

MORPHOPHYSIOLOGICAL CHANGES OF DURUM WHEAT (*TRITICUM DURUM* DESF.) SAMPLES UNDER THE INFLUENCE OF TEMPERATURE

R. Kurbonova, O. Umarova, U. Jumanov, Sh. Payanov

Gulistan State University

Research Institute of Grain and Leguminous Crops,

Surkhandarya Scientific Experimental Station

Abstract. This study investigated the effects of temperature changes during the autumn–winter growing season on the morphophysiological and biochemical characteristics of the winter durum wheat cultivar Makuz and the KGU-561 breeding line. According to the results, decreasing temperature significantly reduced plant height, root length, leaf area, net photosynthetic productivity, and transpiration intensity. From October to December, net photosynthetic productivity decreased from $0.55 \text{ g m}^{-2} \text{ day}^{-1}$ to $0.02 \text{ g m}^{-2} \text{ day}^{-1}$. At the same time, under the influence of cold stress, the content of soluble carbohydrates in leaves increased, reaching 28.9% in the Makuz cultivar and 27.8% in the KGU-561 line in December. Spectrophotometric analyses revealed that decreasing temperature reduced the contents of chlorophyll “a” and chlorophyll “b”, while the content of carotenoids increased. The Makuz cultivar exhibited higher morphological, physiological, and biochemical characteristics compared with the KGU-561 line. The obtained results showed that the Makuz cultivar was more tolerant to cold stress, whereas the KGU-561 line was more sensitive to low-temperature conditions. The findings are important for evaluating cold tolerance in durum wheat genotypes and selecting promising breeding materials.

Keywords: durum wheat, *Triticum durum*, cold stress, temperature, photosynthetic productivity, transpiration, soluble carbohydrates, chlorophyll, carotenoids, cold tolerance, breeding lines, morphophysiological characteristics.

Introduction. The growth and development of durum wheat (*Triticum durum* Desf.) is one of the physiological processes most sensitive to temperature among environmental factors. According to Maccaferri et al. (2019), the optimum growth temperature for winter wheat is 15–20°C, and when the temperature falls below 5°C, cell division and elongation slow down significantly. When the temperature decreases to between –2°C and –5°C, vegetative growth may decline by 40–60%. The authors emphasize that the degree of adaptation to temperature varies among durum wheat genotypes [1].

Under low-temperature conditions, morphological traits such as plant height, root length, and leaf area change considerably. According to Blum (2018), a decrease in temperature from 15°C to 0°C reduced plant height growth by 45–55% and root growth by 25–35% in winter wheat. A reduction in leaf area serves to limit transpiration and maintain water balance. Therefore, cold-tolerant genotypes retain leaf area relatively better under low-temperature conditions [2].

Photosynthesis is one of the physiological processes most sensitive to cold stress. According to Taiz and Zeiger (2015), photosynthetic intensity decreases by 50–70% when temperature declines from 20°C to 5°C. Under cold conditions, enzymatic reactions slow down, CO₂ fixation decreases, and consequently net photosynthetic productivity is reduced. At the same time, transpiration intensity decreases by 40–60%. These changes represent adaptive responses aimed at reducing energy expenditure and conserving water [3].

Numerous studies have shown that the content of soluble carbohydrates in leaves increases under cold conditions. According to Fowler and Limin (2004), the amount of soluble sugars in winter wheat leaves increases by 1.5–2.5 times during cold periods. Soluble carbohydrates increase the concentration of cell sap, limit the formation of ice crystals, and protect cells from cold damage. Therefore, the accumulation of carbohydrates is generally higher in cold-tolerant cultivars [4].

A decrease in temperature negatively affects chlorophyll pigments. According to Ashraf and Harris (2013), the contents of chlorophyll “a” and chlorophyll “b” may decrease by 30–60% under cold conditions. At the same time, carotenoid content increases by 1.5–3.0 times. Carotenoids function as antioxidants and protect chloroplasts from oxidative stress. As a result, the photosynthetic apparatus of plants is partially preserved [5].

An increase in proline, soluble proteins, and antioxidant enzymes is commonly observed in cold-tolerant wheat cultivars. According to Virdis et al. (2020), under low-temperature conditions, proline content increased by 2–3 times, while the activities of superoxide dismutase (SOD) and catalase (CAT) increased by 25–45%. These compounds protect cells from free radical damage and enhance tolerance to cold stress [6].

Materials and Methods. The Makuz cultivar and the KGU-561 breeding line from the durum wheat collection were used for comparative evaluation of physiological and biochemical characteristics. In addition, eleven durum wheat samples were selected as research materials for assessing cold tolerance under field conditions.

Plant height and root length were measured using ten randomly selected seedlings, and average values were calculated. Daily growth rate was determined according to the following formula:

$$K_{\text{growth}} = (L_2 - L_1) / t$$

where: L_1 – initial length (mm), L_2 – subsequent length (mm), t – number of elapsed days.

Leaf area was determined using the paper-weight method, in which the leaf outline was traced and its area was calculated according to the ratio between paper weight and known paper area.

Net photosynthetic productivity (NPP) was determined according to the Nichiporovich method. Dry matter accumulation per unit leaf area was calculated using the following formula:

$$NPP = (W_2 - W_1) / (t \times A)$$

where: W_1 – dry matter mass at the beginning of the experiment (g), W_2 – dry matter mass at the end of the experiment (g), t – period (days), A – leaf area (m^2).

Transpiration was measured using Ivanov's rapid weighing method. Leaves were weighed before measurement, and water loss during a fixed period (1 hour) was determined using an electronic balance and recalculated per 1 m^2 of leaf area.

Photosynthetic pigments (chlorophyll "a", chlorophyll "b", and carotenoids) were determined using a DR-3900 spectrophotometer at wavelengths of 662 nm, 645 nm, and 440 nm, respectively.

Pigment contents were calculated using Arnon's equations:

$$\text{Chl-a} = 11.75 \times E_{662} - 2.35 \times E_{645}$$

$$\text{Chl-b} = 18.61 \times E_{645} - 3.96 \times E_{662}$$

$$\text{Car} = (1000 \times E_{440} - 2.13 \times \text{Chl-a} - 97.63 \times \text{Chl-b}) / 209$$

where E_{662} , E_{645} , and E_{440} are the optical density values at the corresponding wavelengths.

The content of soluble carbohydrates was determined as a percentage using an electronic refractometer based on aqueous extracts prepared from leaf samples. Leaf samples from each cultivar were analyzed in triplicate, and average values were recorded.

Statistical analyses were performed using SPSS-17 software. The results were expressed as mean $\pm$ standard error ($M \pm SE$). Differences among treatments were considered significant at $P < 0.05$.

Assessment of Cold Stress Under Laboratory Conditions

Cold tolerance under field conditions was determined based on the number of surviving seedlings. Laboratory experiments were conducted to evaluate the effect of temperature on germination characteristics. The effects of temperatures of 5, 10, 15, 20, 25, 30, and 35°C on germination percentage and germination duration of the wheat (*Triticum aestivum*) cultivars Andijon-2 and Vassa were studied. The temperatures of 5°C and 10°C were maintained using an MR 5V420 laboratory refrigerator, while the remaining temperatures were established using a Memmert thermostat. Statistical analyses were performed using SPSS-17 software.

Results and Discussion

The effects of temperature changes on the morphophysiological characteristics of seedlings are presented in Table 1. In October, the average plant height was 84.6 mm, root length was 49.3 mm, and leaf area was 4.72 cm² in the Makuz cultivar. In the KGU-561 line, the corresponding values were 83.6 mm, 49.0 mm, and 4.55 cm², respectively, which were lower than those of the Makuz cultivar by 1.0 mm, 0.3 mm, and 0.17 cm².

In November, growth intensity slowed due to decreasing temperature. In December, growth nearly ceased, whereas in January a slight recovery was observed, with an average growth rate of 0.08 mm day⁻¹. These results are associated with the significant slowing of cell division and elongation processes under cold stress conditions.

According to plant height, the Makuz cultivar was 0.2–1.0 mm taller than the KGU-561 cultivar during all observation months. The greatest difference in root length was observed in December, when the Makuz cultivar showed a value 2.2 mm higher than KGU-561. In terms of leaf area, the superiority of the Makuz cultivar increased throughout the vegetation period. In general, the Makuz cultivar showed superiority during the vegetation period in plant height, root system development, and the formation of photosynthetic surface area.

Differences in Net Photosynthetic Productivity and Transpiration Intensity in Durum Wheat Samples According to Temperature and Cultivars

The results of photosynthesis and transpiration are presented in Table 2. In October, the net photosynthetic productivity of the Makuz cultivar was 0.55 g/m²/day, whereas in November it decreased to 0.24 g/m²/day. In December and January, photosynthesis was almost absent due to low temperatures. A similar decreasing trend was observed in the transpiration process. These results are consistent with literature data indicating that cold stress has a strong effect on the photosynthetic apparatus and water exchange processes [8,9].

In October, the net photosynthetic productivity of the KGU-561 line was 0.05 g/m²/day lower than that of the Makuz cultivar, while transpiration intensity was 0.09 g/m²/hour lower. In November, the difference in net photosynthetic productivity was 0.02 g/m²/day, whereas transpiration values were the same. In December, the net photosynthetic productivity in KGU-561 was 0.02 g/m²/day lower than that of the Makuz cultivar, while transpiration values were equal. In January, the net photosynthetic productivity and transpiration values were recorded as the same in both genotypes.

Effect of Temperature Changes During Different Months on Net Photosynthetic Productivity and Transpiration Parameters

Table 2

Month	NPP Makuz	NPP KGU-561	Transpiration Makuz	Transpiration KGU-561
October	0.55	0.50	0.50	0.41
November	0.24	0.22	0.20	0.20
December	0.02	0.00	0.01	0.01
January	0.03	0.03	0.01	0.01

During the autumn vegetation period, the Makuz cultivar showed a slight superiority over the KGU-561 line in terms of net photosynthetic productivity. By the winter period (December–January), the physiological activity of both genotypes decreased sharply, and their values became almost equal.

Changes in Soluble Carbohydrates under the Influence of Temperature and Their Differences Among Samples

It was determined that the content of soluble carbohydrates in leaves increased sharply under low-temperature conditions (Table 3). In the Makuz cultivar, the carbohydrate content, which was 12.8% in October, increased to 28.9% in December. In the KGU-561 line, the carbohydrate content was 11.9% in October and increased to 27.8% when the temperature reached its minimum level. This situation indicates an enhancement of the protection mechanism against ice crystal formation through the accumulation of osmoprotectants in plants.

Table 3

Changes in Soluble Carbohydrate Content in Leaves under Temperature Variations During Different Months (%)

Month Soluble carbohydrates (%)

Table 3

Month	Makuz cultivar	KGU-561 line
October	12.8	11.9
November	19.6	19.5
December	28.9	27.8
January	25.1	24.6

During the autumn–winter vegetation period, the content of soluble carbohydrates increased in both genotypes and reached its maximum value in December. In October, the soluble

carbohydrate content in the KGU-561 line was 0.9% lower than that of the Makuz cultivar; in November, it was 0.1% lower; in December, 1.1% lower; and in January, 0.5% lower. The greatest difference was observed in December, when the soluble carbohydrate content in the KGU-561 line was 1.1% lower than that of the Makuz cultivar. In general, it was determined that the Makuz cultivar accumulated more soluble carbohydrates than the KGU-561 line during all observation months. From these results, it can be concluded that, compared with the Makuz cultivar, the KGU-561 line may be considered a less cold-tolerant line.

Changes in Photosynthetic Pigment Content under the Influence of Temperature and Their Differences Among Samples

According to the results of spectrophotometric analyses, the contents of chlorophyll “a” and chlorophyll “b” in leaves decreased under cold conditions, whereas the content of carotenoids increased (Table 4). This indicates that the protective role of carotenoids is enhanced under cold stress due to their antioxidant properties.

Table 4

Pigment Content During the Autumn–Winter Vegetation Period and Differences Between Cultivars (KGU-561 Relative to the Makuz Cultivar) (mg/g dry weight)

Month	Chl a Makuz	Chl a KGU- 561	Difference	Chl b Makuz	Chl b KGU- 561	Difference	Carotenoids Makuz	Carotenoids KGU-561	Difference
October	2.33	2.29	-0.04	1.49	1.45	-0.04	0.44	0.40	-0.04
November	1.62	1.62	0.00	1.13	1.12	-0.01	0.67	0.62	-0.05
December	0.80	0.77	-0.03	0.59	0.60	+0.01	1.22	1.18	-0.04
January	0.85	0.80	-0.05	1.25	1.22	-0.03	1.16	1.11	-0.05

The change in temperature affected the content of photosynthetic pigments in winter durum wheat seedlings. As temperature decreased, the contents of chlorophyll “a” and chlorophyll “b” decreased quantitatively, whereas the content of carotenoids increased under the influence of low temperatures.

In the Makuz cultivar (KGU-561) (**Note:** the values given in parentheses correspond to the KGU-561 line), during October (15°C), the average content of chlorophyll “a” was 2.33 (2.29) mg/g, chlorophyll “b” was 1.49 (1.45) mg/g, and the carotenoid content was 0.44 (0.40) mg/g.

When the same study was conducted in November (11°C), the chlorophyll “a” content decreased by 29.6% compared with October and amounted to 1.62 (1.62) mg/g. The chlorophyll “b” content was 1.13 (1.12) mg/g, representing a decrease of 27.6%. In contrast, the carotenoid content reached 0.67 (0.62) mg/g, increasing by 0.23 mg/g (54%).

In December (−1.5°C), under the influence of cold weather conditions, the chlorophyll “a” content in the Makuz cultivar (KGU-561) was 0.80 (0.77) mg/g and chlorophyll “b” content was 0.59 (0.60) mg/g. Compared with October, chlorophyll “a” and chlorophyll “b” decreased significantly by 61.5% and 59.7%, respectively. The carotenoid content in this month reached 1.22 (1.18) mg/g, which was three times higher than that recorded in October.

In January (5.7°C), the chlorophyll “a” content was 0.85 (0.80) mg/g, chlorophyll “b” content was 1.25 (1.22) mg/g, and carotenoid content was 1.16 (1.11) mg/g. Although cold weather conditions, snowfall, frost occurrence, and night temperatures as low as −3°C were observed during January, the weather was relatively warmer than in December. This resulted in increases of 3.4% and 5.5% in pigment contents compared with December, respectively, while the carotenoid content decreased by 10%.

When the dynamics of photosynthetic pigments were studied during the autumn–winter vegetation period, a decrease in chlorophyll content and an increase in carotenoid content were observed in both genotypes. Regarding chlorophyll *a* content, the Makuz cultivar showed higher values than the KGU-561 line throughout all observation periods. The greatest difference was recorded in January, when the Makuz cultivar exceeded the KGU-561 line by 0.05 mg/g.

The Makuz cultivar also generally showed superiority in chlorophyll *b* content. Only in December was the chlorophyll *b* content in the KGU-561 line 0.01 mg/g higher than that of the Makuz cultivar. On average, however, the Makuz cultivar accumulated 0.02 mg/g more chlorophyll *b*.

The carotenoid content increased significantly as the wintering period approached. The high accumulation of carotenoids in December and January may be associated with the adaptation of plants to low temperatures and oxidative stress. Throughout all observation periods, the Makuz cultivar accumulated 0.04–0.05 mg/g more carotenoids than the KGU-561 line.

The results analyzed above were obtained from the average monthly values of two winter durum wheat genotypes included in this study and were used to evaluate the effect of temperature on the physiological and biochemical characteristics of wheat. Significant decreases in temperature resulted in differences between the cultivars. In other words, under the same temperature conditions, certain cultivars exhibited different physiological and biochemical responses.

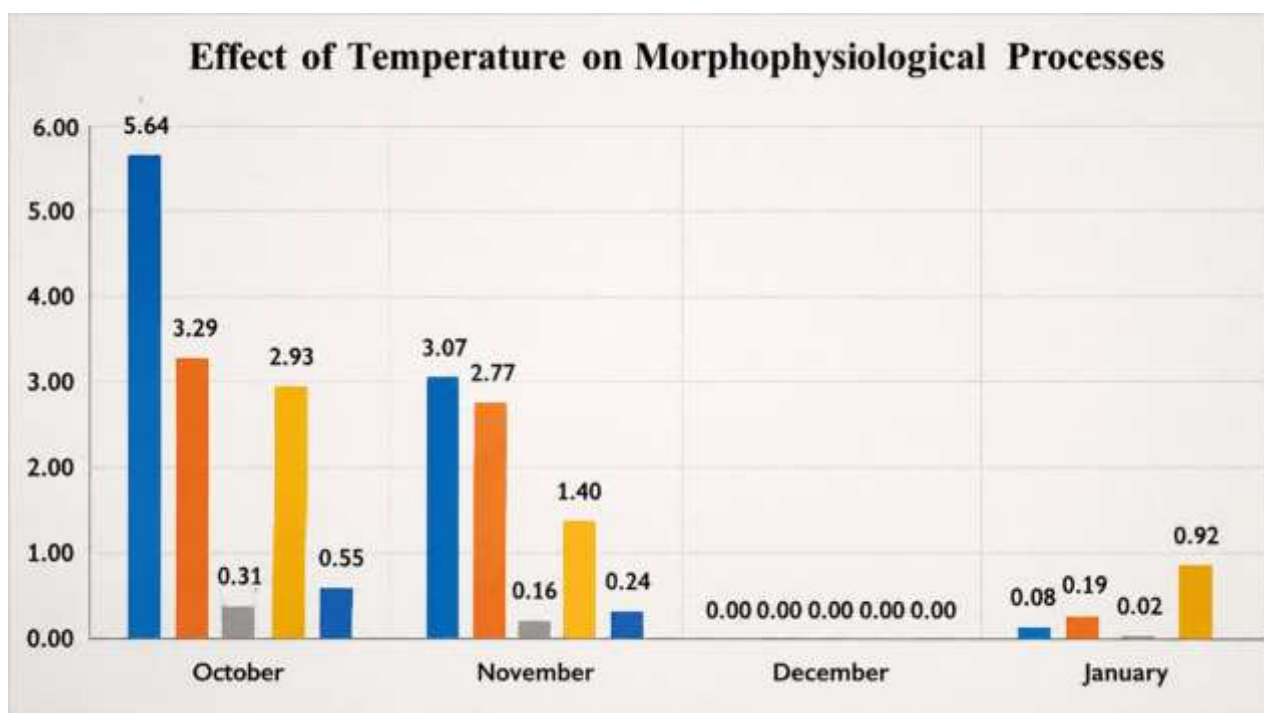


Figure 1. Tendency of decrease in leaf area and photosynthetic productivity in seedlings under cold conditions (using the Makuz cultivar as an example).

Note: From left to right, 1 – Daily growth rate of the plant, mm/day. 2 – Daily growth rate of the root, mm/day. 3 – Daily change in leaf area, cm²/day.
4 – Transpiration intensity. 5 – Net photosynthetic productivity.

The figure clearly shows how the dynamics of leaf area and net photosynthetic productivity of winter durum wheat seedlings change under cold conditions. In October, the expansion of leaf area contributed to a relatively high level of photosynthetic activity. However, in November and especially in December, the sharp decrease in temperature slowed leaf development, leaf area remained almost unchanged, and photosynthetic productivity decreased sharply. In January, although the leaf area increased slightly due to a partial rise in temperature, photosynthetic efficiency remained at a low level.

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