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PROLINE AMINO ACID CONTENT UNDER DIFFERENT WATER REGIMES IN BROWN-FIBER COTTON VARIETIES OF *GOSSYPIUM HIRSUTUM* L.

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Abstract. This article examines the role of proline amino acid in the development of brown-fiber cotton (*Gossypium hirsutum* L.) varieties (*010108, 011250, 04494, 011022, A-800-1, A-2384, A-1025*) under different water regimes. Proline plays a crucial role in enhancing plant stress tolerance, regulating metabolism, and supporting tissue development. As an osmoprotectant, it protects cotton from drought, possesses antioxidant properties, and mitigates oxidative stress. Moreover, proline is involved in energy metabolism, improving fiber length, elasticity, and quality. Its influence on the synthesis of flavonoid and phenolic compounds may also affect fiber pigmentation. Therefore, proline is one of the key factors in ensuring stable growth and high-quality fiber production in cotton.

Keywords: *Gossypium hirsutum* L., proline amino acid, stress, antioxidant, optimal water supply, water deficit.

Introduction. Under water stress conditions, the intensity of various physiological processes, such as leaf growth, stomatal conductance, photosynthesis rate, and nitrogen metabolism, decreases [1]. The study of drought stress effects on physiological processes in plants is often determined by assessing plant water status through tissue water potential measurements. Under these conditions, water potential, cell growth, and protein synthesis decline, while carbon dioxide uptake and leaf transpiration decrease. However, proline accumulation and abscisic acid levels increase [1].

Proline and hydroxyproline belong to the imino acid group because they contain an imino group (-NH-) instead of an amino group ($-NH_2$) and possess an α-carbon atom. Due to these properties, proline differs from conventional amino acids and plays a multifunctional biological role. The

strong interconnection between imino acids and amino acids allows their mutual conversion, enabling imino acids to transform into amino acids [7].

Proline is one of the universal osmolytes in plants and is an organic compound with both hydrophilic and hydrophobic properties, making it highly soluble in water. Under stress conditions, it functions as an osmoregulator, antioxidant, and chemical chaperone, playing a crucial role in protecting plant cells. Proline is transported into mitochondria by two carriers: one exclusively transports proline, while the other, known as the "proline/glutamate" antiporter, participates in proline degradation. Proline enters mitochondria via a specific uniport, and its catabolic rate is directly dependent on transport efficiency [8].

The export of glutamate from mitochondria occurs through the "proline/glutamate" antiporter, which facilitates proline exchange. Initially, proline was only recognized for its osmoregulatory function. However, subsequent research has demonstrated its multifunctional properties [9]. Beyond osmoprotection, proline acts as a "chemical" chaperone by stabilizing membrane structures and proteins under stress conditions. It also serves as a scavenger for reactive oxygen species (ROS), reducing oxidative stress. Additionally, proline plays a significant role in regulating the expression of stress-responsive genes and maintaining cellular pH. During the recovery phase, proline serves as an energy source and provides excess nitrogen and carbon [6].

Initially, proline was thought to be involved solely in osmoregulation, but later studies confirmed its role as a multifunctional metabolite. It stabilizes membrane structures and proteins, acts as an antioxidant by neutralizing reactive oxygen species, regulates pH homeostasis, and controls the expression of stress-related genes. During post-stress recovery, it functions as a source of energy, excess nitrogen, and carbon [3].

The primary pathway for proline biosynthesis originates from glutamate, where the formation of glutamate semialdehyde and 1-pyrroline-5-carboxylate (P5C) is crucial. This process involves the enzymes P5C synthetase and P5C reductase, whose gene expression increases under stress conditions. Particularly, under water deficit, this pathway represents a key adaptive response. Consequently, proline is not only essential for stress tolerance but also plays a significant role in plant development and fiber quality improvement [4].

Proline degradation occurs in mitochondria through the action of proline dehydrogenase (PDH) and P5C

dehydrogenase (P5CDH). Under conditions of elevated intracellular proline concentrations, the activity of these enzymes increases. Research has shown that proline concentration within plant cells strongly influences cellular functions. When proline levels become excessively high, its degradation intensifies, as excessive accumulation can exert toxic effects on cells [5].

Thus, the regulatory network of genes controlling proline biosynthesis and degradation is activated in response to changes in proline concentration within plant cells.

Materials and methods. As the object of the study, the following brown-fiber cotton samples belonging to the medium-fiber *Gossypium hirsutum* L. species were used: 010108, 011250, 04494, 011022, 09965, A-800, and 08492.

Determination of Proline Amino Acid Content in Cotton Leaves: For this experiment, leaf samples were taken from the 3rd-4th leaves (counting from the growth point) of cotton plants grown under field conditions. Each sample, weighing 50 mg, was placed into a test tube. The leaf samples were homogenized in a solution containing 5 mL of a 70:30 mixture of 96% ethanol and distilled water. The homogenate was heated in a water bath at 96°C for 20 minutes. After heating, the samples were centrifuged at 2500 rpm for 5-6 minutes.

The supernatant obtained from the extract was collected, and the remaining portion was mixed with a reagent solution containing 1% ninhydrin, 60% acetic acid, and 20% 96% ethanol. The mixture was further centrifuged at 10,000 rpm for 10 minutes.

The proline content was determined by measuring light absorbance at 520 nm using an Agilent Cary 60 UV-Vis spectrophotometer. The amount of proline in the cotton leaves was calculated using the following equation [2]:

Proline = (Absextract – blank)/slope * Volextract/Volaliquot * 1/FW

Where:

- ($Abs_{extra}c_t$ – Blank) / Slope = Absorbance value of the extract,
- $Vol_{extra}c_t$ = Total volume of the extract,
- $Vol_{ali}q_{uot}$ = Volume used in extraction,
- FW = Weight of the plant material used.

Results and discussion. In our experiments, two different environmental conditions were created during the peak flowering period of the brown-fiber cotton samples. The experimental water regime for cotton was conducted under different conditions. In the first condition (experimental), irrigation was

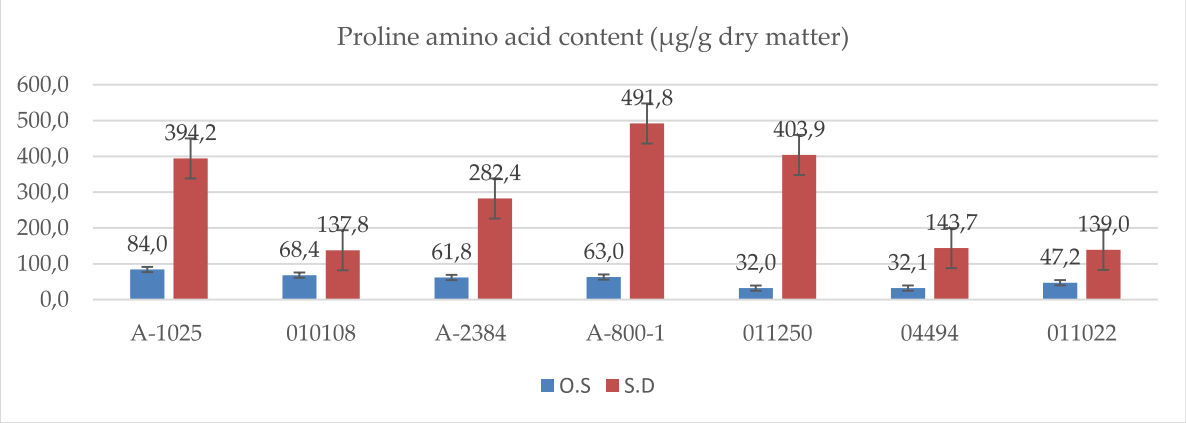
performed twice following a 1:1:0 scheme, with a total water consumption of 2800–3000 m³/ha. That is, during the experimental phase, artificial drought stress was induced by watering once during the budding stage and once again at the beginning of the flowering period. In the control condition (second condition), irrigation was performed four times following a 1:2:1 scheme, with a total water consumption of 4800–5000 m³/ha throughout the vegetation period. The experiments were conducted simultaneously for both conditions.

The proline amino acid content in the leaves of brown-fiber cotton samples was analyzed. Under drought stress conditions, the proline content increased to varying degrees in all studied varieties compared to the optimally watered condition. Under optimal water supply conditions, the highest proline content was observed in the A-1025 sample (84.0 µg/g), while the lowest levels were recorded in the 011250 and 04494 samples (32.0 µg/g and 32.1 µg/g, respectively). The remaining samples showed no significant differences in proline content (Table 1).

Under drought stress conditions, the proline amino acid content in all studied brown-fiber cotton samples increased to varying degrees compared to optimally watered conditions.

The highest proline levels under water stress were observed in the A-800, 011250, and A-1025 samples (491.8 µg/g, 403.9 µg/g, and 394.2 µg/g, respectively), while the lowest levels were detected in the 011022 and 010108 samples (139.0 µg/g and 137.8 µg/g, respectively). The high proline accumulation under drought stress suggests that these samples exhibit greater drought tolerance than others. That is, among the studied brown-fiber cotton samples, A-800, 011250, and A-1025 were identified as the most drought-tolerant varieties.

Table 1



Numerous scientific studies have reported that the accumulation of proline in drought-tolerant plants under water deficit conditions is significantly higher than in drought-sensitive plants [5]. Proline plays a crucial role in osmotic adjustment, stabilizing cellular structures, scavenging reactive oxygen species (ROS), and regulating gene expression related to stress responses. Increased proline levels are considered a key physiological marker of plant adaptation to drought stress.

Our experimental findings confirm that under drought conditions, the proline content in the leaves of the A-800, 011250, and A-1025 cotton samples was significantly higher compared to other samples. This suggests that these genotypes exhibit enhanced drought tolerance by accumulating higher levels of proline, which contributes to their ability to withstand water deficit.

Conclusion. Based on our study, it can be concluded that the proline amino acid content in the leaves of brown-fiber cotton samples increased under drought stress conditions compared to the control. This indicates that the synthesis and accumulation of proline serve as a protective mechanism against drought-induced oxidative stress.

Under drought stress, the activity of endogenous protective enzymes, along with proline accumulation, increased at varying levels across different cotton genotypes. The degree of proline accumulation was found to be closely linked to the plant's genetic potential for drought tolerance. Among the studied cotton samples, A-800, 011250, and A-1025 exhibited higher proline content under water deficit conditions, suggesting that these genotypes possess superior drought resilience.

These findings highlight the importance of proline as a biochemical marker for selecting drought-tolerant cotton varieties. Further research focusing on the genetic regulation of proline metabolism and its interaction with other stress-responsive pathways could provide valuable insights into developing more resilient cotton cultivars for arid and semi-arid regions.

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