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EFFECT OF EXTRA POTASSIUM SUPPLY ON AMINO ACID COMPOSITION OF CORN SEED UNDER THE DEFICIT IRRIGATION CONDITIONS: (SECTION C)

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Abstract

The study investigates the influence of extra potassium application and deficit irrigation on amino acid composition of corn seed. The field experiment was conducted with split plot design with three replications in Aydın location of Turkey in 2013. Parcels of the experiment was established with three different supplies as non-fertilization, standard fertilization [210 kg.ha^{-1} pure N, 60 kg.ha^{-1} pure P, 60 kg.ha^{-1} pure K, 60 kg.ha^{-1} (NH_4NO_3, P_2O_5 and K_2O with 15-15-15 composite before planting and H_2NCONH_2 - 150 kg.ha^{-1} with urea before first water)] and extra potassium (standard fertilization +60 kg.ha^{-1} K_2SO_4) and three irrigation doses (500 mm, 400 mm and 300 mm) during development stages [(8 leaf stage (V8), before Tasseling (VT), after blister, milk stage and dough stage)]. 31G98 corn variety, identified as having the highest yield in previous our paper published Scientific Papers Series A. Agronomy, was used. Amino acids rates (essential, conditionally essential and non-essential) of the corn seeds obtained from the different treatments parcels were measured with HPLC.

According to the results of the experiment, significant differences among treatments and irrigation doses were found. Almost all essential amino acids were increased with standard fertilization and extra potassium supply compared to non-fertilization. Just methionine and threonine in this group were irregular with these treatments. Similarly, almost all essential amino acids values except for histidine, valine and phenylalanine were increased with 400 mm compared to 300 mm, whereas these groups were decreased with 500 mm irrigation dose. As for conditionally essential and non-essential amino acids, glutamic acid and serine were increased with standard fertilization while were decreased with extra potassium supply. Contrary to glutamic acid and serine, glycine was decreased with standard fertilization while was increased with extra potassium supply. The other amino acids in the group were increased with both standard fertilization and extra potassium supply compared to non-fertilization. In terms of irrigation doses, tyrosine and serine values were increased with raising irrigation doses. Proline, arginine, aspartic acid and glutamic acid were increased with 400 mm compared to 300 mm, whereas the amino acids were decreased with 500 mm irrigation dose.

Key words: *corn, amino acid, potassium supply, lysine, HPLC.*

INTRODUCTION

Proteins are one of the most necessary component of the body. They are abundant component after water and they play significant role in the whole cells of body both structural and functional. For example muscle cells make up over 40% of our body protein (Grimble et al., 1992). Moreover hemoglobin contains 16% (Vinton et al., 1986). So we can say that protein has an essential function in every cell including in membranes, transporters, enzymes, components of the immune system, and is also a precursor to hormones (Jerlich et al., 2000).

Proteins are macromolecules, which are constructed in the body from chains of amino acids. Using the 20 primary amino acids, the body constructs an overwhelming abundance of protein chains, each with a different order coded by our genetic code (Raiha et al., 1996). These chains interact and fold into specific three dimensional shapes to give specialized functionality. Throughout human life, there is some speedy growth periods especially in infancy, childhood and teenage (Lourenço, Camilo, 2002), so there are increases in length, mass, development and maturation of function (Vinton et al., 1986). Similarly, some other periods like pregnant or lactating, there are also the demands for net tissue deposit or milk formation. In each of these periods, the needs are for a pattern of amino acids that matches the material being deposited, including extracellular proteins, DNA, RNA, cell membranes etc. (Jerlich et al., 2000). There is good evidence that the pattern of amino acids that is needed to meet these demands is different from that in the basal state. There are some studies about different practices like planting date, genotype selection, reduced tillage, and diversification of crop, organic matter and mineral application to increase defense against drought (Carr, 2010; Allen, 2012). Potassium application is the one of treatments for maximum seed yield and quality against the effects of drought. Because potassium plays a vital role in control of ionic balance and regulation of stomas for water use (Rafat et al., 2012). Besides potassium is a primary regulator for osmotic potential while maintaining low water potential of crop. Therefore, accumulating K^+ in their tissues may play an important role for crops under drought condition (Zare et al., 2014). The study were investigated to influence of standard (60 $kg.ha^{-1}$ K_2SO_4) and extra potassium fertilizer (double potassium rate) on amino acids composition under deficit irrigation conditions.

MATERIALS AND METHODS

The research was carried out in Aydin with typical Mediterranean climate (hot summer and mild winter), located in west Turkey at 37º 44' N 27º 44' E at 65 m above sea level; and was conducted during 2013. Initial result of soil analysis is shown in Table1 (Bouyoucos G.J., 1962; Ayers, Westcot, 1989; Walkley, Black, 1934).

Table 1. Soil texture and chemical analysis

Soil texture[1] (%)			pH[2]	Organic mater[3]
Sand	Silt	Clay		(%)
72.0	16.7	11.3	8.4	1.2

Method of: [1]Bouyoucos; [2]1: 2.5 Saturasyon; [3]Walkley-Black.

The experimental soil in studied field contains with sandy loamy structure with alkaline characteristic and it mixed with quite low organic matters.

Monthly temperature, total rainfall and long term (1975-2013) values in Aydin shown in Table 2. The temperature of 2013 was higher than long term means expect for June and July. Rainfall data analysed that May, July and August were showed lower value than long term means.

Table 2. Monthly temperature and total rainfall during corn growth period and long term mean (1975-2013) in Aydin

Months	Temperature (°C)		Precipitation (mm)	
	2013	Long term	2013	Long term
April	16.1	15.7	42.6	45.5
May	23.2	20.9	1.0	33.5
June	25.3	25.9	18.4	14.0
July	27.9	28.4	2.4	3.5
August	27.8	27.2	0.0	2.2
September	22.6	23.2	22.8	14.4

Experimental design

The experiment was set up as split block experimental design with 3 replications. All parcels were sowed April 26, 2013 and the first seed emergence observation was conducted on May 13, 2013. Each plot area was 28 m^2 (5 m x 5.6 m) and consisted of 8 rows. Distance between rows was 70 cm and intra row spaces were 18 cm. P31G98 which is hybrid (F1) and has single cross corn (*Zea mays* L.) cultivar was used as the crop material. The variety produced by Pioneer Turkey Seed Distribution and Marketing Co. Ltd was identified as having the highest yield and high thousand seed weight in previous our paper published Scientific Papers, Series A, Agronomy. Treatment factors were created out with non-fertilizer, standard fertilizer and extra potassium application. There was not been any applications fertilizers form to non-fertilizer parcels. Standard fertilizer application from soil was applied as 210 $kg.ha^{-1}$ pure nitrogen (NH_4NO_3) (60 $kg.ha^{-1}$ with 15-15-15 composite was applied immediately at the beginning of cultivation - 150 $kg.ha^{-1}$ with urea (H_2NCONH_2) before first water), 60 $kg.ha^{-1}$ phosphor (P_2O_5) and 60 $kg.ha^{-1}$ potassium (K_2O). Extra potassium application was formed to by being added to 60 $kg.ha^{-1}$ K_2SO_4 on to the standard fertilizer application. In other words, it has been said that extra potassium application had only double potassium rate compared to standard fertilization. So we hope that effects of double potassium application on amino acids composition of corn seed under deficit irrigation conditions.

Soil from the field experiment [(0-30 cm, 30-60 cm and 60-90 cm depth (Rd)] was put into pots. The water content of the soil after being saturated by irrigation and allowed to drain is called field capacity (FC). Crop can no longer take up water from the soil is referred wilting point (WP). The water held by the soil between field capacity and the permanent wilting point is considered available water. Corn is capable of using 50% of the available water (AW). Irrigation water requirement (100 mm) was calculated with the following formulas (Martin, Gilley, 1993; Lamm et al., 1994).

$$AW = Rd\,(FC-WP)/100$$

Irrigation doses was formed as standard (5x100 mm) during 5 times (V8, before VT, after blister, milk stage and dough stage), as deficit irrigation (4x100 mm) during 4 times (V8, before VT, after blister and milk stage) and as more deficit irrigation (3x100 mm) during 3 times (V8, before VT and after blister) at corn growth period. All irrigation applied and time were given in Table 3.

Table 3. Irrigation times in corn growing season

Irrigation time	Irrigation rate per plot		
	300 mm	400 mm	500 mm
20th June 2013	**X**	**X**	**X**
04th July 2013	**X**	**X**	**X**
12th July 2013	**X**	**X**	**X**
25th July 2013	-	**X**	**X**
06th August 2013	-	-	**X**
10th September 2013	Harvest time		
X: Applied 100 mm water			

The analysis of amino acid composition of corn seed

On the basis of needs from the diet for nitrogen balance or growth, amino acids were traditionally classified as nutritionally essential or non-essential for humans and animals (Lupi et al., 2008). Essential amino acids are defined as amino acids whose carbon skeletons cannot be synthesized by the body relative to needs (El Idrissi, 2008). Conditionally essential amino acids normally can be synthesized in adequate amounts by the organism, but must be provided from the diet to meet optimal needs under conditions where rates of utilization are greater than rates of synthesis. Non-essential amino acids can be synthesized in adequate amounts by the body to meet optimal requirements (Novelli, Tasker, 2008; Phang et al., 2008).

Sample Preparation

Grain samples from the experiment corn samples were milled and weighed 0.1 g. 5 ml 6 N HCl was added to the sample and respectively 250 µl 2 mM phenol and 0.1 g Na_2SO_3 was added. The sample was placed in an oven at 110°C 24 h and after the time pH level of the sample was adjusted to 6.7-7.3 with 5 N NaOH. The supernatant was centrifuged with 4000 rpm in 5 minutes and then filtered through a filter and fitted to vials.

HPLC Determination

The whole amino acid content of the samples was performed with HPLC system, consisting of a Shimadzu Nexara XR (Shimadzu Corporation, Kyoto, Japan) with Zorbax Eclipse AAA (15 cm x 4.6 mm x 3.5 µm) coloumn. The mobile phase A which consisted of 40 mmol $NaH_2PO_4.2H_2O$ adjusted to pH 7.8 with 5 N NaOH. The mobile phase B was composed of acetonitrile-methanol-water (45: 45: 10, vol. %). Briefly the hydrolyzed samples and solutions the standard amino acid mixture was automatically derivative with OPA and FMOC. Coloumn temperature was 40°C and injection volume was 9 µl. The amino acids were achieved by calibrating with a standard mixture of amino acids. Peak integration accuracy was enhanced by manual establishment of peak baselines using software.

Statistical analysis

The amino acids data collected from the experiment were subjected to analysis of variance (ANOVA) using TOTEM STAT statistical software (Acikgoz et al., 2004). Means among treatments were compared using Least Significant Difference (LSD) at $P \leq 0.05$ probability (Stell et al., 1997).

Table 4. Change of essential amino acids with potassium treatment on deficit irrigation conditions

Treatment (A)	Irrigation Doses (B)	HIS	VAL	MET	THR	ILE	LEU	LYS	PHE
Non-fertilization	300	0,218	0,294	0,198	0,234	0,203	0,606	0,179	0,303
	400	0,218	0,281	0,191	0,213	0,207	0,616	0,183	0,307
	500	0,242	0,305	0,178	0,234	0,210	0,641	0,197	0,316
	average	0,226	0,293	0,189	0,227	0,207	0,621	0,186	0,309
Standard fertilization	300	0,246	0,333	0,176	0,242	0,244	0,782	0,205	0,374
	400	0,245	0,314	0,152	0,282	0,253	0,769	0,236	0,347
	500	0,251	0,307	0,183	0,239	0,225	0,776	0,175	0,354
	average	0,247	0,318	0,170	0,254	0,241	0,776	0,205	0,358
Extra Potassium supply	300	0,243	0,340	0,178	0,243	0,247	0,774	0,224	0,366
	400	0,277	0,374	0,224	0,278	0,267	0,839	0,234	0,385
	500	0,246	0,367	0,229	0,230	0,290	0,766	0,198	0,368
	average	0,255	0,360	0,210	0,250	0,268	0,793	0,219	0,373
Average of Irrigation Doses	300	0,236	0,322	0,184	0,240	0,231	0,721	0,203	0,348
	400	0,247	0,323	0,189	0,258	0,242	0,741	0,218	0,346
	500	0,246	0,326	0,197	0,234	0,242	0,728	0,190	0,346
LSD A*B (0,05)		0,009	0,023	0,011	0,015	0,013	0,047	0,018	0,021
HIS: Histidine, VAL: Valine, MET: Methionine, THR: Threonine, ILE: Isoleucine, LEU: Leucine, LYS: Lysine, PHE: Phenylalanine									

Table 5. Change of conditionally essential and dispensable amino acids with potassium treatment on deficit irrigation conditions

Treatment (A)	Irrigation Doses (B)	GLY	PRO	ARG	TYR	CYS	ASP	GLU	SER
Non-fertilization	300	0,290	0,558	0,360	0,253	0,359	0,416	1,056	0,327
	400	0,283	0,548	0,369	0,258	0,282	0,454	1,068	0,378
	500	0,306	0,592	0,394	0,257	0,315	0,484	1,146	0,377
	average	0,293	0,566	0,374	0,256	0,319	0,451	1,090	0,361
Standard fertilization	300	0,314	0,623	0,410	0,300	0,342	0,538	1,384	0,439
	400	0,262	0,629	0,405	0,290	0,357	0,510	1,313	0,378
	500	0,289	0,597	0,378	0,291	0,257	0,477	1,297	0,430
	average	0,288	0,616	0,398	0,294	0,319	0,508	1,331	0,416
Extra Potassium supply	300	0,311	0,625	0,399	0,303	0,370	0,480	1,243	0,324
	400	0,353	0,680	0,459	0,314	0,419	0,594	1,451	0,455
	500	0,249	0,650	0,411	0,334	0,346	0,482	1,106	0,407
	average	0,304	0,652	0,423	0,317	0,378	0,519	1,267	0,395
Average of Irrigation Doses	300	0,305	0,602	0,390	0,285	0,357	0,478	1,228	0,363
	400	0,299	0,619	0,411	0,287	0,353	0,519	1,277	0,404
	500	0,281	0,613	0,394	0,294	0,306	0,481	1,183	0,405
LSD A*B (0,05)		0,025	ns	0,026	0,013	0,044	0,038	0,088	0,053
GLY: Glycine, PRO: Proline, ARG: Arginine, TYR: Tyrosine, CYS: Cysteine, ASP: Aspartic acid, GLU: Glutamic acid, SER: Serine									

RESULTS AND DISCUSSIONS

Least Square means of amino acid parameters was calculated through variance analysis. According to The results of variance analysis for treatment factors (different fertilizations) and water doses (deficit irrigations) and their interaction are presented in terms of essential, conditionally essential and dispensable amino acids. Irrigation dose x treatment factor interaction was found to be significant in all parameters except for proline. All essential, conditionally essential and dispensable amino acids values were tabulated in Table 4 and Table 5. Moreover LSD (0.05) values about Irrigation dose x treatment interaction were given under the tables.

Histidine, valine, methionine, threonine, isoleucine, leucine, lysine and phenylalanine values were shown as essential amino acids in Table 4. Almost all essential amino acid averages were increased with standard fertilization compared to non-fertilization. Just methionine average under standard fertilization condition was decreased. Similarly, almost all essential amino acid averages were increased with extra potassium supply compared to standard fertilization. Only threonine under extra potassium supply condition was decreased. So we can say that a dose potassium (60 kg/ha) with standard fertilization and double potassium dose (120 kg/ha) with extra potassium supply treatment effected positively almost all essential amino acids.

According to irrigation doses results, some essential amino acid averages were increased with increasing irrigation dose but, others were decreased or didn't change. Valine, methionine and isoleucine averages were increased with increasing irrigation doses (only isoleucine stable under 500 ml). Histidine, threonine, leucine and lysine averages were increased with 400 ml and then the averages decreased with 500 ml. just phenylalanine value was decreased with increasing irrigation doses. Thus, we can say that drought stress (300 ml) affected negatively almost all essential amino acid averages expect for phenylalanine. And increase water doses once (400 ml) or twice (500 ml) into irrigation period could block the negative effect on some essential amino acid averages in some degree.

Glycine, proline, arginine, tyrosine, cysteine, aspartic acid, glutamic acid and serine values were shown as conditionally and non-essential amino acids in Table 5. Almost all conditionally essential and non-essential amino acid averages were increased with standard fertilization compared to non-fertilization. Just glycine was decreased and cysteine wasn't change under standard fertilization condition. Similarly, almost all conditionally essential and non-essential amino acid averages were increased with extra potassium supply compared to standard fertilization. Only glutamic acid and serine averages were decreased under extra potassium supply condition. So we can say that a dose potassium (60 kg/ha) with standard fertilization and double potassium dose (120 kg/ha) with extra potassium supply treatment effected positively or nerveless almost all conditionally essential and non-essential amino acids.

In accordance with irrigation doses results, some conditionally essential and non-essential amino acid averages were increased with increasing irrigation dose but, others were decreased or didn't change. Serine and tyrosine were increased with increasing irrigation doses. Proline, arginine, aspartic acid and glutamic acid were increased with 400 ml and then the averages decreased with 500 ml. Glycine and cysteine values were decreased with increasing irrigation doses. So, we can say that drought stress (300 ml) affected negatively on almost all conditionally essential and non-essential amino acid averages expect for glycine and cysteine. And increase water doses once (400 ml) or twice (500 ml) into irrigation period could comparatively block the negative effect.

CONCLUSIONS

Our results from the study given below:

- Except for methionine and threonine, all essential amino acids were increased with treatments (standard fertilization and extra potassium supply). So we can say that a dose potassium (60 kg/ha) with standard fertilization and double potassium dose (120 kg/ha) with extra potassium supply treatment effected positively almost all essential amino acids;
- Except for phenylalanine, drought stress (300 ml) affected negatively all essential amino acid averages. And we can say that increase water doses either once (400 ml) or twice (500 ml) into irrigation period blocked the negative effect on essential amino acid averages in some degree;
- There were found different results about potassium treatments effects on conditionally essential and non-essential amino acid averages. Glycine and cysteine averages under standard fertilization and glutamic acid and serine averages under extra potassium supply decreased. So we can say that a dose potassium

(60 kg/ha) with standard fertilization and double potassium dose (120 kg/ha) with extra potassium supply treatment effected positively or nerveless almost all conditionally essential and non-essential amino acids;

- Expect for glycine and cysteine, drought stress (300 ml) affected negatively on almost all conditionally essential and non-essential amino acid averages. Increase water doses either once (400 ml) or twice (500 ml) into irrigation period could comparatively block the negative effect.

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