Original article

Fluctuation in amino acids content in *Triticum aestivum* L. cultivars as an indicator on the impact of post-emergence herbicides in controlling weeds

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A B S T R A C T

This study was carried out in a demonstrated field in El-Sharkia Governorate, Egypt, during the winter of season 2020 to evaluate the leverage of four post-emergence herbicides i.e., tribenuron-methyl, clodinafop-propargyl, pyroxsulam and pinoxaden compared to control on total protein and amino acid contents in three wheat cultivars (Shandwel 1, Giza 171, and Sakha 95). Generally, the use of foliar herbicides led to a significant decrease in essential, non-essential amino acids and protein contents. However, tribenuran-methyl herbicide significantly increased the levels of proline, glycine, arginine, and histidine, but cystine and threonine not affected as compared to control. On the other hand, foliar herbicide application was significantly increased physiological, biochemical parameters and yield of Shandweel cultivar as compared to the other varieties. The physiological and biochemical models of dual-herbicide-tolerant wheat cultivars add to our understanding of the crop. In recent agricultural systems, herbicide tolerant plants are important for long-term weed management. Therefore, the study recommended the safely usage of Tribenuran-methyl as foliar herbicide in weed management.

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1. Introduction

Herbicides inhibiting the biosynthesis of amino acids are effective tools for weed control and have succeeded because of their low toxicity in mammals. These herbicides block pathways that mammals lack. Many herbicides have primary modes of action associated with the complex suppression of enzymatic activity in amino acid biosynthesis pathways (Hall et al., 2020). Compounds that inhibit the biosynthesis of branched-chain amino acids (valine, leucine, and isoleucine) by suppressing acetolactate are a form of herbicide (Zulet et al. 2013). The mechanism of herbicides is blocking biochemical and physiological pathways, reducing weed biomass. A relatively new class of sulfonylurea herbicides is the most common and abundant (Kieloch et al., 2013). Excessive addition of chemical herbicides alters the amino acid content in plants by interacting with other metabolic processes, where they indirectly affect nitrogen metabolism, inhibiting amino acid biosynthesis, consequently the amino acid content in plant tissues (Radhakrishnan et al., 2018). Proteins consist of amino acids, which are categorized to essential or non-essential depending on how they are synthesized in humans. Plants are the only ones that can synthesize essential amino acids (leucine, isoleucine, methionine, phenylalanine, arginine, histidine, tryptophan, valine, threonine, and lysine). However, non-essential amino acids (alanine, -alanine, asparagine, cysteine, glutamine, aspartic acid, glycine, proline, serine, and tyrosine) are synthesised by both plants and humans (Kumar et al., 2017). In most plants, amino acids are active

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compounds in which nitrogen is transported (Tegeder 2014). Because of their primary function in nitrogen metabolism, their biosynthesis, degradation, and transport are all tightly controlled to meet demand in response to nitrogen and carbon availability (Pratelli and Pilot 2014). Membrane proteins facilitate transporting amino acids into and out of plant cells, and within organelles. While several putative amino acid transporters have been identified, only a few proteins have been shown to play a role in plant nitrogen transport. In the rhizosphere, amino acids are linked to plant root development, as well as microbial colonization, symbiotic interactions, and pathogenesis (Moe 2013). In wheat plants, the relationship between amino acid uptake and inorganic nitrogen was studied (Gioseffi et al., 2012). Amino acids are essential for metabolic processes, transportation and storage of all nutrients, including carbohydrates, proteins, vitamins, minerals, water, and fats. Plant-based proteins contain more than 60% of the needed proteins for human growth, development, and antioxidant properties. Free amino acids are essential for secondary plant metabolism and the biosynthesis of compounds like phenolics and glucosinolates, which play important roles in the interaction of the environment and human health, either directly or indirectly (Abudayeh et al. 2016). The aim of this study was to investigate the effect of four post-emergence herbicides, pinoxaden, tribenuron-methyl, pyroxsulam, and clodinafop-propargyl, on % N, amino acids, total protein contents in wheat grains as indicator on controlling weeds.

2. Materials and methods

2.1. Materials

2.1.1. Wheat cultivars:

Wheat cultivars (Shandwel 1, Giza 171, and Sakha 95) were obtained from Agriculture Research Center, Cairo. The three cultivars were sown in sandy clay soil in the winter seasons of 2020. The physical and chemical composition of soil was analysed at the Agricultural Research Center, Ministry of Agriculture, Egypt, according to Page et al. (1982), and the results shown in Table 1.

2.1.2. Herbicides

Four post-emergence herbicides were used against weeds field, the characteristics of these herbicides are presented in Table 2.

2.2. The experimental design

The experiment was carried out in a randomized, complete block design with three replicates, the experimental block size was 160 m², with an individual plot size of 16 m² (5.34 m length x 3.0 width). The herbicides doses / hectare were calculated according to the surface of feddan (4200 m²). Pinoxaden in emulsifiable concentration form (4.5% EC) at 550 cm³/ fed., tribenuron-methyl in Dry Flowables form (75% DF) at 8 gm./fed, and pyroxsulam in dust form (4.5% OD) at 160 cm³/ fed. The herbicides were solubilized in a suitable amount of water (200 L/fad.) to spraying also to the plot area (16 m²) (El-Kholy, 2013; Abouziena et al., 2008). The experimental farm is located at 30° 42′ 0 N, 31° 48′ 0 E. Each plot received herbicide solution as recommended doses by the U.S.A Environmental Protection Agency.

2.3. Sample preparation

Wheat plants were sprayed at the age of the fourth leaf (Tottman, 1987), then after 48 h from spraying, twelve plants were randomly selected from each plot, which were pulled up then put in icebox with dry ice until analysis.

2.4. Estimation of total protein content and amino acids

The wheat leaves were randomly collected from each plot after 3 day of herbicide foliar application to determine total crude protein content (A.O.A.C., 2000). Ethyl alcohol (80%) was used to extract amino acids from oven-dried leaves. The determination of quantitative and qualitative amino acids was done according to (Christias et al.,1975).

2.5. Statistical analysis

ANOVA was used to statistically analysed the significant differences between treatments. The variations between treatments were compared using LSD at the 0.05 level. The triplicate data means were subjected to proper statistical analysis of variance according to (Snedecor and Cochran, 1980).

3. Results

3.1. Effect of the four herbicides at the recommended dose in total protein, total nitrogen percentages in the grain of some wheat cultivars

Data presented in Table 3 illustrate the effect of four herbicides on grain nitrogenous content and total protein of three wheat cultivars Shandwel 1, Giza 171, and Sakha 95. There were significant differences between all herbicide treatments. The results cleared that herbicides significantly affected the nitrogen content and total protein of grains. Between grains, Shandwel was the most sensitive to all herbicides however, Sakha 95 was the lowest one compared with control. The pinoxaden was the highest effect on nitrogen percentage and total protein in wheat grains of the three cultivars, Shandwel 1, Giza 171, and Sakha 95 (0.672, 3.954%), respectively. Nevertheless, tribenuron-methyl was the lowest effective on nitrogen and total protein (0.756, 4.447%), respectively compared with control, while pyroxsulam and clodinafop-propargyl were significantly decreased nitrogen content and total protein and have medium values between herbicides in wheat grains.

| Table 1 |
| --- |
| Physical and chemical properties of the experimental soil before sowing. |
| Soil properties | Values |
| Mechanical-Clay % | 9.80 |
| Silt % | 17.30 |
| Sand % | 72.90 |
| Texture | Sandy-Clay |
| Organic Matter (O.M) (g/kg) | 8.7 |
| pH (1:2.5 soil suspension) | 8.20 |
| Ec (ds.m⁻¹·(1:5)) | 1.20 |
| Total N (mg/kg) | 4.35 |
| Available K (mg/kg) | 210 |
| Soluble anions, meq.L⁻¹: |
| HCO₃⁻ | 0.10 mg kg⁻¹soil |
| Cl⁻ | 3.00 mg kg⁻¹soil |
| SO₄²⁻ | 4.61 mg kg⁻¹soil |
| Soluble Cations, meq.L⁻¹: |
| Ca²⁺ | 2.00 mg kg⁻¹soil |
| Mg²⁺ | 2.00 mg kg⁻¹soil |
| Na⁺ | 4.35 mg kg⁻¹soil |
| K⁺ | 0.26 mg kg⁻¹soil |
3.2. Effect of herbicides on essential amino acids and non-essential amino acids in wheat cultivars

3.2.1. Effect of Tribenuron-methyl herbicide

In this study, data presented the effect of tribenuron-methyl herbicide on the essential amino acids i.e. lysine, methionine, cysteine, threonine, isoleucine, leucine, tyrosine, valine, phenylalanine and non-essential amino acids (aspartic acid, serine, proline, glycine, alanine, histidine, glutamic acid, and arginine). Data in Table 4 showed the amounts of amino acids in control treatments where results showed high concentration in glutamic, aspartic acid, lysine, and proline. The increase in protein causes an increase in amounts of amino acids. On the other hands, Table 5 showed the amino acids content in wheat cultivars treated with tribenuron-methyl, the total increase in amino acids was found to be directly related to the increase of total proteins in the control sample. Proline, glycine, arginine, and histidine contents were increased, while no influence was observed on essential amino acids cysteine and threonine compared with control. Slight decrease in amounts of methionine and valine; however, considerable decrease in leucine,
lysine, isoleucine, phenylalanine, tyrosine, aspartic acids, serine and glutamic acid contents confirms adverse effects of tribenuron- methyl on these amino acids.

It may be inferred from the analytical data that the amino acids leucine, lysine, isoleucine, phenylalanine, tyrosine, aspartic acids, serine, and glutamic acid have undergone similar adverse effects with the tested herbicide while proline, glycine, arginine, and histidine were increased. In addition, it is important to note that, the Shandwel 1 cv. was the most varieties affected by herbicides; however, Sakha 95 was the lowest.

3.2.2. Effect of pyroxsulam herbicide

Data in Table 6 cleared that phenylalanine and aspartic acid exhibited higher values than other amino acids in all wheat cultivars, however, a decrease in values of rest amino acids compared with control treatments in all cultivars. A decrease in total protein causes a decrease in all amino acids except phenylalanine and aspartic acid. No significant differences between the effect of pyroxsulam on tested wheat cultivars.

3.2.3. Effect of clodinafop- propargyl herbicide

The results in Table 7 showed the same route in the previous treatments where, essential and non-essential amino acids values decreased except cysteine do not affected by the clodinafop-propargyl treatment as compared to control. Shandweel cv. was the most varieties affected by herbicide but Sakha 95 was the lowest.

3.2.4. Effect of pinoxaden herbicide

The foliar application of pinoxaden herbicide on wheat cultivars adversely affected the values of leucine, lysine, isoleucine, phenylalanine, tyrosine, aspartic acids, serine and glutamic acid; however, methionine and valine were not affected and cysteine was increased (Table 8). It noticed that Shandwel 1 cv. was the most affected cultivar.

Table 5
Effect of tribenuron- methyl herbicide on essential and non-essential amino acids of wheat cultivars.

| Amino acids (A)                  | Wheat cultivars         | Means (A) |
|----------------------------------|-------------------------|-----------|
|                                  | Shandawel 1 | Giza 171 | Sakha 95 |
| Essential amino acids            |             |          |          |
| Lysine                           | 7.44gh     | 7.40 h   | 7.33i    | 7.39D    |
| Methionine                       | 0.73zh     | 0.69zj   | 0.56zj   | 0.66P    |
| Cystine                          | 0.13zk     | 0.12zk   | 0.12zk   | 0.12Q    |
| Threonine                        | 3.86iu     | 3.81v    | 3.75w    | 3.80 J    |
| Isoleucine                       | 7.18j      | 7.13 k   | 7.07 L   | 7.12E    |
| Leucine                          | 5.45o      | 5.40p    | 5.37p    | 5.40G    |
| Phenylalanine                    | 4.22q      | 4.20q    | 4.14r    | 4.18H    |
| Tyrosine                         | 2.34f      | 2.30 fg  | 2.29 g   | 2.31O    |
| Valine                           | 4.11r      | 4.03 s   | 3.96 t   | 4.03I    |
| Non-essential amino acids        |             |          |          |
| Aspartic acid                    | 11.66d     | 11.60e   | 11.50f   | 11.58B   |
| Serine                           | 3.40y      | 3.34a    | 3.31a    | 3.35 L    |
| Glutamic acid                    | 12.94a     | 12.86b   | 12.76c   | 12.85A   |
| Proline                          | 7.46 g     | 7.47 g   | 7.41 h   | 7.44C    |
| Glycine                          | 2.86zd     | 2.84zd   | 2.77ze   | 2.82 N    |
| Alanine                          | 3.05zb     | 3.04zb   | 2.96zc   | 3.02 M    |
| Histidine                        | 3.48x      | 3.45x    | 3.37yz   | 3.43 K    |
| Arginine                         | 5.74 m     | 5.76n    | 5.74n    | 5.78F    |
| Means (B)                        | 5.07 A     | 5.02B    | 4.96C    |
| LSD at 5%                        | For (A) = 0.001 | For (B) = 0.003 | For (A × B) = 0.001 |

Table 6
Effect of pyroxsulam herbicide on essential and non-essential amino acids of wheat cultivars.

| Amino acids (A)                  | Wheat cultivars         | Means (A) |
|----------------------------------|-------------------------|-----------|
|                                  | Shandawel 1 | Giza 171 | Sakha 95 |
| Essential amino acids            |             |          |          |
| Lysine                           | 7.38c       | 7.30 cd  | 7.25cde  | 7.31C    |
| Methionine                       | 0.67u       | 0.65u    | 0.60u    | 0.64 N    |
| Cystine                          | 0.12v       | 0.11v    | 0.10v    | 0.11O    |
| Threonine                        | 3.78mn      | 3.66m    | 3.61m    | 3.68I    |
| Isoleucine                       | 7.10def     | 7.04ef   | 6.96ef   | 7.03D    |
| Leucine                          | 5.39hi      | 5.31i    | 5.31i    | 5.34F    |
| Phenylalanine                    | 4.67j       | 4.10 k   | 4.04k    | 4.27G    |
| Tyrosine                         | 2.29 l      | 2.24 t   | 2.20 t   | 2.24 M    |
| Valine                           | 3.97km      | 3.84mn   | 3.84mn   | 3.88H    |
| Non-essential amino acids        |             |          |          |
| Aspartic acid                    | 11.50b      | 11.41b   | 11.41b   | 11.44B   |
| Serine                           | 3.33o       | 3.30o    | 3.30o    | 3.31 J    |
| Glutamic acid                    | 12.86a      | 12.84a   | 12.85a   | 12.85A   |
| Proline                          | 7.40c       | 7.30 cd  | 7.27cde  | 7.32C    |
| Glycine                          | 2.75ps      | 2.67s    | 2.67 s   | 2.69L    |
| Alanine                          | 2.97p       | 2.94pq   | 2.91pq   | 2.94 K    |
| Histidine                        | 3.33o       | 3.34o    | 3.31o    | 3.32 J    |
| Arginine                         | 5.74 g      | 5.65 g   | 5.58gh   | 5.66E    |
| Means (B)                        | 5.02 A      | 4.92 A   | 4.89 A   |
| LSD at 5%                        | For (A) = N.S. | For (B) = 0.12 | For (A × B) = 0.24 |
sensitive varieties to herbicides; however, Sakha 95 was the resistant.

4. Discussion

In most plants, amino acids are the most active compounds for metabolic processes, transportation and storage of all nutrients, including carbohydrates, proteins, vitamins, minerals, water, and fats. Plant-based proteins contain more than 60% of the proteins needed for human growth, development, and antioxidant properties. Free amino acids are essential for secondary plant metabolism and the biosynthesis of compounds like phenolics and glucosinolates, which play important roles in the interaction of the environment and human health, either directly or indirectly. Wheat is a major source of carbohydrates and energy; it is valued grain for health, which contains protein, vitamins, fibers and phytochemicals (Desoky et al., 2020a,b, El-Saadony et al., 2021a), and this composition differ based on herbicide treatments, and the herbicides had a significant effect on crude protein and fat content (Mehmeti et al., 2016). They also reported that when plots were treated with triasulfuron + dicamba, the content of cured protein in wheat grain increased (15.25%) higher than all other treatments, while the lowest content of crude protein in wheat grain was in the plot was treated with mechanical control (13.72%), additionally, they reported that plots were treated with tribenuron-methyl significantly differed from other treatments.

Also, (Kryeziu, 2008) found that the crude protein contents of winter wheat estimated by two methods; Kjeldahl (11.63%) or the Near-infrared spectroscopy (NIRS) apparatus (11.66%). According to Ehsan Ali et al., (2014), the crude protein content of wheat grain varieties ranged from 7.8% to 12.88%. Pirsabak cultivar has the highest protein content and Khattakwal has the lowest. The same matters were observed in rice cultivars; the herbicides damages were discovered when treated with azimsulfuron (Morais et al., 2011; Singh et al. 2016); where Souahiet al. (2016) stated

Table 7
Effect of clodinafop- propargyl herbicide on essential and non-essential amino acids of wheat cultivars.

| Amino acids (A) | Wheat cultivars | Means (A) |
|----------------|----------------|----------|
|                | Shandawel 1    | Giza 171 | Sakha 95 |
| Essential amino acids | | | |
| Lysine | 7.31e | 7.23f | 7.16 fg | 7.23C |
| Methionine | 0.63ze | 0.59zef | 0.54zfg | 0.59P |
| Cystine | 0.13zg | 0.12zg | 0.11zg | 0.12Q |
| Threonine | 3.70st | 3.64 t | 3.55u | 3.63 J |
| Isoleucine | 6.94j | 6.86 | 6.80j | 6.87E |
| Leucine | 5.29m | 5.23mn | 5.20m | 5.24G |
| Phenylalanine | 4.14o | 4.10o | 3.99p | 4.07H |
| Tyrosine | 2.24zc | 2.20zcd | 2.14zd | 2.19O |
| Valine | 3.86q | 3.80qr | 3.74rs | 3.80I |
| Non-essential amino acids | | | |
| Aspartic acid | 11.30c | 11.19d | 11.16d | 11.22B |
| Serine | 3.27wx | 3.25x | 3.16y | 3.22L |
| Glutamic acid | 12.67a | 12.60a | 12.50b | 12.59A |
| Proline | 7.24f | 7.16gh | 7.03u | 7.16D |
| Glycine | 2.67a | 2.64a | 2.56b | 2.63N |
| Alamine | 2.83z | 2.76z | 2.75z | 2.78 M |
| Histidine | 3.33v | 3.30wv | 3.21x | 3.28 K |
| Arginine | 5.60 k | 5.51 l | 5.46 L | 5.52F |
| Means (B) | 4.89 A | 4.83 AB | 4.77B | |
| LSD at 5% | For (A) = 0.07 | For (B) = 0.02 | For (A × B) = 0.08 |

Table 8
Effect of pinoxaden on essential and non-essential amino acids of wheat cultivars.

| Amino acids (A) | Wheat cultivars | Means (A) |
|----------------|----------------|----------|
|                | Shandawel 1    | Giza 171 | Sakha 95 |
| Essential amino acids | | | |
| Lysine | 7.17 g | 7.10 h | 7.05i | 7.11C |
| Methionine | 0.57zk | 0.55zkl | 0.52zl | 0.54P |
| Cystine | 0.14zm | 0.13zm | 0.12zm | 0.13Q |
| Threonine | 3.50k | 3.45y | 3.40z | 3.45 J |
| Isoleucine | 6.84 k | 6.78 l | 6.67 m | 6.76E |
| Leucine | 5.19p | 5.17pq | 5.14q | 5.16G |
| Phenylalanine | 4.05s | 3.98 s | 3.94 t | 3.99H |
| Tyrosine | 2.15i | 2.14j | 2.11j | 2.13O |
| Valine | 3.80u | 3.70v | 3.65w | 3.72I |
| Non-essential amino acids | | | |
| Aspartic acid | 13.13d | 11.06e | 10.98f | 11.05B |
| Serine | 3.17tb | 3.14tb | 3.08tc | 3.13L |
| Glutamic acid | 12.55a | 12.51b | 12.45c | 12.50A |
| Proline | 7.13 h | 7.06i | 6.98j | 7.05D |
| Glycine | 2.51 | 2.46 | 2.41 | 2.46 N |
| Alamine | 2.75zd | 2.70ze | 2.67ze | 2.71 M |
| Histidine | 3.27a | 3.23a | 3.17b | 3.22 K |
| Arginine | 5.42n | 5.36o | 5.36o | 5.38F |
| Means (B) | 4.73 A | 4.69B | 4.78C | |
| LSD at 5% | For (A) = 0.02 | For (B) = 0.02 | For (A × B) = 0.04 |

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that decrease in protein content is an indication of a reduction in plant growth. Protein and proline contents differ based on rice varieties (Zayed et al., 2014; Zhou et al., 2018). (Verma et al., 2007) found that wheat cultivar PBW-343 had higher protein content i.e., 10.39% followed by NW-1014 (10.37%), and these were statistically superior to the cultivars HP-2733 (10.31 percentage), HP-1731 (10.13 percentage), and K-9107 (10.16%), respectively. They also, discovered that the protein content in wheat treated with sulfofuran and Fenoxaprop herbicides was was better than isoproturon and tordonphenilamin treatments. On the other hands, Delchev et al., (2015) showed that protein percentage increased when treated with fertilizer complex of lactofol and Terra-sorb organic fertilizer Humustim were combined with herbicides Axial one, Hussar max, and place, and their tank mixtures. They also, stated that the increase of protein percentage is the highest at combinations of herbicide Axial one with foliar fertilizer Terra-sorb (13.52%), 13.71% with Terra-sorb + Hussar max OD, and 13.33% with Terra-sorb + Palace 75 WG. Furthermore, they show that the protein percentage ranged from 11.49% with the control treatment, up to 13.52% with the combination of axial one + Terra-sorb foliar fertilizer.

Herbicides can affect physiological processes and cause biotic stresses in plant as heavy metals and salinity (El-Saadony et al., 2021a,b; El-Saody et al., 2021b); however, weeds herbicides used to combat weeds affect primary and secondary metabolisms in the tested crops (Souahi et al. 2016; Fernández-Aparicio et al., 2017). The highest levels of herbicides affect the contents of alanine, cysteine, histidine, isoleucine, leucine, methionine, and tyrosine, with the inhibition percentage varying depending on the plant species and growth stage (Souahi et al., 2016). A decline in protein content is an indicator of a reduction in plant development (Zayed et al., 2014; Zhou et al., 2018). The japonica group had a slightly lower ratio of aspartate-derived concentration to glutamate-derived free amino acid than the indica group (Kamara et al., 2010).

5. Conclusion

The exposure to the herbicides causes significant variation in essential, non-essential amino acids, protein content. Also, post-emergence herbicides tolerant varieties are not always available for many crop species. Therefore, tribenuran-methyl can be used safely as herbicide as foliar on wheat cultivars because it didn’t affect the amino acids and protein contents in wheat cultivars; however, pinoxaden herbicide cause more adverse than other tested herbicides and cause deficiency of amino acids while studied pyroxysulam and clodinafop-propargyl have medium effects.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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