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Journal of Agricultural Sciences
Journal homepage:
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Effect of Different Levels of Micronutrients Fertilizer on Protein and Basic and Acidic Amino Acids Contents of Grains of Sorghum Cultivars

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ARTICLE INFO
Research Article DOI: 10.1501/Tarimbil_0000001317
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Received: 03 October 2013, Received in Revised Form: 15 May 2014, Accepted: 28 May 2014

ABSTRACT

Grains of four sorghum cultivars (Tabat, Tetrom, WadAhmed and Gadambellia) were grown for two consecutive seasons under different levels of micronutrients fertilization (0, 2, 4 and 8 g 5kg^{-1} soil). The protein and basic and acidic amino acids of the grains were investigated. The protein content of both seasons was significantly ($P \leq 0.05$) increased with increase in micronutrients dose. However, the grains harvested during the second season had significantly ($P \leq 0.05$) higher protein content than that harvested during the first season. The protein content of the cultivar Tabat was increased by 55% as a maximum value during the first season but during the second season it increased by 71% for the cultivar Gadambalia. The basic essential amino acids (histidine, lysine and arginine) and acidic (aspartic and glutamic acid) were significantly ($P \leq 0.05$) increased during both seasons with micronutrients dose. Lysine was increased by >100% during both seasons for the cultivar Gadambalia.

Keywords: Amino acids; Fertilization; Micronutrients; Protein; Sorghum

Değişik Düzeylerde Mikro Besin Maddesi ile Gübrelemenin Sorgum Çeşitlerinde Tanenin Protein, Bazik ve Asidik Amino Asit İçeriğine Etkisi

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ÖZET

Dört sorgum çeşidinin (Tabat, Tetrom, WadAhmed ve Gadambellia) tohumları, değişik seviyelerde mikro besin maddesi (0, 2, 4 ve 8 g $5kg^{-1}$ toprak) ile gübreleme uygulamasıyla ardaşık iki büyüme sezonu boyunca yetiştirilmiştir. Tanelerdeki protein, mutlak gerekli ve asidik amino asitler incelenmiştir. Protein içeriği her iki yetiştirme sezonunda da, mikro besin maddesi gübrelemesine bağlı olarak önemli ($P \leq 0.05$) derecede artmıştır. Öte yandan, ikinci büyüme sezonunda hasat edilen taneler, birinci büyüme sezonundaki tanelerden ($P \leq 0.05$) daha yüksek protein içeriğine sahip olmuşlardır. Birinci yılda Tabat çeşidi % 55 peotein içeriği ile en yüksek değeri alırken, ikinci yılda Gadambalia çeşidi % 71 protein içeriği artışıyla en yüksek değere sahip olmuştur. Mutlak gerekli temel amino asitler (histidin, lisin ve arginin)ve asitik amino asitler (aspartik ve glutamik asitler) mikro besin maddesi gübrelenmesine bağlı olarak her iki büyüme sezonunda da artmışlardır. Gadambalia çeşidinde, lisin oranı her iki yılda da % 100'den fazla artmıştır

Anahtar Kelimeler: Amino asit; Gübreleme; Mikro besin maddesi; Protein; Sorgum

1. Introduction

Sorghum *Sorghum bicolor* (L) Moench is the fifth most important cereal crop after wheat, rice, maize, and barley in terms of production (FAO 2005). Grain sorghum provides the staple food for a large population of Africa, India and the semi-arid parts of the tropics. It is consumed by the poor peoples of many countries as a major source of proteins and calories in the diet in India and Africa. Besides being a staple food, it is also used as feed for animals and it is an industrial raw material; its stalk provides fodder, fuel, shelter and syrup. Grain sorghum is the leader cereal crop in the Sudan. It is the main staple food, prevailing throughout the country and covering more than 60% of the total cultivated cereals area, with an annual production of about 4.0 million tons (FAO 2005). Sorghum acts as a principal source of energy, protein, vitamins and minerals for millions of the poorest people living in Africa, Asia and the semi-arid tropics worldwide (Klopfesntein & Hoseney 1995). Most of sorghum cultivars grown in Sudan contained only 9 to 11% protein content and most of the amino acids are limited (Elbashir et al 2008). Amino acid composition is an important feature in determining the nutritional value of sorghum grain for human and animal diets. It is evident that the nutritional importance of a given food/feed stuff depends not only on nutrient composition of raw foodstuff but also on the amount utilized (Vijayakumari et al 1998).

Some of the adverse effects of micronutrient deficiency-induced stress in plants include low crop yield and quality. Soil pH influences solubility, concentration in soil solution, ionic form, and mobility of micronutrients in soil, and consequently acquisition of these elements by plants (Fageria et al 1990; Fageria et al 1997). The deficiency of essential micronutrients induces abnormal pigmentation, size, and shape of plant tissues, reduces leaf photosynthetic rates, and leads to various detrimental conditions such as yield and grains composition (Masoni et al 1996). Today, although the production of energy and protein appears to be adequate to feed the developed world, agriculture systems in many developing countries still do not provide enough nutrients to meet human needs (Welch & Graham 2004). Different approaches have been tried to improve the nutritional quality of sorghum grain including: fermentation and malt pretreatment (Abdelhaleem et al 2008), supplementation with legumes (Asma et al 2006), cluster bean (ELbashir et al 2008) and pigeon pea (Abdallah et al 2010) as well as conventional breeding (Abdelseed et al 2011). Moreover, previous research focused only on the effect of micronutrients fertilization on total yield. Therefore, in this study, we would like to investigate the effect of micronutrients fertilization on protein and basic essential and acidic amino acids composition of four sorghum cultivars grains under controlled conditions for two consecutive growing seasons.

2. Material and Methods

2.1. Materials and plant experiments

Grains of sorghum (Sorghum biocolor L. Monech) cultivars namely; Tabat, Tetron, Wad Ahmed and Gadamblia were obtained from the Department of Agronomy, Faculty of Agriculture, University of Khartoum, Shambat, Sudan. Micronutrients blend (MB) was obtained as a mixture of 14% water soluble Mo + 0.3% water soluble Mn + 0.3% water soluble B + 1.2% FeS + 0.02% Cu_2SO_4 + 0.02% $ZnSO_4$ + 0.004% $(NH4)_6[Mo_7O_{24}].4H_2O$, and macronutrients fertilizers (MF) ($N–P_2O_5–K_2O$) were donated by the Arid Land Research Center, Tottori University, Japan. Unless otherwise stated all the reagents used in this study were of analytical grade. A trial experiment was carried out to optimize the conditions suitable for minerals absorption. The conditions tested are pH, environment and soil temperature and soil type. After adjustment of the conditions, the final experiments were carried out (2010 and 2011) at the Experimental Station of the Faculty of Agriculture, University of Khartoum, Shambat (latitude 15°400/N and longitude 32°320/E). The soil was sandy clay (82% sand and 18% clay) with pH of 7.2. The grains of the cultivar were seeded in pots with three grains per pot and after germination, were reduced to one plant per pot. Four doses (0, 2, 4 and 8 g $5kg^{-1}$ soil) of MB were applied to each pot. Beside micronutrients, all treatments received MF ($N–P_2O_5–K_2O$) at a fixed dose of 6 g $5kg^{-1}$ soil. After addition of fertilizer and germination of the seeds, the pH of the soil dropped to 5.7 and temperature between 20 and 25 °C. To avoid water loss, the pots were watered under controlled conditions (weight difference). Each experiment was arranged in a factorial design with four replicates. At the end of each season, the grains were collected, sun dried, cleaned from dirt and broken grains and then ground to pass a 0.15 mm screen and stored at 4 °C.

2.2. Protein determination

The protein content of sorghum grains was determined by micro-Kjeldahl method, as Kjeldahl nitrogen multiplied by 6.25 (AOAC 1995).

2.3. Amino acids determination

The amino acids composition of the samples was measured on hydrolysates using amino acids analyzer (Sykam-S7130, Tokyo, Japan) based on high performance liquid chromatography technique. Sample hydrolysates were prepared following the method of Moore and Stain (1963). About 200 mg of the sample was taken in a hydrolysis tube. Then 5 mL of 6 N HCl was added to and the tube tightly closed and incubated at 110 °C for 24 h. After incubation, the solution was filtered and 200 mL of the filtrate was evaporated to dryness at 140 °C for 1 h. The hydrolysates after dryness were diluted with 1.0 mL of 0.12 N citrate buffer (pH 2.2). Aliquot of 150 µL of the sample hydrolysates was injected in an action separation column at 130 °C. Ninhydrin solution and an eluent buffer (solvent A, pH 3.45 and solvent B, pH 10.85) were delivered simultaneously into a high temperature reactor coil (16 m length) at a flow rate of 0.7 mL min^{-1}. The buffer⁄ninhydrin mixture was heated in the reactor at 130 °C for 2 min to accelerate chemical reaction of amino acids with ninhydrin. The products of the reaction mixture were detected at wavelengths of 570 and 440 nm on a dual channel photometer. The amino acids composition was calculated from the areas of standards obtained from the integrator and expressed as mg 100 g^{-1} sample.

2.4. Statistical analysis

Each determination was carried out on three separate samples and analyzed in triplicate on dry weight basis; the figures were then averaged. Data were assessed by the analysis of variance (Snedecor & Cochran 1987). Comparisons of means for treatments were made using Duncan's multiple range tests. Significance was accepted at $P \leq 0.05$.

3. Results and Discussion

Figure 1 shows the protein content of sorghum grains of four cultivars (Tabat, Tetrom, WadAhmed and Gadambellia) grown under different levels of micronutrients (0, 2, 4 and 8 g 5 kg^{-1} soil) and a constant dose (6 g 5 kg^{-1} soil) of compound macronutrients for two consecutive growing seasons. The protein content of the grains before fertilization was 10.34, 10.34, 10.30 and 10.37% for the cultivars Tabat, Tetron, WadAhmed and Gadambalia, respectively during the first growing season while during the second growing season was 10.05, 10.54, 10.40 and 10.34% for the cultivars, respectively. For all cultivars, the protein content of the grains harvested during the first growing season was increased with increase in fertilizer dose. However, the percent increase in protein content was higher during the second growing season. During the first growing season, the maximum rate of increment in protein content was 55, 42, 35 and 41% at a fertilizer dose of 8 g 5kg^{-1} soil for the cultivars Tabat, Tetron, WadAhmed and Gadambalia, respectively, while during the second growing season was 61, 53, 52 and 71% for the cultivars, respectively.

The results obtained for protein content of sorghum grains indicated that micronutrients fertilization is an effective method in improving storage protein in sorghum grain. It has been reported that micronutrients fertilization at low pH enhanced protein synthesis in cereal crops (Fageria et al 2002). In addition, the application of micronutrient-enriched NPK fertilizers improved the nutritional quality by increasing the quantity of protein of the harvested grains, since micronutrient-enriched NPK fertilizers also increase the concentration of micronutrients in grain (Malakouti 2008). As shown in Figure 2, histidine content of the cultivars grains was increased significantly ($P \leq 0.05$) with micronutrients dose especially for the grains harvested during the second growing season. Histidine content of the grains before fertilization was 87.34, 54.54, 57.56 and 56.37 mg 100 g^{-1} for the cultivars Tabat, Tetron, WadAhmed and Gadambalia, respectively during the first growing season while during the second growing season was 83.19, 60.20, 67.77 and 97.03 mg 100 g^{-1} for the cultivars, respectively. The cultivar Gadambalia recorded higher percent increase in

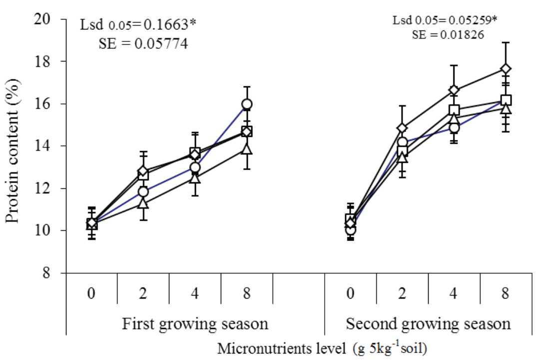

Figure 1- Protein content (%) of grain sorghum cultivars (○, Tabat; □, Tetron; Δ, WadAhmed; ◊, Gadambalia) grown under different levels of micronutrients fertilizer

Şekil 1- Değişik mikrobesin maddesi gübrelemesi ile yetiştirilen tane sorgum çeşitlerinin (○, Tabat; □, Tetron; Δ, WadAhmed; ◊, Gadambalia) protein içerikleri

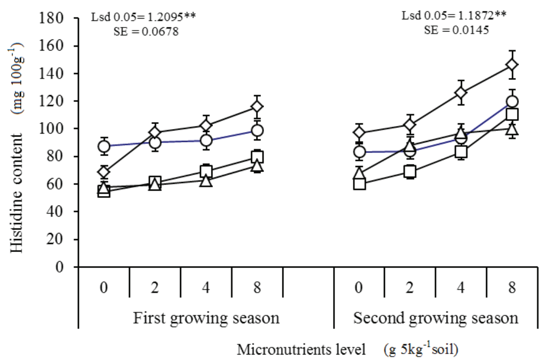

Figure 2- Histidine content (mg 100g^{-1}) of grain sorghum cultivars (○, Tabat; □, Tetron; Δ, WadAhmed; ◊, Gadambalia) grown under different levels of micronutrients fertilizer

Şekil 2- Değişik mikrobesin maddesi gübrelemesi ile yetiştirilen tane sorgum çeşitlerinin (○, Tabat; □, Tetron; Δ, WadAhmed; ◊, Gadambalia) histidin içerikleri (mg 100g^{-1})

histidine (69%) followed by Tetron (46%) during the first growing season. However, during the second growing season, Tetron recorded higher percent increase (84%) followed by Gadambalia (51%) when the plants grown under 8 g 5 kg^{-1} soil. The rate of increment in histidine was significantly ($P \leq 0.05$) higher during the second growing season for all cultivars compared to the first growing season. Lysine content of the cultivars grains was increased significantly ($P \leq 0.05$) with micronutrients dose especially for the grains harvested during the second growing season (Figure 3).

Lysine content of the grains before fertilization was 108.87, 71.29, 98.81 and 95.91 mg 100 g^{-1} for the cultivars Tabat, Tetron, WadAhmed and Gadambalia, respectively during the first growing season while during the second growing season was 120.75, 98.93, 114.12 and 115.28 mg 100 g^{-1} for the cultivars, respectively. Fertilization of the plant with 8 gm 5 kg^{-1} soil during both growing season significantly ($P \leq 0.05$) increased lysine with a maximum rate of increment obtained for the cultivar Gadambalia which exceeded 100% followed by Tabat cultivar (94%) during the first season.

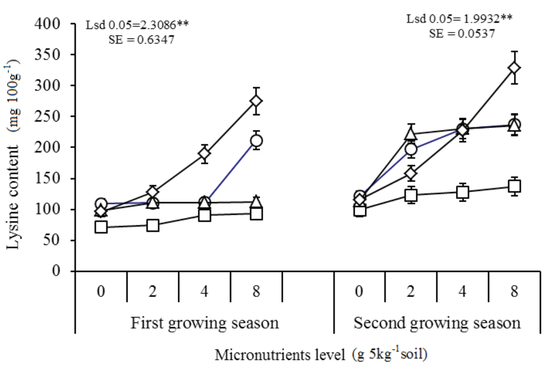

Figure 3- Lysine content (mg 100g^{-1}) of grain sorghum cultivars (○, Tabat; □, Tetron; Δ, WadAhmed; ◊, Gadambalia) grown under different levels of micronutrients fertilizer

Şekil 3- Değişik mikrobesin maddesi gübrelemesi ile yetiştirilen tane sorgum çeşitlerinin (○, Tabat; □, Tetron; Δ, WadAhmed; ◊, Gadambalia) lisin içerikleri (mg 100 g^{-1})

The rate of increment in lysine was significantly ($P \leq 0.05$) higher during the second growing season for all cultivars compared to the first growing season. It was observed that fertilization of the grains of the cultivar Gadabalia with 8 gm 5 kg^{-1} soil during both seasons significantly ($P \leq 0.05$) increased lysine content and the rate of increment exceeded 100%. As shown in Figure 4, arginine content of the grains before fertilization was 163.85, 122.07, 124.18 and 218.72 mg 100 g^{-1} for the cultivars Tabat, Tetron, WadAhmed and Gadambalia, respectively during the first growing season while during the second growing season was 186.71, 200.29, 195.19 and 226.14 mg 100 g^{-1} for the cultivars, respectively. Fertilization of the plant with 8 gm 5 kg^{-1} soil during the first growing season significantly ($P \leq 0.05$) increased arginine content with a maximum rate of increment observed for the cultivar WadAhmed (39%) followed by the cultivar Tetron (38%). The rate of increment in arginine was significantly ($P \leq 0.05$) higher during the second growing season compared to the first growing season with a maximum rate of increase observed for the cultivar Tabat (62%) followed by Gadabalia (40%). Aspartic acid (aspartate) content of the grains before fertilization was 316.65, 381.25, 337.92 and 400.00 mg 100 g^{-1} for the cultivars Tabat, Tetron, WadAhmed and Gadambalia, respectively during the first growing season while during the second growing season was 295.66, 328.05, 325.29 and 448.04 mg 100 g^{-1} for the cultivars, respectively (Figure 5). Fertilization of the plant with 8 gm 5 kg^{-1} soil during the first growing season significantly ($P \leq 0.05$) increased aspartat content with a maximum rate of increment observed for the cultivar Gadambalaia (47%) followed by the cultivar Tetron (29%). The rate of increment in aspartate was significantly ($P \leq 0.05$) higher during the second growing season with a maximum rate of increase observed for the cultivar Tabat (61%) followed by Gadambalia (58%). Figure 6 shows the effect of micronutrients fertilization on glutamic acid content of four sorghum cultivars grains. Glutamic acid (glutamate) content of the grains before fertilization was 1254.67, 1334.69, 1310.67 and 1298.71 mg 100 g^{-1} for the cultivars Tabat, Tetron, WadAhmed and Gadambalia, respectively during the first growing season while during the second growing season was 1300.23, 1300.92, 1026 and 1340.04 mg 100 g^{-1} for the cultivars, respectively.

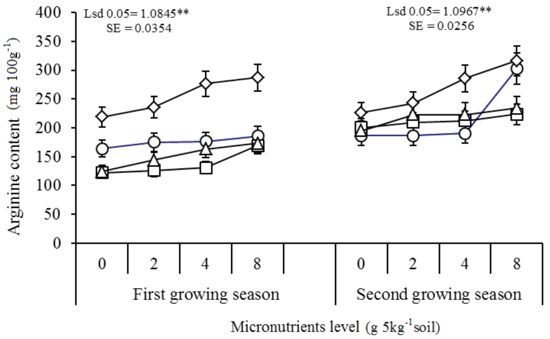

Figure 4- Arginine content (mg 100 g^{-1}) of grain sorghum cultivars (○, Tabat; □, Tetron; Δ, WadAhmed; ◊, Gadambalia) grown under different levels of micronutrients fertilizer

Şekil 4- Değişik mikrobesin maddesi gübrelemesi ile yetiştirilen tane sorgum çeşitlerinin (○, Tabat; □, Tetron; Δ, WadAhmed; ◊, Gadambalia) arginin içerikleri (mg 100 g^{-1})

Fertilization of the plant with 8 g 5 kg^{-1} soil during the first growing season significantly ($P \leq 0.05$) increased glutamate content with a maximum rate of increment observed for the cultivar Gadambalaia (100%) followed by the cultivar Tetron (29%). During the second growing season, the rate of increment in glutamate was significantly ($P \leq 0.05$) higher with a maximum rate of increase observed for the cultivar Gadambalaia (120%) followed by Tabat (24%). The results revealed that micronutrients fertilization had increased the amino acids contents which could be attributed to the increase in protein content of all cultivars after micronutrients treatments. The effect of micronutrients fertilization on protein content and amino acids composition was found to be varied between the cultivars and also seasonal variations were observed in protein and amino acids contents for each cultivar. The differences among plant cultivars have been attributed to genetics, physiological/biochemical mechanisms, responses to climate variables, tolerance to pest and diseases, and responses to agronomic management practices. Genetic variations in plant acquisition of micronutrients have been reviewed (Duncan & Carrow 1999). The development of cultivars effective in the acquisition and use of micronutrients and with the desired agronomic characteristics is vital for improving yields and achieving genotypic adaptation to diversified environmental conditions and increased resistance to pests (Baligar et al 1998). The results revealed that the cultivars Tabat and Gadambalia highly respond to micronutrients fertilization and the protein content significantly ($P \leq 0.05$) increased during both growing seasons. The results obtained for protein content of sorghum grains indicated that micronutrients fertilization is an effective method in improving raw protein of sorghum. It has been reported that micronutrients fertilization at low pH enhanced protein synthesis in cereal crops (Fageria et al 2002**).** Moreover, the application of micronutrient-enriched NPK fertilizers improved the nutritional quality by improving the quantity of protein of the harvested grains, since micronutrient-enriched NPK fertilizers also increase the concentration of micronutrients in grain (Malakouti 2008). The increment in raw protein accompanied by a significant ($P \leq 0.05$) increase in both basic and acidic amino acids which indicated positive correlation between raw protein and amino acid content. An increase in basic amino acids is very important from nutritional point of view because they are consider as an essential amino acids especially arginine for infants and growing children. Variations in amino acids content between the growing seasons and the cultivars could be attributed to variation in protein content of the cultivars. Although it has been reported that macro- and micro-nutrients interact with each other forming complexes and reduced the acquisition of such minerals (Fageria et al 2002). In the present study we optimize the conditions that enhance the accessibility of minerals as indicated by an increase in protein content which accompanied by an increase in both basic and acidic amino acids composition.

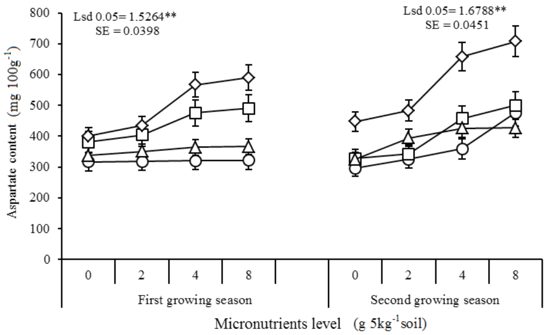

Figure 5- Aspartic acid content (mg 100 g^{-1}) of grain sorghum cultivars (○, Tabat; □, Tetron; ∆, WadAhmed; ◊, Gadambalia) grown under different levels of micronutrients fertilizer

Şekil 5- Değişik mikrobesin maddesi gübrelemesi ile yetiştirilen tane sorgum çeşitlerinin (○, Tabat; □, Tetron; ∆, WadAhmed; ◊,Gadambalia) aspartik asit içerikleri (mg 100 g^{-1})

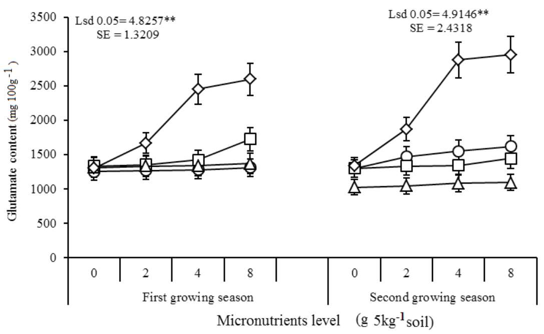

Figure 6- Glutamic acid content (mg 100 g^{-1}) of grain sorghum cultivars (○, Tabat; □, Tetron; ∆, WadAhmed; ◊, Gadambalia) grown under different levels of micronutrients fertilizer

Şekil 6- Değişik mikrobesin maddesi gübrelemesi ile yetiştirilen tane sorgum çeşitlerinin (○, Tabat; □, Tetron; ∆, WadAhmed; ◊,Gadambalia) glutamik asit içerikleri (mg 100 g^{-1})

4. Conclusions

The observations about protein and basic essential and acidic amino acid composition in the studied samples tend to suggest that the application of micronutrient-enriched NPK fertilizers improved the protein and amino acids content of sorghum grains since micronutrient-enriched NPK fertilizers also increase the concentration of micronutrients in grain. Moreover, micronutrients fertilization when compared with chemicals or heat treatment as means to improve the nutritional quality emerges as an attractive and healthy alternative.

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