

Dynamics of accumulation of phenolic compounds, anthocyanins, and photosynthetic pigments in the vegetative organs of the wheat plants under continuous drought stress

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Phenolic compounds play an important role in photosynthesis and respiration processes, growth and development, reproduction, and immunity of plants. Although progress is gained in the research of the chemical composition and biosynthesis of phenolic compounds and their intracellular localization, the role of phenolic compounds in the protection of plants from adverse environmental factors, including drought, has not been fully studied. This problem is still relevant today, as a number of important crops, including durum wheat (*Triticum durum* Desf.), are exposed to soil and atmospheric drought during their development. The main purpose of the research was to determine the role of phenolic compounds, anthocyanins, and photosynthetic pigments in the protection of wheat from drought. For analysis, plant samples (Barakatli 95 (drought-tolerant) and Garagylchyg 2 (drought-sensitive)) were taken during the generative development of the plant - flowering, milk ripeness, and wax ripeness. Root and leaf samples were crushed in liquid nitrogen, then the amount of dissolved phenolic compounds was determined based on the interaction of phenols with the Folin-Ciocalteu reagent. The amount of anthocyanins and photosynthetic pigments decreased, the phenolic compounds reached the maximum at the end of the milk ripeness phase and then decreased relatively, while this parameter remained higher than in the watered variant. The amount of phenolic compounds in the leaves of both genotypes was found to increase under drought. In this case, more phenolic compounds were synthesized in the tolerant genotype compared to the sensitive genotype. Besides, under drought conditions, wheat leaves were found to be able to synthesize more phenolic compounds than roots.

Keywords: *Triticum durum* Desf., drought, phenolic compounds, photosynthetic pigments

INTRODUCTION

Drought tolerance of plants is regulated by several physiological and biochemical processes. Drought stress causes the formation of reactive oxygen species, which lead to destructive changes in cells, lipid peroxidation, protein oxidation, etc. As a result of drought, some compounds such as proline, α -tocopherol, and polyphenols are accumulated in plant cells, which in turn protect the plant from oxidative damage and maintain the turgor pressure of cells (Faize et al., 2011). Non-enzymatic antioxidants such as pigments, ascorbic acid, carotenoids, anthocyanins, phenols, and flavonoids play a major defense role in the neutralization of reactive oxygen species (ROS). In the studies conducted by Šircelj and co-authors (2005), 3 main types of plant responses to drought stress are noted: prevention of water loss (e.g. osmotic regulation), protection of cell components (e.g. quantitative and qualitative changes in pigments), repair of oxidative damage (e.g.

antioxidant defense systems). Drought stress disrupts the balance between the generation and utilization of reactive oxygen species in plant cells, resulting in oxidative stress (Neill et al., 2002). To reduce the damage caused by ROS, plants have a strong antioxidant defense system, which includes both antioxidant enzymes and low-molecular-weight secondary metabolites - ascorbic acid, glutathione, tocopherols, carotenoids, and phenolic compounds (Posmyk et al., 2005). Another characteristic feature of higher plants is their ability to synthesize a large amount of secondary compounds under stress conditions (Wink, 2003). These compounds differ from each other both in their structure and function. Such substances include phenolic compounds, terpenoids, alkaloids, and saponins. Phenolic compounds, unlike other compounds of secondary metabolism, are universal components of plant tissues (Harborne, 1980). They play an important role in the processes of photosynthesis and respiration, growth and development, reproduction, and

immunity in plants. In addition, phenolic compounds are also of practical importance (Harborne, 1980; Dixon, Pariva 1995). They are used both in the food industry and in medicine as important drugs and vitamin P preparations (Cheynier, 2005; Zeynalova and Novruzov, 2018). The research shows that wheat grains are rich in phenolic compounds that are beneficial for human health. The most common phenolic compounds in wheat are phenolic acids and flavonoids located in the outer layer of the grain. Many of the phenolic compounds in bran participate in gastrointestinal digestion together with carbohydrates, where they ensure antioxidant protection. Currently, during the creation of modern wheat varieties, preference is given to genotypes with a high amount of phenolic compounds - phenolic acids, flavonoids, anthocyanins, coumarins, and lignans (Shahidi and Ambigaipalan, 2015). Various phenolic compounds are synthesized in wheat, as well as in other higher plants. These compounds are found both in monomeric (phenylpropanoids, flavonoids) and polymeric (lignin) forms. Although phenylpropanoids are found in all plant organs, flavonoids are synthesized only in leaves. This may be attributed to the localization of chloroplasts in the leaf, which is the main synthesis site of those compounds. According to the literature data, more polyphenols are accumulated in the green tissues of the plant. The study of the amount of soluble phenolic compounds in the leaves of different wheat varieties showed that, although in most cases, winter wheat and spring wheat have similar phenolic metabolism, the varietal diversity is mainly manifested in the level of flavonoids. According to literature data, the activation of phenolic metabolism in wheat leaves at low temperatures indicates that secondary metabolites play an important role in plant adaptation to stress (Posmyk et al., 2005). Polyphenols are known to be low-molecular-weight antioxidants protecting plants from oxidative stress under stress conditions (Larson 1988; Wingsle et al., 1999). Due to their high reactivity, the presence of aromatic rings, and free hydroxyl groups in their structure, these compounds easily participate in free radical reactions and utilize reactive oxygen species and peroxide radicals under stress conditions (Zhao, Zou, 2002). At this time, both phenylpropanoids and flavonoids act as antioxidants. Apigenin, a phenolic compound found in wheat leaves, has an antioxidant capacity similar to ascorbic acid (Lin et al., 2002). At low temperatures, the antioxidant properties of phenolic compounds are of particular importance for leaves, because leaves are more exposed to the effects of heat and frost compared to other organs. Besides, phenolic compounds act as substrates for peroxidases localized in the vacuole and, together with peroxidases, participate in the protection of the

cell from reactive oxygen species (Janas et al., 2002). The activity of soluble peroxidases in wheat tissues is inversely related to the dynamics of accumulation of polyphenols, which can regulate peroxidation processes in the plant. Phenolic compounds together with other soluble metabolites accumulated in cells (first of all, sugars and soluble proteins) play the role of cryoprotectors, regulate the temperature inside the cell and protect the plant from freezing by increasing its frost tolerance. It should be noted that, unlike other secondary metabolites, phenolic compounds can be easily oxidized and have the ability to react with proteins due to hydrogen bonds. Therefore, some phenolic compounds are involved in the regulation of enzyme activities. Studies show that daphnetin (7,8-dihydroxycoumarin) as an inhibitor of protein kinases is involved in the transduction of signals of various natures (Ndong et al., 2003). Although there have been enough achievements in the study of the chemical composition and biosynthesis of phenolic compounds, and their intracellular localization in the modern period, the role of polyphenols in the life of plants has not been fully studied. The share of phenolic compounds in the protection of plants from adverse environmental factors, including drought, has not been fully studied. This problem is still relevant today, because a number of agriculturally important plants, including durum wheat (*Triticum durum* Desf.), are exposed to soil and atmospheric drought during their development. Although there is enough information about the effect of temperature on lipids, proteins, and carbohydrate metabolism in plant cells (Levitt, 1980; Chirstiansen, 1985). there is very little information about their effect on phenol metabolism. The main goal of the research work was the study of the dynamics of the accumulation of soluble phenolic compounds, photosynthetic pigments, and anthocyanins in wheat plants exposed to drought during the generative development period.

MATERIALS AND METHODS

The objects of the research. Barakatli 95 (drought-tolerant) and Garagylchyg 2 (drought-sensitive) varieties of local durum wheat (*Triticum durum* Desf.) with contrasting tolerance and productivity obtained from the Genbank of the Research Institute of Crop Husbandry of the Ministry of Agriculture of the Republic of Azerbaijan were used as the research objects. The wheat seeds used in the research were planted in 2016-2019 in the experimental field of Plant Physiology and Biotechnology Department of Absheron Experimental Station (AES) of the Research Institute of Crop Husbandry of the

Ministry of Agriculture of the Republic of Azerbaijan, in 3 replications, with 400 seeds per 1 m².

Quantification of soluble phenolic compounds. The amount of soluble phenolic compounds was determined by the interaction of phenols with the Folin-Ciocalteu reagent (Naczka and Shahidi, 1989). After incubating 50 mg of dried leaf samples in 80% ethanol in a water bath with a temperature of 80°C, the obtained extract is centrifuged at a speed of 12,000 g for 10 min. After adding 0.5-0.7 ml of 80% ethanol to the obtained supernatant, it is mixed and centrifuged again under the same conditions. After adding 2.5 ml of diluted Folin-Ciocalteu and 2.0 ml of Na₂CO₃ (75 g/l) solution to 0.5 ml of supernatant, it is incubated for 2 hours with continuous stirring, then the optical density was measured at 765 nm (Thermo Scientific Evolution 350 UV-Vis Spectrophotometer (UK, England)). The calibration curve was constructed based on different concentrations of gallic acid in ethanol. The amount of soluble phenolic compounds was determined by the following formula:

$$F = (C \cdot V_{\text{extract}}) / m \cdot 1000,$$

where: F – total amount of phenolic compounds (mg Gallic acid/g dry biomass); C – the value obtained from the calibration curve; V – total volume of the extract; m – leaf mass; 1000 – the coefficient to convert liter to ml.

Quantification of photosynthetic pigments and anthocyanins. Wheat leaf samples were extracted with 80% acetone/Tris (80:20; pH 7.8) solution (Sims, Gammon, 2002). The amounts of photosynthetic pigments and anthocyanins were determined spectrophotometrically (Thermo Scientific Evolution 350 UV-Vis Spectrophotometer (UK, England)), optical density was measured and the following formulas were used for calculations:

$$\text{Chl } a \text{ (}\mu\text{mol/ml)} = 0.01373 A_{663} - 0.000897 A_{537} - 0.003046 A_{647}$$

$$\text{Chl } b \text{ (}\mu\text{mol/ml)} = 0.02405 A_{647} - 0.004305 A_{537} - 0.005507 A_{663}$$

$$\text{anthocyanins (}\mu\text{mol/ml)} = 0.08173 A_{537} - 0.00697 A_{647} - 0.002228 A_{663}$$

$$\text{carotenoids (}\mu\text{mol/ml)} = (A_{470} - (17.1 \times (\text{Chl } a + \text{Chl } b) - 9.479 \times \text{anthocyanin})) / 119.26$$

Statistical analysis: All experiments were performed in 3 replicates and errors were calculated using Student's t-test statistical analysis program. When the value of P is <0.01, 0.05, the differences between the mean values were considered significant.

RESULTS AND DISCUSSION

Drought is one of the main environmental factors that negatively affect the development and

productivity of plants and inhibit the process of photosynthesis. The effect of drought caused by high temperature on grain yield depends on the drought tolerance of the genotype, the intensity and duration of the stress, and the reproductive development phase of the plant. Studies show that long-term soil drought seriously damages the photosynthetic apparatus and photosynthetic pigments, as a result of which the assimilation of carbon dioxide by the plant weakens and the amount of chlorophyll decreases (Saeidi and Abdoli, 2015). The decrease in the amount of total chlorophyll and the ratio of chlorophyll a/b is more noticeable in sensitive genotypes than in tolerant genotypes. The samples from plants grown under field conditions and exposed to long-term soil drought were taken in the generative development phase of the plant - flowering, milk ripeness, wax ripeness phases, and the amounts of anthocyanins and photosynthetic pigments were measured after the leaf samples were crushed and frozen in liquid nitrogen. The amount of chlorophyll was found to decrease significantly in both tolerant and sensitive genotypes under drought compared to the watered variant. This decrease was more significant in Garagylchyg 2 (Fig. 1).

As seen in the Fig. 1, as drought deepens, the ratio chl a/b decreases. The obtained results are consistent with the literature data. Research shows that the amount of photosynthetic pigments (Chl a and b, carotenoids) decreases under water shortage conditions, and plants having more chlorophyll are characterized as drought tolerant (Farshadfar and Amiri, 2015). Drought causes changes in the rate of photosynthesis and the permeability of chloroplast membranes. This can be explained by inhibition of chlorophyll degradation or synthesis, as well as disruption of the thylakoid membrane integrity (Tabaeizadeh, 1998). In our studies, the chl a/b ratio decreased in both genotypes exposed to drought: in the Barakatli 95 genotype, the ratio amounted to 3.08 in the flowering phase, 2.49 in the milk ripeness phase, and 2.23 in the wax ripeness phase. For Garagylchyg 2, these parameters were 2.61, 2.38, and 2.19, respectively. The Chl a/b ratio is more sensitive to drought, which is attributed to faster degradation of chlorophyll a compared to chlorophyll b under the effect of a stressor (Fang et al., 1998). During drought, the destruction of pigments and destabilization of the photosynthetic membrane lead to a decrease in the amount of chlorophyll (Saeidi and Abdoli, 2015). Ciscato et al. (1997) noted that the decrease in chlorophyll content and the chl a/b ratio in maize may be related to a direct effect of drought on the light-harvesting complex of PS2.

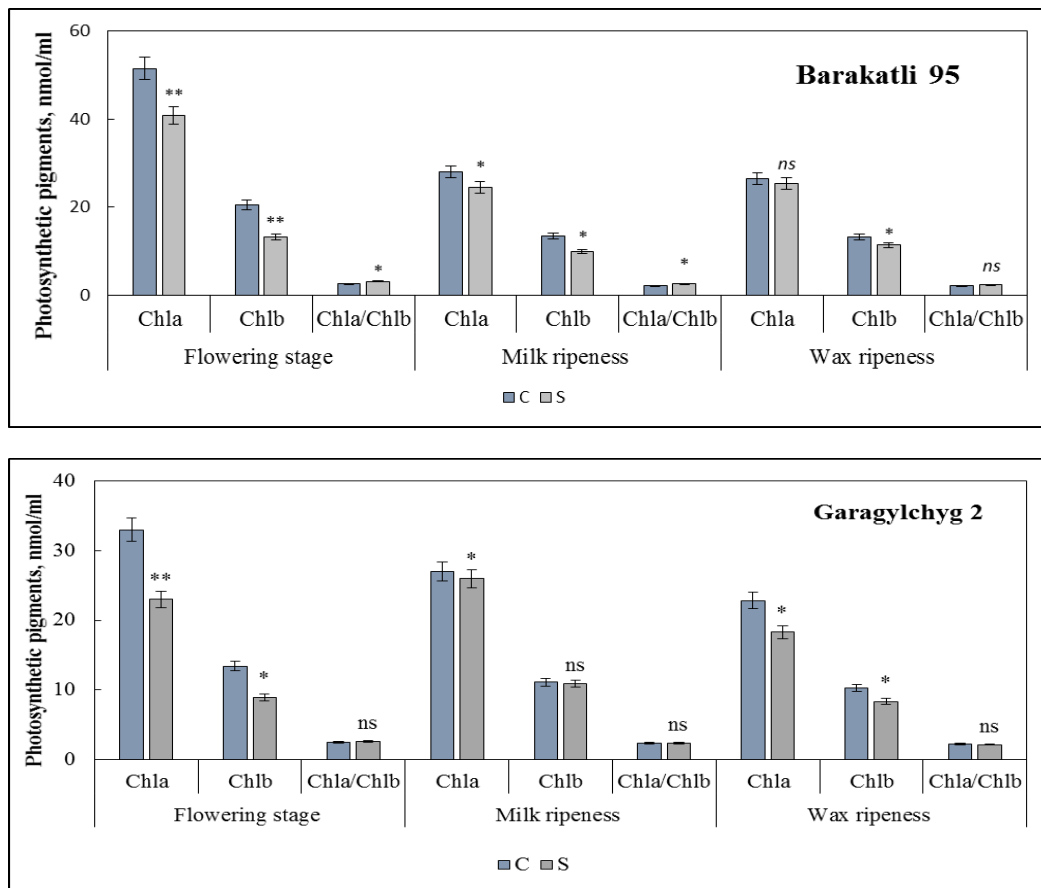


Fig. 1. The amount of photosynthetic pigments in leaves of durum wheat genotypes during the generative development period (nmol/ml). Statistical analyses were performed according to Student's t-test: **, * - respectively, <0.01, <0.05 significant at probability levels, ns - not significant.

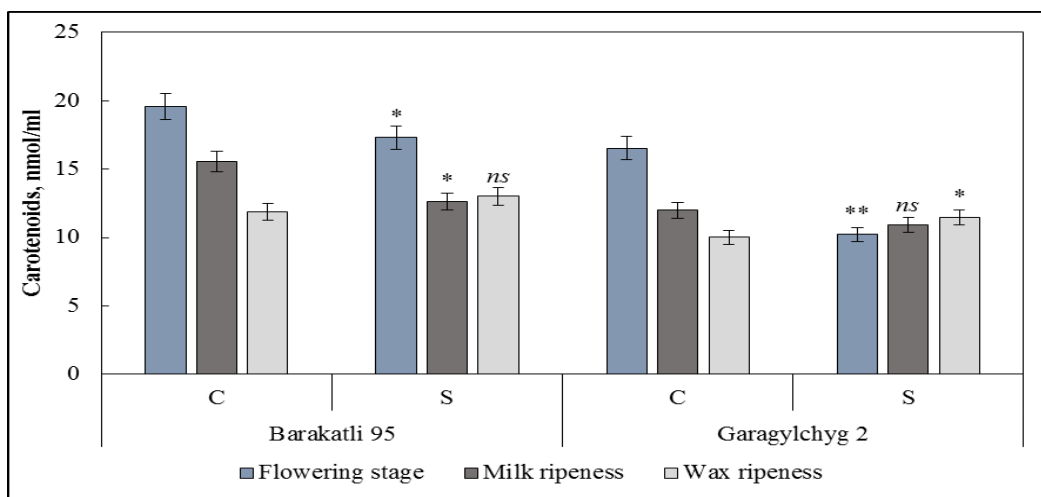


Fig. 2. The amount of carotenoids in leaves of durum wheat genotypes during generative development (nmol/ml). Statistical analyses were performed according to Student's t-test: **, * - respectively, <0.01, <0.05 significant at probability levels, ns - not significant.

Carotenoids are low-molecular-mass antioxidants involved in the plant defense against stress. In our experiments, a decrease in the amount of carotenoids was observed in the stress variants compared to the control during the flowering and milk ripeness phases of both genotypes. It is interesting that in the deeper phase of drought - the

wax ripeness phase, the amount of these substances increases in both Barakatli 95 and Garagylchyg 2 genotypes in the stressed variants compared to the normally watered variant (Fig. 2). This shows that carotenoids play a protective role against reactive oxygen species under stress conditions.

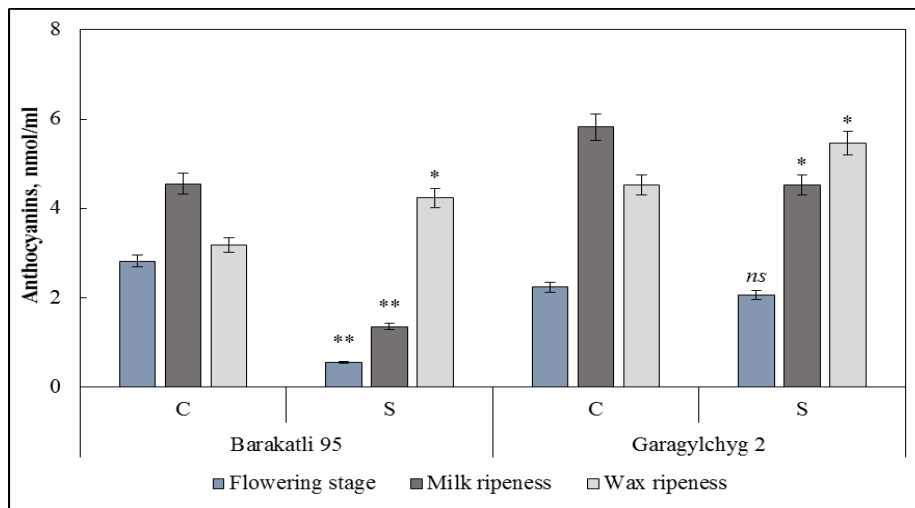


Fig. 3. The amount of anthocyanins in the leaves of durum wheat genotypes during the generative development period (nmol/ml). Statistical analyses were performed according to Student's t-test: **, * - respectively, <0.01, <0.05 significant at probability levels, ns - not significant.

Anthocyanins and flavonoids reduce the risk of photooxidative damage to cells by absorbing excess solar energy (Landi et al., 2015; Cirillo et al., 2021). Various types of stress activate the synthesis of anthocyanins. Synthesis of anthocyanins in plant cells increases intensively under conditions of soil drought, solar radiation, and water scarcity, which ensures their adaptation to unfavorable environmental conditions. In our studies, the amount of anthocyanins increased in the flowering and milk ripeness phases, and a decrease was observed in the wax ripeness phase of the control variants (Fig. 3). In drought-exposed genotypes, the synthesis of these secondary metabolites was stimulated in all studied phases, increased more sharply when the drought deepened, and this increase was statistically significant in the tolerant variety compared to the sensitive variety. Thus, compared to the flowering phase, in the milk ripeness phase, a 2.5-fold increase was observed in the Barakatli 95 genotype and a 2.2-fold increase in the Garagylchyg 2 genotype. In the wax ripeness phase, in the Barakatli 95 genotype, the synthesis increased 7.7-fold, and in Garagylchyg 2 genotype this increase was 2.6-fold.

During the generative development stage of ontogenesis, the dynamics of the accumulation of soluble phenolic compounds in the roots and leaves of both normally watered and drought-exposed durum wheat genotypes were comparatively studied. It is known that plant tissues are more sensitive to drought stress in these phases of development. Phenolic compounds, which are secondary metabolites, perform various defense

functions in plant organs during stress. Studies conducted by Okello et al. (2017) showed that *Solanum scabrum* and *Solanum villosum* plants respond differently to water deficiency. As a result of stress, phenolic compounds accumulate more in the roots of *S. scabrum* and the leaves of *S. villosum*. The authors explain this by the differential distribution of phenolic compounds in different plant species. The phenols accumulated in the roots act as osmolytes, participate in the maintenance of turgor in the root cells and increase the osmotic potential. In the leaves, phenolic compounds participate in the defense of enzymes under stress, protection of the structure and integrity of the membrane, and utilization of reactive oxygen species (Okello et al., 2017).

The results of our experiments showed that the amount of phenolic compounds was higher in the leaves as well as roots of both genotypes during the flowering stage under drought compared to the watered variant. Thus, in the flowering phase, 62.36 mg/g of total phenolic compounds were synthesized in the leaves of the Barakatli 95 genotype exposed to drought, and 80.53 mg/g in the milk ripeness phase (Fig. 4). For the sensitive Garagylchyg 2 genotype, this parameter was 66.79 and 71.42 mg/g of dry biomass, respectively. In drought-exposed plants, the amount of phenolic compounds reached the maximum in the milk ripeness phase. Although in the deeper phases of drought, the synthesis of soluble total phenolic compounds in both leaves and roots was inhibited, it was higher compared to the watered variants (Fig. 4).

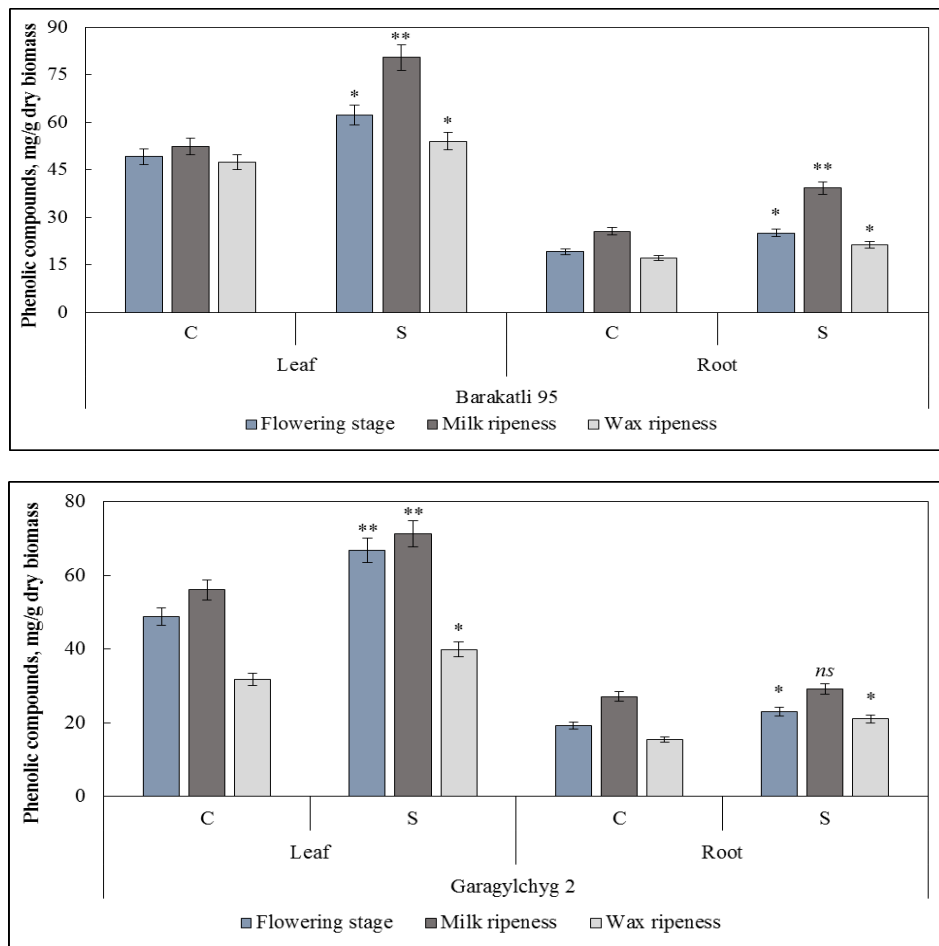


Fig. 4. The amount of soluble phenolic compounds in the leaves of durum wheat genotypes during the generative development period (mg/g dry biomass). Statistical analyses were performed according to Student's t-test: **, * - respectively, <0.01, <0.05 significant at probability levels, ns - not significant.

Thus, in the wax ripeness phase, the amount of total phenolic compounds decreased 1.5-fold in the leaves of the tolerant genotype and 1.8-fold in the sensitive genotype compared to the milk ripeness phase. The same trend was observed in the roots. The decrease in the amount of phenolic compounds as a result of long-term stress is attributed to the weakening of the expression of genes responsible for their synthesis. It is noted that the expression of many genes is enhanced during the first 12 hours of stress as a result of the effect of the stressor on the plant (Kilian et al., 2007). This process requires energy consumption. However, the energy capacity of plants is limited. Kilian and co-authors show that after 24 hours, the expression of most genes is inhibited or substantially attenuated. Xiong and co-authors (2006) noted that the activity of the enzyme phenylalanine ammonia-lyase (PAL) in the roots of *Arabidopsis thaliana* was significantly weakened during long-term drought. PAL (EC 4.3.1.5) is one of the main enzymes controlling the synthesis of phenolic compounds in plants (Szafran'ska et al., 2002). The analyses showed that more phenolic compounds were synthesized in the roots of tolerant

genotypes compared to sensitive genotypes.

There are numerous reports on the stimulation of the synthesis of phenolic compounds by drought (Ayaz et al., 2000; Alexieva et al., 2001). Many authors concluded that the synthesis of phenols in plant tissues increases under abiotic stress (Dixon and Paiva, 1995; Janas et al., 2002; Wrobel et al., 2005; Weidner et al., 2009a). Similar results were also observed in the experiments conducted with the *Amaranthus tricolor* plant exposed to drought stress. It was found that the dry biomass of the plant, leaf area, relative water content (RWC), the amount of photosynthetic pigments and soluble proteins decreased under drought, while the amount of malondialdehyde (MDA), hydrogen peroxide (H_2O_2), proline, carotenoids, ascorbic acid, polyphenols, flavonoids as well as total antioxidant activity increased (Sarker and Oba, 2020). Based on such positive correlation observed between MDA, H_2O_2 , osmoprotectants, and non-enzymatic antioxidants, the authors concluded that these compounds have an important vital function in the detoxification of ROS in *A. Tricolor* under drought. Chung et al. (2006) showed that stress caused by

frost and water deficit leads to an increase in total phenolic compounds in *Rehmannia glutinosa*. These results were also confirmed in studies conducted with soybean plants exposed to low-temperature stress (Posmyk et al., 2005). On the other hand, some researchers have observed a completely opposite effect. Thus, the research conducted on *V. vinifera* seedlings exposed to cold stress revealed that the amounts of total phenolic compounds, tannins, and phenolic acids were lower in the stressed samples compared to the control (Weidner et al., 2009b). In studies conducted by Amarowicz and co-authors (2010), the same trend was observed in leaves of *V. vinifera* exposed to low-temperature stress. Such inconsistencies observed in the experimental results may depend on the type of stress, its intensity, duration, plant developmental stages as well as biological material (e.g. whole plant or different parts of plants, roots, or leaves) (Weidner et al., 2009a).

In conclusion, a less decrease in carotenoids and chlorophyll content and a greater increase in the content of phenolic compounds and anthocyanins occurred in the tolerant wheat genotype (Barakatli 95) under continuous drought stress. Under drought conditions, wheat leaves were found to be able to synthesize more phenolic compounds than roots.

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Davamlı quraqlıq stresinə məruz qalmış buğda bitkisinin vegetativ orqanlarında fenol birləşmələrin, antosianların və fotosintetik piqmentlərin toplanma dinamikası

Əliyeva Durna Rəfael qızı

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Fenol birləşmələr bitkilərdə fotosintez və tənəffüs proseslərində, böyümə və inkişafda, reproduksiya və immunitətdə mühüm rola malikdirlər. Muasir dövrdə fenol birləşmələrin kimyəvi tərkibinin və biosintezinin, hüceyrədaxili lokalizasiyasının tədqiqində kifayət qədər nailiyyətlər əldə olunsada, bitkilərin ətraf mühitin qeyri-əlverişli amillərindən, o cümlədən quraqlıqdan müdafiəsində fenol birləşmələrin rolu tam öyrənilməmişdir. Bu problem bu gün də aktualdır, çünki bir sıra kənd təsərrüfatı əhəmiyyətli bitkilər, o cümlədən bərk buğda (*Triticum durum* Desf.) öz inkişafı dövründə torpaq və atmosfer quraqlığına məruz qalır. Aparılan tədqiqat işinin əsas məqsədi buğda bitkisinin quraqlıqdan müdafiəsində fenol birləşmələrin, antosianların və fotosintetik piqmentlərin rolunun müəyyənəşdirilməsindən ibarətdir. Analizlər üçün bitki nümunələri (Bərəkətli-95 (quraqlığa davamlı), Qaraqılçığı-2 (quraqlığa həssas)) bitkinin generativ inkişaf mərhələsində - çiçəkləmə, süd yetişmə, mum yetişmə fazalarında götürülmüş, kök və yarpaq nümunələri maye azotda xırdalandıqdan sonra həll olan fenol birləşmələrin miqdarı fenolların Folin-Çokalteu reaktivi ilə qarşılıqlı təsirinə əsasən təyin edilmişdir. Quraqlığa məruz qalmış bitkilərdə antosianların, xla, xlb və karotinoidlərin miqdarı quraqlığın təsirindən azalmış, fenol birləşmələrin miqdarı süd yetişmə fazasının sonunda maksimum həddə çatmış, sonrakı fazalarda nisbətən azalsada, suvarılan variantlarla müqayisədə yuxarı olmuşdur. Müəyyən olunmuşdur ki, hər iki genotipin yarpaqlarında quraqlığın təsirindən fenolu birləşmələrin miqdarı artır. Bu zaman davamlı genotiplərdə həssas genotiplərlə müqayisədə daha çox fenol birləşmələri sintez olunur. Eyni zamanda, quraqlıq zamanı buğda yarpaqlarının köklərlə müqayisədə daha çox fenol birləşmələri sintez etmək qabiliyyətinə malik olduğu qənaətinə gəlinmişdir.

Açar sözlər: *Triticum durum* Desf., quraqlıq stressi, fenol birləşmələr, fotosintetik piqmentlər