

Determination of Chlorophyll Content in Leaves of *G. Hirsutum* L. Species in Conditions of Water Shortage

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Abstract: *In this experiment, the amount of chlorophyll in the leaves of Ishonch and Tashkent-6 varieties of cotton was studied under optimal and low water conditions. Compared to optimal conditions, the amount of chlorophyll "a", chlorophyll "b", total chlorophyll and carotenoids of both varieties decreased to different degrees in conditions of water shortage. The chlorophyll stability index in the trust variety was 85.9%, and in the Tashkent-6 variety it was 83.8%.*

1. Introduction

Currently, there is a shortage of water in various parts of the world. According to an FAO (2005) analysis, by 2050, drought and salinity could lead to a sharp deterioration in land quality in more than 50% of many regions of the world. Scientists point out that by 2050, the amount of water on Earth could be halved (S.M. Vicente-Serrano, C. Gouveiab et al., 2013).

It has been found that lack of water in plants has a negative impact on their development and productivity (A. A. Apchelimov., O. P. Soldatova., 2009). According to Wang et al. (2003), drought has reduced crop yields by up to 50%.

Abiotic stresses lead to a number of morphological, physiological, biochemical and molecular changes and affect productivity (Z. Davlatnazarova., K.A. Aliev., 1997). Stress reduces photosynthetic activity. It affects the permeability of membranes and the function of enzymes, accelerates the metabolic process and leads to the accumulation of active oxygen radicals. In this case, premature cell death has been identified (B.A. Beknazarov., 2009).

Chlorophyll is one of the main constituents of chloroplasts. Chlorophyll "a" and "b" in chlorophyll play an important role in the process of photosynthesis and ultimately affect plant growth and development (L. Taiz and E. Zieger, 2006). The decrease in photosynthesis is due to the main components of the chloroplast, which may directly limit the photosynthetic potential (M. Maisura et al., 2014).

Drought stress is one of the factors influencing the ratio of chlorophyll "a" and "b" to total chlorophyll and "a / b" (M. Havaux., 1998; S. Delfine et al., 1998; M. Ashraf and S. .Ahmad., 2000; S. Kiani et al., 2008; A. Massacci et al., 2008; M. Hamayun et al., 2010). In drought-tolerant varieties of chlorophyll, chlorophyll "a", chlorophyll "b" and total chlorophyll content were significantly reduced in drought conditions (P. Manivannan et al., 2007b). When two olive varieties were grown under drought conditions, total chlorophyll content was found to decrease from 29% to 42% (M. Guerfel et al., 2009). The cotton plant is characterized by a decrease in chlorophyll in drought conditions (A. Massacci et al., 2008).

The chlorophyll stability index is the ratio of the total chlorophyll content of a plant grown in optimal conditions to the total chlorophyll content of a plant in a drought environment. A high chlorophyll stability index is one of the hallmarks of drought tolerance (R. K. Sairam et al., 1997). It is effective to pay attention to this sign in determining the drought tolerance of plants (D.A. Johnson., 1980).

Decreases in chlorophyll levels under drought stress have been reported to be associated with chlorophyll degradation during photo-oxidation (S. Delfine et al., 1998; M. Ashraf., 2009; and M. Hamayun et al., 2010). Both chlorophyll "a" and "b" change in drought environments (M. Farooq et al., 2009).

The main reason for the decrease in chlorophyll content under drought stress is the slowing down of photosynthetic activity. In addition, chlorophyll loss in plant tissue in a water-deficient environment results in swelling of the chloroplast shells, disruption of lamella vesiculation, and accumulation of lipid droplets (W.M. Kaiser et al., 1981).

The low concentration of photosynthetic pigments and the decrease in photosynthetic potential limit its ability to produce basic products. The amount of chlorophyll in the leaf is one of the important parameters from a physiological point of view. Loss of chlorophyll content in a water-deficient environment has been reported to occur with the destruction of masked cells in plants (A. A. Shakeel et al., 2011).

2. Research Methods

In our research, Ishonch and Tashkent-6 varieties belonging to *G. hirsutum* L. were grown in different water regimes (optimal water supply and modeled drought). Ishonch type F3 [*G. barbadense* (C-6037) x Tashkent-6] x is based on the L-27 ridge from the Tashkent-6 combination, while the Tashkent-6 variety is based on the combination of {(C-4727 x *G. hirsutum* ssp. *mexicanum*) x S-4227}. Under lysimeter conditions the seeds were sown in the scheme 90x20x1, at a depth of 4-5 cm above ground level. In both backgrounds, the varieties were placed in three returns, with 12 cell rows in each return in a randomized manner. Irrigation in the scheme of 1-2-1 under the optimal water regime (total water content 4800-5000 m³ / ha), and against the background of

artificial drought in the scheme 1-1-0 (total water content 2800-3000 m³ / ha) was held. The same agro-technical work was carried out on both backgrounds.

To determine the amount of chlorophyll "a", chlorophyll "b" and carotenoids, samples were taken from leaves 3-4, counting from the point of growth of the cotton plant. Each leaf was washed twice and placed in 3 test tubes of 50 g. 80 ml of acetone was added to 5 ml of each solution, and the leaf samples were homogenized and centrifuged at 5,000 rpm for 10 min. At 470 nm, Agilent Cary was detected on a 60 UV-Vis brand spectrophotometer. Chlorophyll "a", chlorophyll "b" and carotenoids were determined using the equation (K. H. Lichtenthaler., 1983; Nayek Sumanta., 2014), the chlorophyll stability index was determined by R.K. Sairam (1997):

$$\text{Chl "a"} [\text{mg/l}] = 12,25 * A_{663,2} - 2,79 * A_{646,8}$$

$$\text{Chl "b"} [\text{mg/l}] = 21,5 * A_{646,8} - 5,1 * A_{663,2}$$

$$\text{car} [\text{mg/l}] = ((1000 * A_{470}) - (1,82 * \text{Chla}) - (85,02 * \text{Chl b})) / 198$$

$$F [\text{mg/g}] = (V \cdot C) / P$$

F— pigment content in plant leaf samples [mg/g];

V — liquid volume, [ml];

C — pigment concentration, [mg/l];

P — weight of the plant tissue, [g]

Chlorophyll Stability Index (CSI)

$$CSI = \frac{\text{total chlorophyll under water stress}}{\text{total chlorophyll under normal condition}} * 100\%$$

3. Research Results

A number of scholars (JL Araus et al., 1998; F. Anjum et al., 2003; S. Kiani et al., 2008; A. Massacci et al., 2008 and M. Hamayun et al., 2010) Under the influence of drought, the amount of chlorophyll "a", "b" and total chlorophyll in the leaves of the plant decreases.

According to the analysis of the results of our research, the difference between the levels of chlorophyll "a", chlorophyll "b", total chlorophyll and carotenoids in the leaves of Ishonch and Tashkent-6 varieties is reliable in terms of optimal water supply and water scarcity (Table 1).

At the same time, under the conditions of optimal water supply (control option), the average value of chlorophyll "a" in the Trust variety was 1.61 ± 0.05 mg / g, and in the absence of water - 1.40 ± 0.06 mg / g. In the Tashkent-6 variety, the mean value was 1.70 ± 0.02 mg / g in the control variant, and 1.41 ± 0.03 mg / g in the water shortage.

The decrease in the amount of chlorophyll "a" during drought may be related to the inhibition of the oxidant in the photo-oxidation process (V. Verma et al., 2004; M. Farooq et al., 2009).

In the control (optimal water regime) variant, the average content of chlorophyll "b" in the leaves of the Trust variety was 0.59 ± 0.02 mg / g, in the experimental (water deficiency) variant 0.49 ± 0.02 mg / g, and in the Tashkent-6 variety it was 0.59 ± 0.01 mg / g and 0.51 ± 0.01 mg / g, respectively (Table 1).

It should be noted that some studies have shown a slight increase in the amount of chlorophyll "b" in resistant samples under water shortages compared to resistant samples (M. Muhamad., 2014).

The value of the chlorophyll a / b ratio depends on the amount of chlorophyll "a" and chlorophyll "b" (M. Maisura et al., 2014). In the experiments of F. Anjum and M. Farooq, changes in the ratio of chlorophyll a / b and the amount of carotenoids in plants under conditions of low water supply compared to the optimal water regime were observed (F. Anjum et al., 2003; M. Farooq et al., 2009).

Analysis of our results on this indicator 2.86 ± 0.054 in the case of low water supply (experimental variant), 2.73 ± 0.071 in the control variant, and 2.76 ± 0.015 in the Tashkent-6 variety, respectively and showed that it was 2.88 ± 0.038 . (Table 1). It has been found that drought stress causes severe damage to the activity of photosynthetic reaction centers, which leads to an increase in the amount of chlorophyll "b" and a decrease in the ratio of chlorophyll a / b (A. A. Mir., 2013).

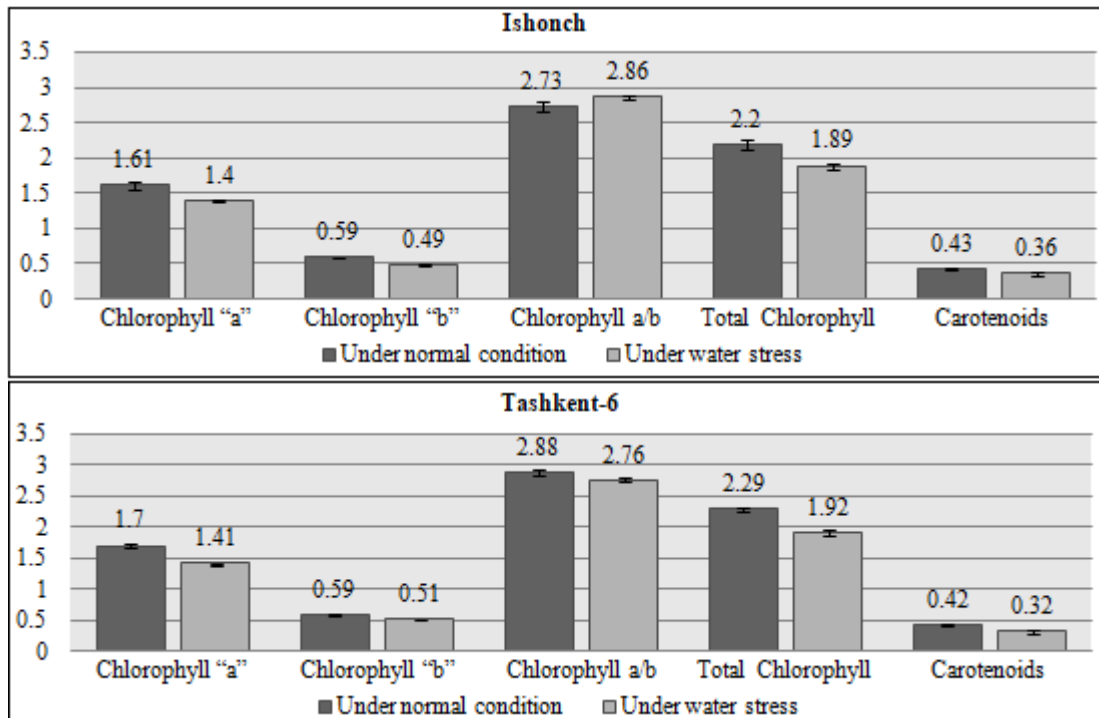
The total chlorophyll content in the control variant of Ishonch and Tashkent-6 cotton varieties was 2.20 ± 0.06 and 2.29 ± 0.03 mg / g, respectively, and in the experimental variant 1.89 ± 0.06 mg / g. and 1.92 ± 0.04 mg / g. (Table 1). M. D. Patil et al. (2011) found that the chlorophyll stability index was higher in drought-tolerant plant genotypes than in non-drought-tolerant genotypes. In our experiment, the chlorophyll stability index was 85.9% in the Trust variety and 83.8% in the Tashkent-6 variety. It was noted that the amount of chlorophyll in the leaves of Ishonch plant is relatively stable compared to the amount of chlorophyll in the leaves of Tashkent-6 plant in the conditions of water shortage.

Carotenoids can protect plants from photo-oxidation (A. A. Mir et al., 2013). In the experiments of A. K. Parida (2007), the content of chlorophyll and carotenoids in cotton genotypes decreased in a low-water environment and an increase in the amount of chlorophyll and carotenoids through re-irrigation.

In caution, the amount of carotenoids was 0.36 ± 0.02 mg / g under low water conditions (experimental variant) and 0.43 ± 0.02 mg / g under normal water regime conditions (control variant). formed. These values are 0.32 ± 0.01 mg / g and 0.42 ± 0.01 mg / g, respectively, in the Tashkent-6 variety, and the amount of carotenoids in water shortages is higher than in the vulnerable variety - Tashkent-6.

4. Conclusion

Changes in the amount of chlorophyll and carotenoids in the leaves of cotton plants were found under conditions of low water supply compared to the optimal water regime. Under conditions of low water supply, the total chlorophyll, chlorophyll a / b and carotenoids in the Ishonch variety showed less change than in the Tashkent-6 variety. In our experience, the Trust variety can be used as a primary source for obtaining drought-resistant genotypes.



References

- [1] Anjum, F., Yaseen M., Rasule., Wahid A and Anjum S (2003). Water stress in barley (*Hordeum vulgare* L.). I. Effect on chemical composition and chlorophyll contents. *Pakistan J. Agric. Sci.*, (40): 45–49.
- [2] Araus, J. L., Amaro T., Voltas J., Nakkoul H and Nachit M. M (1998). Chlorophyll fluorescence as a selection criterion for grain yield in durum wheat under Mediterranean conditions. *Field Crops Research*, (55): 209-223.
- [3] Ashraf, M. (2009). Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotechnol Adv.*, (27): 84–93.
- [4] Ashraf, M. and S. Ahmad (2000). Influence of sodium chloride on ion accumulation, yield components and fibre characteristics in salt tolerant and salt-sensitive lines of cotton (*Gossypium hirsutum* L.). *Field Crop Res.*, (66): 115–127.
- [5] B.O. Beknazarov. "O'simliklar fiziologiyasi" Toshkent – "Aloqachi" – 2009. 424-443-betlar.
- [6] Delfine, S., Alvino A., Zacchini M and Loreto F (1998). Consequences of salt stress on diffusive conductance, Rubisco characteristics and anatomy of spinach leaves. *Aust J. Plant Physiol.*, (25): 395–402.
- [7] Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA (2009). Plant drought stress: effects, mechanisms and management. *Agron. Sustain. Dev.*, 29: 185-212.
- [8] Guerfel M, Baccouri O, Boujnah D, Chaibi W, Zarrouk M (2009). Impacts of water stress on gas exchange, water relations, chlorophyll content and leaf structure in the two main Tunisian olive (*Olea europaea* L.) cultivars. *Sci. Horticult.*, 119: 257-263
- [9] Hamayun, M.; S. A. Khan; Z. K. Shinwari; A. L. Khan; N. Ahmad and I. J. Lee (2010). Effect of polyethylene glycol induced drought stress on physiological attributes of soybean. *Pakistan J. Bot.*, (42): 977–986.
- [10] Havaux, M. (1998). Carotenoids as membrane stabilizers in chloroplasts. *Trends Plant Sci.*, (3): 147–151.
- [11] Johnson, D. A. (1980). Improvement of perennial herbaceous plants for drought-stressed western rangelands. p. 419-433. In N.C. Turner and P.J. Kramer (ed.) *Adaptation of plants to water and high temperature stress*. John Wiley & Sons, New York.
- [12] Kaiser W M, Kaiser G, Schöner S, Neimanis S (1981). Photosynthesis under osmotic stress. Differential recovery of photosynthetic activities of stroma enzymes, intact chloroplasts and leaf slices after exposure to high solute concentrations. *Planta*, 153: 430-435.
- [13] Kiani, S.P., Maury P., Sarrafi A and Grieu P. (2008). QTL analysis of chlorophyll fluorescence parameters in sunflower (*Helianthus annuus* L.) under well-watered and water-stressed conditions. *Plant Sci.*, (175): 565–573.
- [14] Lichtenthaler H. K. and Wellburn, A. R., Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents, *Biochem. Soc. Trans.*, 11, 591–592 (1983)
- [15] Maisura Muhammad, Achmad Chozin, Iskandar Lubis, Ahmad Junaedi and Hiroshi Ehara, "Some physiological character responses of rice under drought conditions in a paddy system" *J. ISSAAS* Vol. 20, No. 1: 104-114 (2014)
- [16] Manivannan P, Jaleel CA, Kishorekumar A, Sankar B, Somasundaram R, Sridharan R, Panneerselvam R (2007a). Changes in antioxidant metabolism of *Vigna unguiculata* L. Walp. by propanil under water deficit stress. *Colloids Surf B: Biointerf.*, 57: 69-74.
- [17] Manivannan P, Jaleel CA, Sankar B, Kishorekumar A, Somasundaram R, Alagu Lakshmanan GM, Panneerselvam R (2007b). Growth, biochemical modifications and proline metabolism in *Helianthus*

- annuus L. as induced by drought stress. *Colloids Surf. B: Biointerf.*, 59: 141-149
- [18] Massacci A., Nabiev S.M., Pietrosanti L., Nematov S.K., Chernikova T.N., Thor K and Leipner J., 2008. Response of the photosynthetic apparatus of cotton (*Gossypiumhirsutum*) to the onset of drought stress under field conditions studied by gas-exchange analysis and chlorophyll fluorescence imaging. *Plant Physiol. Biochem.*, 46: 189– 195
- [19] Mir Aafaq Ahmad., Murali P.V and Panneerselvam R. Drought stress induced biochemical alterations in two varieties of *Paspalumscrobiculatum* L. *INT J CURR SCI* 2013, 7: E 80-96
- [20] NayekSumanta, ChoudhuryImranulHaque, JaisheeNishika and Roy Suprakash. Spectrophotometric Analysis of Chlorophylls and Carotenoids from Commonly Grown Fern Species by Using Various Extracting Solvents. *International Science Congress. Journal of Chemical Sciences.* 63-69 september(2014)
- [21] Patil M. D., Biradar D. P., Patil V. C. and Janagoudar B. S., (2011). Response of cotton genotypes to drought mitigation practices Am-Euras. *J. Agric. & Environ. Sci.*, 11 (3): 360-364
- [22] Parida, A. K., Dagaonkar, V. S., Phalak, M. S., Umalkar, G. V., L. P. and Aurangabadkar, L. P. 2007. Alterations in Photosynthetic Pigments, Protein and Osmotic Components in Cotton Genotypes Subjected to Short-term Drought Stress Followed by Recovery. *Plant Biotechnol. Rep.*, 1: 37–48
- [23] Sairam R. K, Deshmukh P. S, Shukla D. S (1997). Tolerance of drought and temperature stress in relation to increased antioxidant enzyme activity in wheat. *J agron Crop Sci* 178:171-178
- [24] Shakeel Ahmad Anjum , Xiao-yu Xie1 , Long-chang Wang , Muhammad FarrukhSaleem , Chen Man and Wang Lei(2011). Morphological, physiological and biochemical responses of plants to drought stress. *African Journal of Agricultural Research* Vol. 6(9), pp. 2026-2032, 4 May, 2011
- [25] Taiz, L. and Zeiger E. (2006). *Plant Physiology*, 4th Ed., Sinauer Associates Inc. Publishers, Massachusetts.
- [26] Vicente-Serrano SM, Gouveiab C, Camarero JJ, Begueríae S, Trigob R, López-Morenoa JI, Azorín-Molinaa C, Pashoa E, Lorenzo-Lacruza J, Revueltoa J, Morán-Tejedaa E, Sanchez-Lorenzoga A . Response of vegetation to drought time-scales across global land biomes. *Proceedings of the National Academy of Sciences.* 2013;110:52–57. DOI: 10.1073/pnas.1207068110
- [27] Verma, V., M.J. Foulces, A. J. Worland, S. R. Bradley, P.D.S. Caligari and J.W. Snape. 2004. Mapping quantitative trait loci for flag leaf senescence as a yield determinant in winter wheat under optimal and drought-stressed environments. *Euphytica*, 135:255-263.
- [28] Wang W, Vinocur B, Altman A . Plant responses to drought, salinity and extremetemperatures: towards genetic engineering for stress tolerance. *Planta.* 2003;218:1-14.DOI: 10.1007/s00425-003-1105-5
- [29] Апчелимов А.А., Солдатова О.П. Ген ATASE2 контролирует устойчивость растений *Arabidopsistraliana* к гербициду ацифлюорфену. Съезд генетиков и селекционеров, посвященный 200-летию со дня рождения Чарлз Дарвина. V-съезд Вав.общ.ген.сел. –Москва. 2009, С. 172.
- [30] Давлатназарова З., Алиев К.А. (1997). Морфологических особенности картофеля в условиях абиотического стресса. стр 27-30.