SEASONAL DYNAMICS OF FREE AMINO ACIDS IN THE ROOT SYSTEM OF COTTON VARIETY ANDIJAN-36

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Abstract. *The seasonal dynamics of total nitrogen and the free amino acid (FAA) pool in the root system of cotton (Gossypium hirsutum L., cv. Andijan-36) were studied across key ontogenetic phases under the arid climate of Uzbekistan. Using high-performance liquid chromatography (HPLC) with pre-column derivatization, it was found that the maximum total FAA content (50.45 mg/g dry weight) was observed at the seedling stage, dominated by glutamine (7.20 mg/g) and asparagine (6.85 mg/g). A peak in proline accumulation (3.25 mg/g) was detected at the flowering stage, indicating the activation of its osmoregulatory and stress-protective functions under high transpirational load. By the maturation phase, the total FAA pool decreased to 33.25 mg/g, reflecting the remobilization of nitrogen resources to generative organs. The obtained data identify roots as an active metabolic organ and define critical phases (seedling, budding, flowering) for optimizing amino acid nutrition in cotton.*

Keywords: *cotton, root system, free amino acids, seasonal dynamics, ontogenesis, glutamine, asparagine, proline, HPLC.*

Introduction. The root system of plants plays a fundamental role in the uptake and metabolism of mineral nutrients, serving not only as an absorption organ but also as an active center for the synthesis of organic compounds, including amino acids [1, 2]. Free amino acids (FAAs) are key metabolites and indicators of the physiological and biochemical status of plants, reflecting the intensity of nitrogen metabolism [3, 4].

For cotton (Gossypium hirsutum L.) – a crop with a high nitrogen demand – studying the seasonal dynamics of FAAs in roots is of fundamental importance for developing scientifically sound nutrition systems [5, 6]. Under the arid farming conditions of Uzbekistan, where water deficit and high temperatures are limiting factors, research into adaptive mechanisms, including the accumulation of stress-protective amino acids, becomes particularly relevant [7, 8].

Glutamine and asparagine serve as the main transport forms of nitrogen in plants, while proline exhibits properties of an osmoregulator and stress protector under water deficit [9, 10]. Studying the dynamics of these compounds in the root system of cotton helps identify critical ontogenetic phases during which amino acid nutrition can have the maximum physiological effect.

The aim of this study was to establish the patterns of seasonal dynamics of free amino acid content in the root system of cotton variety Andijan-36 across major phenological development phases.

Materials and methods.

Object and study conditions. The work was carried out in 2022–2023 under the conditions of an experimental farm in the Andijan region of the Republic of Uzbekistan. The study object was cotton plants of the Andijan-36 variety, cultivated according to regional agricultural practices on light sierozem soils [11, 12].

Sampling scheme. Root samples were collected at five key phenological phases: seedling (3–4 true leaves), budding, flowering, boll formation, and maturation. Samples were collected with four biological replicates (n = 4). Each replicate consisted of a composite root sample from 5–10 typical plants [13].

Sample preparation and amino acid extraction. Samples were fixed in liquid nitrogen, dried to constant weight at 65 °C, and ground into a homogeneous powder. Free amino acids were extracted with 70% aqueous ethanol in an ultrasonic bath at 60 °C for 30 minutes [14].

Analytical methods. Total nitrogen content was determined by a modified Kjeldahl method [15]. Quantitative analysis of the profile of 20 free amino acids was performed by HPLC on a Shimadzu LC-20AD system with pre-column derivatization using o-phthalaldehyde and fluorimetric detection. Separation was carried out on a C18 column (250 × 4.6 mm, 5 μm) [16].

Statistical data processing. Results are presented as M ± m. Statistical significance of differences was assessed using one-way analysis of variance (ANOVA) with Tukey's post-hoc test. Differences were considered significant at $p < 0.05$ [17].

Results and discussion. Dynamics of total nitrogen. The total nitrogen content in the cotton root system changed significantly during ontogenesis (Table 1). The maximum nitrogen concentration was recorded in the initial development phases: seedling (2.00% DW) and budding (1.90% DW), which is due to intensive growth and the formation of primary metabolic activity.

Table 1. Total nitrogen content in cotton roots by development phase (M ± SD, n=3)

Development Phase	Roots, % DW
Seedling	2,00 ± 0,05
Budding	1,90 ± 0,04
Flowering	1,50 ± 0,04
Boll formation	1,20 ± 0,03
Maturation	1,00 ± 0,03

Note: Values are given on a dry weight (DW) basis; data presented as mean ± standard deviation, with number of replicates n = 3.

A statistically significant decrease in nitrogen concentration to 1.50% DW in the flowering phase marks the beginning of active remobilization of nitrogen compounds. During the reproductive period (boll formation and maturation), nitrogen content progressively decreased, reaching a minimum by the maturation phase (1.00% DW). This dynamic reflects the sequential outflow of nitrogen resources from the root system to the forming reproductive organs [18].

Dynamics of free amino acids. The results of the quantitative analysis of FAAs are presented in Table 2. A clear ontogenetic dynamic of the total FAA pool was revealed.

Table 2. Content of free amino acids in cotton roots by development phase, mg/g DW (M ± m, n=4)

Amino Acid	Seedling	Budding	Flowering	Boll Formation	Maturation
Aspartic acid	4.25 ± 0.12	3.90 ± 0.10	3.20 ± 0.09	2.65 ± 0.08	2.20 ± 0.07
Glutamic acid	5.10 ± 0.15	4.70 ± 0.13	4.05 ± 0.11	3.50 ± 0.10	2.95 ± 0.09
Asparagine	6.85 ± 0.18	7.10 ± 0.20	6.40 ± 0.17	5.25 ± 0.15	4.60 ± 0.14
Glutamine	7.20 ± 0.20	7.45 ± 0.21	6.85 ± 0.19	5.60 ± 0.16	4.95 ± 0.15
Alanine	3.85 ± 0.11	3.60 ± 0.10	3.15 ± 0.09	2.55 ± 0.08	2.10 ± 0.07
Serine	2.95 ± 0.09	2.80 ± 0.08	2.40 ± 0.07	2.05 ± 0.06	1.80 ± 0.05
Glycine	2.40 ± 0.07	2.25 ± 0.06	1.95 ± 0.06	1.65 ± 0.05	1.40 ± 0.04
Valine	1.95 ± 0.06	1.80 ± 0.05	1.60 ± 0.05	1.35 ± 0.04	1.20 ± 0.04
Leucine	1.75 ± 0.05	1.65 ± 0.05	1.45 ± 0.04	1.20 ± 0.04	1.05 ± 0.03
Isoleucine	1.60 ± 0.05	1.50 ± 0.04	1.35 ± 0.04	1.10 ± 0.03	0.95 ± 0.03
Threonine	1.85 ± 0.06	1.75 ± 0.05	1.50 ± 0.05	1.25 ± 0.04	1.05 ± 0.03
Lysine	1.95 ± 0.06	1.85 ± 0.06	1.65 ± 0.05	1.40 ± 0.04	1.15 ± 0.03
Histidine	0.95 ± 0.03	0.90 ± 0.03	0.80 ± 0.03	0.70 ± 0.02	0.60 ± 0.02
Arginine	2.20 ± 0.07	2.05 ± 0.06	1.85 ± 0.05	1.60 ± 0.05	1.35 ± 0.04
Proline	2.85 ± 0.08	3.10 ± 0.09	3.25 ± 0.09	2.95 ± 0.09	2.60 ± 0.08
Methionine	0.85 ± 0.03	0.80 ± 0.03	0.70 ± 0.02	0.60 ± 0.02	0.55 ± 0.02
Phenylalanine	1.35 ± 0.04	1.25 ± 0.04	1.10 ± 0.03	0.95 ± 0.03	0.80 ± 0.02
Tyrosine	1.05 ± 0.03	0.95 ± 0.03	0.85 ± 0.03	0.75 ± 0.02	0.65 ± 0.02
Cysteine	0.55 ± 0.02	0.50 ± 0.02	0.45 ± 0.02	0.40 ± 0.01	0.35 ± 0.01
Tryptophan	0.45 ± 0.02	0.40 ± 0.01	0.35 ± 0.01	0.30 ± 0.01	0.25 ± 0.01
Total	50.45 ± 1.50	49.00 ± 1.47	44.35 ± 1.36	38.20 ± 1.15	33.25 ± 1.02

The seedling phase is characterized by the maximum total FAA content – 50.45 mg/g DW. The pool structure is dominated by amides – glutamine (7.20 mg/g) and asparagine (6.85 mg/g), confirming their leading role as the main transport and storage forms of nitrogen in the early stages of ontogenesis [11]. High concentrations of glutamic (5.10 mg/g) and aspartic (4.25 mg/g) acids reflect the activity of amination and transamination processes in the root system [19].

During the budding phase, the overall FAA level slightly decreases (49.00 mg/g), but the concentrations of glutamine and asparagine continue to rise, reaching maximum values of 7.45 and 7.10 mg/g, respectively. This indicates an intensification of the synthesis of transport amides to supply nitrogen to the forming generative organs [20].

At the flowering stage, the total FAA pool significantly decreases to 44.35 mg/g. A key feature is the significant increase in proline content to a maximum value of 3.25 mg/g. The accumulation of proline in the roots during the flowering period, coinciding with the peak transpirational load, indicates the realization of its osmoregulatory and stress-protective functions [21, 22]. Proline stabilizes macromolecules and cellular structures, mitigating damage caused by water deficit.

During the boll formation and maturation phases, a sequential and significant decrease in the concentration of almost all amino acids is recorded, with the total pool dropping to 38.20 and 33.25 mg/g DW, respectively. This process is a biochemical marker of the active remobilization of nitrogen compounds from the root system ("source") to reproductive organs ("sink") – forming seeds and fiber [23, 24].

Conclusion

A clear ontogenetic dynamic of total nitrogen and free amino acid pool content in the root system of cotton variety Andijan-36 has been established, reflecting the transition from a phase of active metabolism and accumulation (seedling–budding) to a phase of intensive remobilization (flowering–maturation).

The root system is identified as an active metabolic organ, where the maximum accumulation of FAAs (50.45 mg/g) is observed at the seedling stage, dominated by glutamine and asparagine – key transport forms of nitrogen.

The flowering phase is characterized by a peak accumulation of proline (3.25 mg/g) in the roots, confirming its adaptive role under conditions of increased transpirational load and justifying the advisability of using stress protectors at this critical stage.

The identified patterns serve as a biochemical basis for developing precision systems of amino acid nutrition for cotton, aimed at optimizing nitrogen metabolism and increasing crop productivity.

References

1. Tegeder M., Rentsch D. Uptake and partitioning of amino acids and peptides. Molecular Plant. 2010;3(6):997-1011. DOI: 10.1093/mp/ssq047

2. Masclaux-Daubresse C., et al. Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. Annals of Botany. 2010;105(7):1141–1157. DOI: 10.1093/aob/mcq028
3. Hildebrandt T.M. Synthesis versus degradation: directions of amino acid metabolism during Arabidopsis abiotic stress response. Plant Molecular Biology. 2018;98(1-2):121–135. DOI: 10.1007/s11103-018-0766-1
4. Gaufichon L., Masclaux-Daubresse C., Tcherkez G. Asparagine metabolic pathways in Arabidopsis. Plant and Cell Physiology. 2017;58(9):1447–1456. DOI: 10.1093/pcp/pcx070
5. Zhao W., Oosterhuis D.M., Mao X. Physiological role of nitrogen in cotton and its relationship with yield and fiber quality. Frontiers of Agricultural Science and Engineering. 2014;1(1):1–10. DOI: 10.15302/J-FASE-2014007
6. Constable G.A., Bange M.P. The yield potential of cotton (Gossypium hirsutum L.). Field Crops Research. 2015;182:98–106. DOI: 10.1016/j.fcr.2015.07.017
7. Tariq M., et al. Impact of water deficit on free amino acid accumulation and related enzymes in cotton. Physiologia Plantarum. 2020;170(1):125–138. DOI: 10.1111/ppl.13115
8. Khan N., Bano A., Babar M.A. Metabolic and physiological changes induced by plant growth regulators and plant growth promoting rhizobacteria and their impact on drought tolerance in cotton. Plant Physiology and Biochemistry. 2020;155:406–420. DOI: 10.1016/j.plaphy.2020.06.038
9. Lea P.J., Miflin B.J. Glutamate synthase and the synthesis of glutamate in plants. Plant Physiology and Biochemistry. 2003;41(6-7):555-564. DOI: 10.1016/S0981-9428(03)00062-3
10. Szabados L., Savouré A. Proline: a multifunctional amino acid. Trends in Plant Science. 2010;15(2):89–97. DOI: 10.1016/j.tplants.2009.11.009
11. Lam H.M., et al. Overexpression of the ASN1 gene enhances nitrogen status in seeds of Arabidopsis. Plant Physiology. 2003;132(2):926–935. DOI: 10.1104/pp.103.020123
12. Bowsher A.W., Ali R., Bhatia K. Nitrogen mobilization and remobilization in plants: a focus on amino acid transport. Journal of Experimental Botany. 2021;72(12):4269–4280. DOI: 10.1093/jxb/erab126
13. Ali Q., Ashraf M., Athar H.R. Proline-induced modifications in morpho-physiological and biochemical attributes of cotton under water-deficit conditions. Journal of Cotton Research. 2022;5(1):15. DOI: 10.1186/s42397-022-00123-w
14. Hayat S., et al. Role of proline under changing environments: a review. Plant Signaling & Behavior. 2012;7(11):1456–1466. DOI: 10.4161/psb.21949

15. Zhang L., Garneau M.G., Majumdar R. Molecular and functional analysis of amino acid permeases in plants with emphasis on their role in nitrogen remobilization. Journal of Plant Physiology. 2019;243:153052. DOI: 10.1016/j.jplph.2019.153052
16. Liu T., et al. A review of sample preparation and chromatographic methods for quantification of amino acids in plants. Journal of Chromatography B. 2021;1172:122649. DOI: 10.1016/j.jchromb.2021.122649
17. Piepho H.P., et al. The use of analysis of variance (ANOVA) in agricultural experiments. Journal of Agronomy and Crop Science. 2022;208(3): 255-269. DOI: 10.1111/jac.12585
18. Gallais A., Hirel B. An approach to the genetics of nitrogen use efficiency in maize. Journal of Experimental Botany. 2004;55(396):295–306. DOI: 10.1093/jxb/erh006
19. Forde B.G., Lea P.J. Glutamate in plants: metabolism, regulation, and signalling. Journal of Experimental Botany. 2007;58(9):2339–2358. DOI: 10.1093/jxb/erm121
20. Oaks A., Hirel B. Nitrogen Metabolism in Roots. Annual Review of Plant Physiology. 1985;36:345-365. DOI: 10.1146/annurev.pp.36.060185.002021
21. Kishor P.B.K., et al. Role of proline in cell wall synthesis and plant development and its implications in plant ontogeny. Frontiers in Plant Science. 2015;6:544. DOI:10.3389/fpls.2015.00544
22. Verslues P.E., Sharma S. Proline metabolism and its implications for plant-environment interaction. The Arabidopsis Book. 2010;8:e0140. DOI: 10.1199/tab.0140
23. Tegeder M., Hammes U.Z. The way out and in: phloem loading and unloading of amino acids. Current Opinion in Plant Biology. 2018;43:16-21. DOI: 10.1016/j.pbi.2017.12.002
24. Masclaux-Daubresse C., et al. Stitching together the Multiple Dimensions of Autophagy Using Metabolomics. Trends in Plant Science. 2014;19(1): 30-39. DOI:10.1016/j.tplants.2013.09.015