treatments were statistically significant ($P<0.01$) and yield decreased with deficit irrigation [12]. The grain yield, 1000grains weights and some qualities characteristics reduced under water stress at the flowering and grain filling stages. A significant grain yield reduction (to 37.6%) was observed after stress during the flowering period at early ear shoot and ovule development than cut Irrigation in vegetative. However, Seed protein Percentage increased. Changes in proteins results from a variety of environmental stresses such as water deficit stress reported by Yordanova *et al.*, (2004)[22]. Accumulation of proline was reported in many plant species under diverse a biotic stress conditions [3].The Number of grains per spike reduced at cut Irrigation flowering and grain filling stage, the reason seems to be shortage of assimilate; because the leaf surface is lower than cut Irrigation in vegetative treatment. Amino Acid foliar application before water deficit stress was caused the negative effects of stress can be reduced and grain yield was less declined than control and Amino Acid foliar after water deficit stress. Although Cut Irrigation in Vegetative with Amino Acid foliar before water deficit stress was highest grain yield but this adjective reduced Cut Irrigation in flowering and Cut Irrigation in grain filling stages, hardly. Accumulation of praline under water deficit stress showed the most correlation with lignin, it had high negative correlation with chlorophyll a too. Water stress at tassel initiation showed greater influence on proline and ABA levels and yield than that at an thesis. Moreover, it was found that proline and ABA levels accumulated under water stress conditions were negatively correlated with corn yield [21].Osborne *et al,* in 2002 also reported that biomass was reduced by moisture stress [11]. Stone *et al.* in 2001which stated that yield was related strongly to biomass especially that accumulated after silking. Biomass also was reduced by water deficit [17]. Rivera- Hernandez *et al.* in 2010 which suggested that although significant differences were observed among irrigation treatments for a variable number of rows per spike, this was the least affected by the rise in soil moisture tension [15].

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