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ASSESSMENT OF HEAT TOLERANCE IN WHEAT (*TRITICUM AESTIVUM* L.) AT THE SEEDLING STAGE

G.F. MAMATKULOVA[1*], D.SH. ERJIGITOV[1], O.A. MUKHAMMADIEV[1], D.B. SOKIBOYEVA[1], M.D. KHOLOVA[1], B.B. ORIPOVA[1,5], D.K. ERNAZAROVA[1,3], S.K. BABOEV[1], O.S. TURAEV[1,2], and F.N. KUSHANOV[1,3,4*]

[1]Institute of Genetics and Plant Experimental Biology, Academy of Sciences, Uzbekistan
[2]Research Institute of Plant Genetic Resources, National Center for Knowledge and Innovation in Agriculture, Tashkent, Uzbekistan
[3]Department of Genetics, National University of Uzbekistan, Tashkent, Uzbekistan
[4]Department of Genetics and Biotechnology, Samarkand State University, Samarkand, Uzbekistan
[5]Tashkent State Dental Institute, Tashkent, Uzbekistan
*Corresponding authors' emails: gavhar0411@gmail.com, fakhriddinkushanov@gmail.com
Email addresses of co-authors: dostonerjigitov68@gmail.com, oybekmuhammadiyev12@gmail.com, durdonasoqiboyeva@gmail.com, mxolova107@gmail.com, barnooripova127@gmail.com, edilrabo64@gmail.com, sai-baboev@yandex.ru

SUMMARY

Wheat (*Triticum aestivum* L.) is a primary food crop globally, susceptible to environmental fluctuations, particularly the heat stress. With climate change and its increasing threats, wheat's response evaluation to the elevated temperatures during early growth stages is crucial for securing the crop and its yield. The presented study sought to assess the heat tolerance in 15 common wheat cultivars at the seedling stage under controlled conditions. Growing wheat cultivars ensued at the optimal (25 °C) and heat-stressed (35 °C) conditions. After seven days of growth, the seedling height, root length, and root number's measurement underwent assessment for heat stress effects. The quantification of the degree of plants damaged used the Damage Index (DI), with heat tolerance evaluated using the Heat Tolerance Index (HTI). The results showed significant variations among the wheat cultivars for heat tolerance, and the two cultivars, Tuyatish and Nodir, displayed minimal growth reduction under the highest temperature, achieving maximum HTI values (98.3% and 89.6%, respectively). The cultivar FAWWON-IRR-D-77 exhibited a substantial damage and low tolerance under high-temperature stress. This study provides essential insights into heat tolerance mechanisms in wheat, forming a foundation for future breeding and molecular studies aimed at improving heat resilience in crops.

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Keywords: Common wheat (*T. aestivum* L.), heat stress, seedling stage, early growth stage, heat tolerance, damage index, heat tolerance index

Key findings: This study identified significant variability among the common wheat (*T. aestivum* L.) genotypes for heat tolerance at the seedling stage. Cultivars Tuyatish and Nodir demonstrated the highest heat tolerance with minimal damage. The line FAWWON-IRR-D-77 exhibited substantial damage and low tolerance to high-temperature stress.

INTRODUCTION

Wheat (*Triticum* spp.) cultivation has a long historical approach, being one of the earliest domesticated food crops. Wheat is the most widely cultivated staple crop and continues to serve as a crucial food source for human consumption (Turaev *et al.,* 2023). In developing countries, wheat-based food products provide over 40% of the essential protein and calorie intake in the human diet. Plant breeders are intensively conducting research to enhance the productivity of cereal crops, including wheat, to ensure food security worldwide (Mammadova *et al.,* 2024a). In this regard, DNA marker technologies' utilization intensifies to develop new wheat cultivars with valuable agronomic traits (Adylova *et al.,* 2018). These efforts include analyzing the correlation coefficient between productivity and bread-making quality in newly developed wheat cultivars, evaluating their adaptability to the soil and climatic conditions of specific regions, and assessing their resistance to biotic and abiotic stress conditions (Bakhadirov *et al.,* 2024).

Bread wheat (*T. aestivum* L.) remains vulnerable to environmental fluctuations, particularly to heat stress. A continuous rise in global temperature has been evident in past years, posing significant challenges to crop plants (Mammadova *et al.,* 2024b). The elevated temperature negatively affects plant growth by shortening developmental phases and reducing crop yields (Kim *et al.,* 2015). With heat stress, wheat relies on its internal regulatory systems, which include physiological and biochemical defense mechanisms, to adapt to unfavorable environmental conditions.

Previous studies reported that a 1 °C increase in temperature could lead to a 6% reduction in global wheat production (Asseng *et al.,* 2015). Tewolde *et al.'s* (2006) investigations enunciated the heat stress alters the grain yield by reducing pollen viability, which hinders the pollination process and, consequently, reduces the grain number. Moreover, heat stress also affects the root system in crop plants. Wheat roots are more sensitive to high temperature than the aboveground biomass, with slow root growth and halting under heat stress conditions (Wasson *et al.,* 2012). All these circumstances limit the plant's ability to absorb water and nutrients, further compounding the adverse effects of heat stress (Wasson *et al.,* 2012).

Negative effects of high temperature were visible on the wheat photosynthesis (Cossani and Reynolds, 2012). According to their analysis, an increase in temperature leads to the denaturation of photosynthetic enzymes, which reduces the photosynthesis process. As a result of the stomata closure in plant leaves, the intake of carbon dioxide become limited, resulting in a decrease in the overall assimilation ability of plants. Djanaguiraman *et al.* (2020) reported the high-temperature stress leads to lessened chlorophyll content and premature leaf senescence in wheat. Reducing chlorophyll directly decreases the photosynthetic process, and, eventually, negatively influencing plant growth and development.

Earlier research revealed that among the wheat genotypes, the amount of chlorophyll varies due to environmental influences, regardless of the type of genotype (Baboeva *et al.,* 2023). Additionally, high-temperature stress shortens the grain-filling period, decreases the photosynthetic capacity and assimilation rate, and accelerates leaf senescence. In turn, this lowers the chlorophyll content, reduces the number of grains, and consequently, causing a remarkable decline in wheat grain yield (Pandey *et al.,* 2019).

In response to the rising global demand for wheat, it is pivotal to investigate the molecular mechanisms underlying heat tolerance to improve the wheat production and quality. Understanding these mechanisms is essential for breeding heat-tolerant wheat cultivars to ensure future food security (Adylova *et al.,* 2018). Based on the above discussion, this study sought to evaluate the cultivated wheat cultivars in Uzbekistan, as well as, their collection of genotypes, for heat tolerance at the seedling stage. This screening process hopes to identify the wheat genotypes that can withstand heat stress, providing a basis for future breeding efforts.

MATERIALS AND METHODS

Plant material

Fifteen common wheat cultivars, including local and international genotypes, were chosen samples for this study. These cultivars came from the wheat breeding programs in Uzbekistan, Russia, Belarus, and the International Maize and Wheat Improvement Center (CIMMYT). The full list of samples appear in Table 1.

Experimental procedure

Growing wheat seedlings under controlled conditions occurred in a plant growth chamber (FPG-450). This experiment proceeded following the methodology of Alsamadany (2016), comprising pre-soaking of seeds overnight in distilled water and then placing in Petri dishes lined with filter paper. Growing the seedlings consisted of two different temperature regimes, i.e., non-stress (optimal temperature at 25 °C as the control) and stressed (35 °C) conditions. The photoperiod setting to 12 hours had a light intensity of 200 μmol $m^{-2}s^{-1}$, with the relative humidity maintained at 70%–75%. The plants received watering with a temperature-adjusted water.

After seven days, the harvest of seedlings took place, with measurements taken for seedling length, root length, and the number of roots. Based on the seedling lengths in the control and heat-stressed conditions, the calculation of the Damage Index (DI) and Heat Tolerance Index (HTI) used the following formulas:

$$DI = \frac{mean\ of\ SL\ at\ 25°C - mean\ of\ SL\ at\ 35°C}{mean\ of\ SL\ at\ 25°C}$$

$$HTI = \frac{mean\ of\ SL\ at\ 35°C * 100}{mean\ of\ SL\ at\ 25°C}$$

Table 1. Common wheat cultivars and lines used in this research.

No.	Samples	Origin	Originator
1	Ilg'or	Uzbekistan	Institute of Genetics and Plant Experimental Biology
2	Jasmina	Uzbekistan	Samarkand Agricultural Institute
3	Asr	Uzbekistan	Research Institute of Cereals and Legume Crops
4	Baxmal-97	Uzbekistan	Research Institute of Rainfed Agriculture
5	Navbahor	Uzbekistan	Research Institute of Cereals and Legume Crops
6	Janub Gavhari	Uzbekistan	Research Institute of Agriculture in the Southern Regions
7	Buxor bobo	Uzbekistan	Landrace wheat
8	Tuyatish	Uzbekistan	Landrace wheat
9	Nodir	Uzbekistan	Research Institute of Cereals and Legume Crops
10	Qayroqtosh	Uzbekistan	Institute of Genetics and Plant Experimental Biology
11	Bardosh	Uzbekistan	Institute of Genetics and Plant Experimental Biology
12	Alekseyvich	Russia	P. P. Lukyanenko National Grain Center
13	FAWWON-IRR-D-77	Turkey	CIMMYT, ICARDA
14	FAWWON-SA-D-85	Turkey	CIMMYT, ICARDA
15	KB-20-YT-IR-9808	Turkey	CIMMYT, ICARDA

Statistical analysis

All data underwent a one-way analysis of seedling length, root length, and number of roots (ANOVA), followed by means comparison using Tukey's test ($P < 0.05$). The means comparison utilized standard error measurements.

RESULTS

The analysis revealed the wheat cultivars exhibited varying degrees of damage under high-temperature stress (Figure 1). However, among the cultivars, Tuyatish showed minimal damage, with a nonsignificant difference in seedling lengths between the control and stressed conditions (18.1 and 17.8 cm at 25 °C and 35 °C, respectively) (Table 2). The Damage Index (DI) of cultivar Tuyatish was the lowest (0.01), while its Heat Tolerance Index (HTI) was the highest (98.3%), demonstrating considerable heat tolerance compared with other wheat cultivars.

Similarly, the cultivar Nodir also gave a minimal variation in seedling lengths under normal and stressed conditions, with 16.5 and 14.8 cm at 25 °C and 35 °C, respectively. The DI of cultivar Nodir was 0.10, and its HTI was

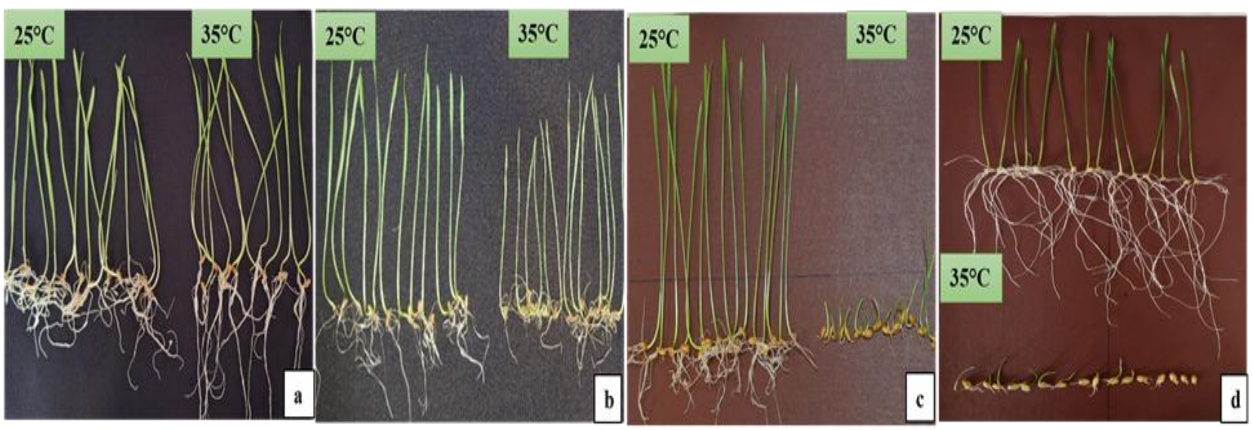

Figure 1. Effect of high temperatures on wheat cultivars. Heat-tolerant cultivars (a. Tuyatish, and b. Nodir), Heat-susceptible cultivars (c. FAWWON-IRR-D-77, and d. KB-20-YT-IR-9808).

Table 2. Seedling length, DI, and HTI values in wheat seedlings at 25 °C and 35 °C.

No.	Wheat genotypes	Seedling length (cm)*		Damage index (DI)	Heat tolerance index (HTI) (%)
		25 °C	35 °C		
1	Ilg'or	11.2±0.2	10±0.3	0.11	88.9
2	Jasmina	12.5±0.2	10.7±0.3	0.13	86.1
3	Asr	13.5±0.2	10.4±0.2	0.22	77.5
4	Baxmal-97	12.2±0.2	8.8±0.2	0.27	72.2
5	Navbahor	11.2±0.1	9.3±0.1	0.17	82.8
6	Alekseyvich	10.8±0.2	3.2±0.2	0.69	30.3
7	Janub Gavhari	12.1±0.24	9.5±0.2	0.21	78.8
8	Buxor bobo	11.8±0.2	8.4±0.2	0.29	70.7
9	Tuyatish	18.1±0.4	17.8±0.2	0.01	98.3
10	FAWWON-IRR-D-77	15.3±0.3	2.6±0.2	0.82	17.4
11	FAWWON-SA-D-85	11.9±0.2	1.7±0.2	0.85	14.2
12	KB-20-YT-IR-9808	9.9±0.3	1.6±0.3	0.83	16.4
13	Nodir	16.5±0.2	14.8±0.2	0.10	89.6
14	Qayroqtosh	10.7±0.2	8.7±0.6	0.18	81.5
15	Bardosh	12.4±0.2	9.3±0.3	0.24	75.1

* The values are the means ± standard errors.

89.6%, categorizing it as relatively heat-tolerant versus other wheat cultivars. Other wheat cultivars, including Ilg'or (11.2 cm at 25 °C and 10 cm at 35 °C), Jasmina (15.5 cm at 25 °C and 10.7 cm at 35 °C), Qayroqtosh (10.7 cm at 25 °C and 8.7 cm at 35 °C), and Navbahor (11.2 cm at 25 °C and 9.3 cm at 35 °C), also showed minimal differences in seedling lengths under two temperature conditions. Their HTI values were above 80%. These cultivars bore similar assessment as heat tolerant under high-temperature stress condition (Table 2).

Some wheat cultivars exhibited moderate levels of damage with the high-temperature stress. The differences in seedling length between the control and stressed conditions were not substantial in these cultivars. For instance, the cultivar Janub Gavhari had 12.1 cm at 25 °C and 9.5 ± 0.2 cm at 35 °C for seedling length, while cultivar Asr had the seedling length at 13.5 cm at 25 °C and 10.4 cm at 35 °C. The cultivar Baxmal-97 had seedling lengths of 12.2 cm at 25 °C and 8.8 cm at 35 °C, and genotype Bardosh measured 12.4 cm at 25 °C and 9.3 cm at 35 °C. These results indicated these wheat cultivars experienced some stress levels; however, their growth reduction was relatively partial.

Analysis of the DI and HTI revealed the wheat cultivar Janub Gavhari had a DI of 0.21 and HTI of 78.8%. The cultivar Asr exhibited a mean DI of 0.22 and HTI of 77.5% and genotype Nodir a DI of 0.27 and HTI of 72.2%, while the cultivar Bardosh demonstrated a mean DI of 0.24 and HTI of 75.1%. The results signified the heat tolerance of these wheat cultivars was at a moderate level under high-temperature stress.

Some wheat cultivars displayed a high level of damage under high-temperature stress. For example, for the line FAWWON-SA-D-85, significant differences were evident in seedling lengths of control and stressed conditions (11.9 and 1.7 cm at 25 °C and 35 °C, respectively), genotype KB-20-YT-IR-9808 (9.9 and 1.6 cm at 25 °C and 35 °C, respectively), and cultivar FAWWON-IRR-D-77 (15.3 and 2.6 cm at 25 °C and 35 °C, respectively). These lines were also distinct with the highest DI values, i.e., FAWWON-SA-D-85 (0.85), KB-20-YT-IR-9808 (0.83), and FAWWON-IRR-D-77 (0.82). Conversely, the HTI values were low in the lines FAWWON-SA-D-85 (14.2%) and KB-20-YT-IR-9808 (16.4%) (Figure 2). The results authenticated these wheat lines were seemingly susceptible to high-temperature stress conditions.

High temperatures diminish plant growth and highlight the impact of heat stress on physiological parameters of crop plants. Under heat stress conditions, cellular water loss occurs, slowing low photosynthesis and other metabolic processes, and directly affecting the seedling length. Aside from seedling length, root length and the number of roots gained evaluation in response to high temperatures. The number of roots' calculation occurred for various wheat cultivars under normal and stressed conditions (Figure 3).

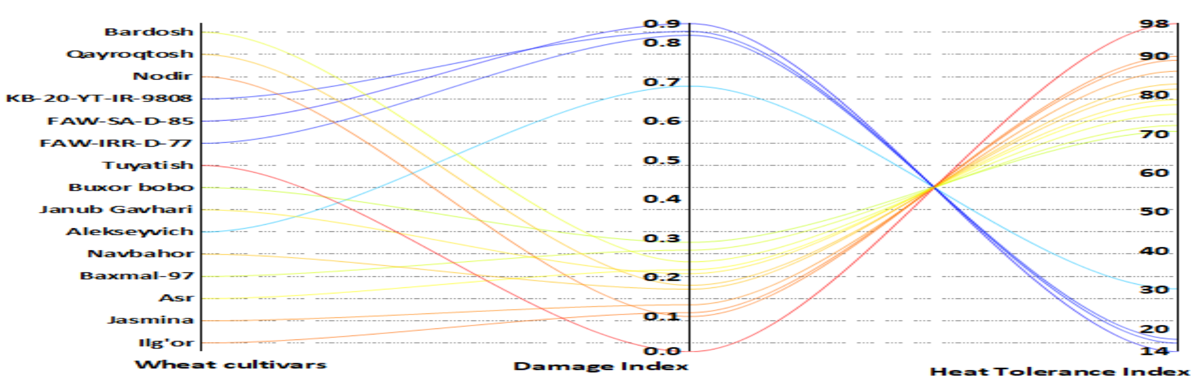

Figure 2. Damage index and heat tolerance index in wheat seedlings under high-temperature stress.

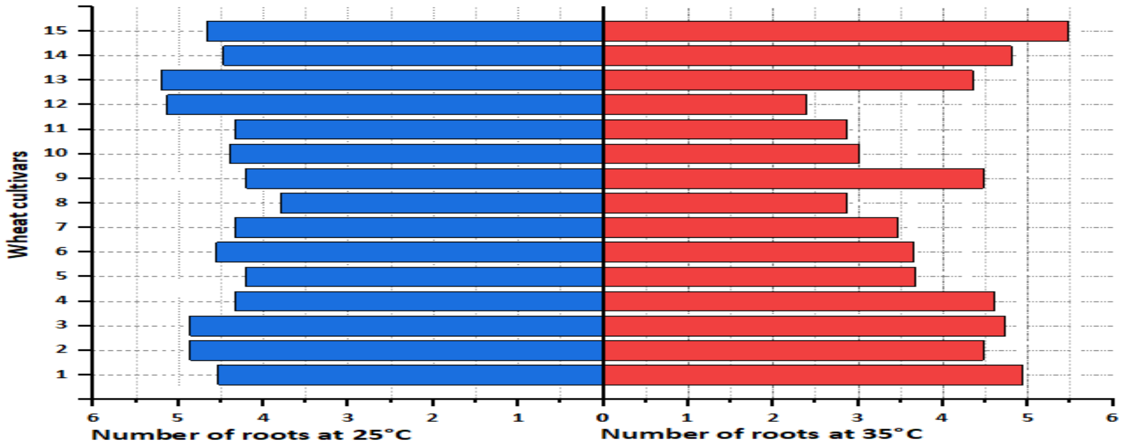

Figure 3. Number of roots in wheat seedlings at 25 °C and 35 °C. 1: Ilg'or, 2: Jasmina, 3: Asr, 4: Nodir, 5: Navbahor, 6: Alekseyvich, 7: Janub Gavhari, 8: Buxor bobo, 9: Tuyatish, 10: FAWWON-IRR-D-77, 11: FAWWON-SA-D-85, 12: KB-20-YT-IR-9808, 13: Baxmal-97, 14: Qayroqtosh, and 15: Bardosh.

The presented study analyzed the effects of temperature conditions on the root systems of wheat plants. At the optimal growth temperature (25 °C), several cultivars exhibited robust root development. However, three cultivars, Ilg'or, Nodir, and Tuyatish, produced many roots under optimal temperature conditions. It indicates their ability to develop effective root systems and optimal plant growth. In contrast, the high temperature (35 °C) induces stress in most wheat cultivar plants. It was notable in wheat cultivar Alekseyvich and FAWWON-IRR-D-77 the significant decrease in the number of roots at the high-temperature stress condition. However, heat-tolerant cultivars like Nodir, Tuyatish, and Bardosh, maintained many roots even under heat stress condition, demonstrating their resilience to high temperatures.

Overall, analyzing how wheat cultivars respond to temperature stress is crucial. Observing root development under optimal and high-temperature conditions helps identify tolerant and adaptable cultivars to temperature stress. The effect of heat stress on wheat root length as investigated revealed the following results. Variations in root length among the wheat cultivars were prominent in response to temperature changes. Plants grown at 25 °C, considered the optimal temperature for wheat, displayed well-developed root systems in most cultivars. Under normal temperature conditions, wheat cultivars, such as Asr, Jasmina, and line KB-20-YT-IR-9808, exhibited the longest roots.

In contrast, the 35 °C temperature, classified as a stressful condition, proved unfavorable for wheat development. Most wheat cultivars showed a significant reduction in their root length at the high-temperature stress condition. Notably, wheat cultivar and lines Alekseyvich, FAWWON-SA-D-85, and KB-20-YT-IR-9808 had relatively shorter root lengths. However, the cultivars Tuyatish, Navbahor, and Nodir maintained comparatively longer roots even under heat stress condition, and were assumedly heat-tolerant wheat genotypes (Figure 4).

DISCUSSION

The promising findings revealed significant variations among the wheat cultivars for heat tolerance under high-temperature stress. The heat-tolerant cultivars, such as Tuyatish and Nodir, showed minimal reduction in seedling and root lengths compared with other wheat cultivars. These results align with previous studies authenticating intrinsic physiological mechanisms as crucial for plant adaptation to

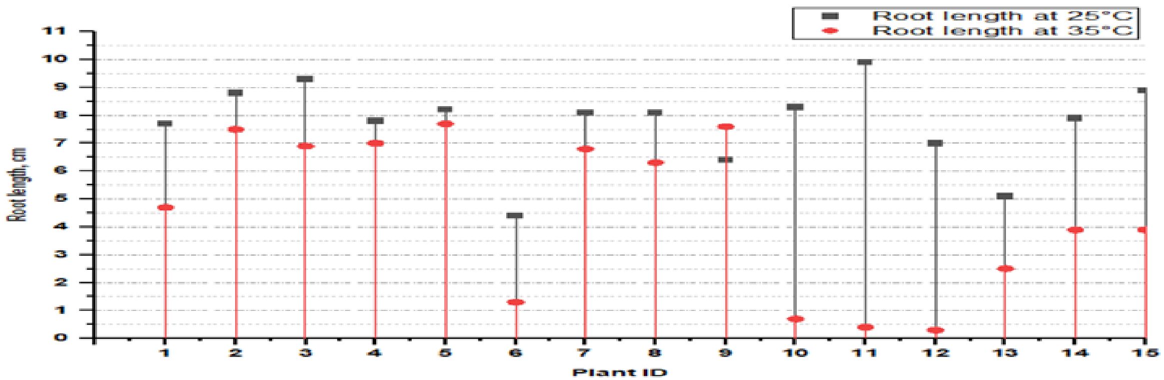

Figure 4. Root length in wheat seedlings at 25 °C and 35 °C. 1: Ilg'or, 2: Jasmina, 3: Asr, 4: Nodir, 5: Navbahor, 6: Alekseyvich, 7: Janub Gavhari, 8: Buxor bobo, 9: Tuyatish, 10: FAWWON-IRR-D-77, 11: FAWWON-SA-D-85, 12: KB-20-YT-IR-9808, 13: Baxmal-97, 14: Qayroqtosh, and 15: Bardosh.

heat stress. Asseng *et al.'s* (2015) results demonstrated the elevated temperature negatively influence the wheat growth by reducing developmental phases, leading to lower grain yield. The presented outcomes corroborate their findings, particularly for lines with low Heat Tolerance Index (HTI) values, such as FAWWON-SA-D-85 and KB-20-YT-IR-9808. They exhibited reduced growth under heat-stress conditions.

High-temperature stress negatively affects the seed germination and plant growth and development in various crops, including wheat (Hossain *et al*., 2013). Wheat plants are highly sensitive to heat stress, which inhibits its growth and triggers morphophysiological variations. Heat stress influences the above-ground biomass of the plant and significantly slows down the root growth and development. These adverse effects ultimately result in substantial yield losses in crop plants (McClung and Davis, 2010; Huang *et al*., 2012). In wheat plants, the extent of damage depends upon the developmental stage where exposure of plants occur to extreme temperature. For instance, after seven days of germination, the wheat plants' exposure to 45 °C for two hours significantly reduced shoot and root lengths, dry mass, chlorophyll content, and membrane stability index (Gupta *et al.,* 2013). Similarly, a 24-hour exposure to 42 °C inhibited root and early leaf growth and enhanced the reactive oxygen species and lipid peroxidation products in coleoptiles and seedlings of wheat (Savicka and Škute, 2010).

Factually, heat stress affects photosynthesis and chlorophyll content and serves as critical factors for plant growth and, eventually, the yield. Cossani and Reynolds (2012) emphasized high temperatures denature photosynthetic enzymes, impairing the photosynthetic process in the wheat crop. These findings were also consistent with the study results of reduced seedling and root growth under high-temperature stress conditions in wheat. Additionally, Djanaguiraman *et al.* (2020) reported the high-temperature stress leads to chlorophyll degradation and premature leaf senescence in wheat, resulting in lower photosynthetic efficiency. This aligns with the reduced growth observed in susceptible wheat cultivars, particularly those found with high Damage Index (DI) values.

Root development plays a vital role in heat tolerance, as it directly impacts water and nutrient uptake. Wasson *et al.* (2012) highlighted high temperatures hinder root growth, limiting the plant's ability to adapt to stress conditions. The presented study also supports these observations, with heat-tolerant wheat cultivars like Tuyatish maintaining a robust root system even under heat stress conditions, whereas the susceptible cultivars exhibited significantly reduced root length. The results suggested root resilience under heat stress conditions can work as a key trait for identifying the heat-tolerant wheat genotypes.

Moreover, the accelerated leaf senescence and shortened grain-filling periods under heat stress, as described by Pandey *et al.* (2019), were also noteworthy in the latest findings. These physiological responses, along with reduced chlorophyll content, contribute to the lower photosynthetic and assimilatory capacities in heat-susceptible cultivars. Interestingly, Baboeva *et al.* (2023) noted environmental factors significantly affect the chlorophyll content in wheat genotypes regardless of their type. These observations support the variability in HTI values across the studied wheat cultivars, suggesting that environmental factors are critical in determining heat tolerance. For example, wheat cultivars Navbahor and Bardosh demonstrated moderate heat tolerance, maintaining considerable root and seedling lengths under high-temperature stress conditions.

On growth and physiological traits, the variability observed among the studied wheat cultivars highlights the importance of breeding genotypes with superior heat tolerance. Modern plant breeding efforts, as noted by Adylova *et al.* (2018), leverage DNA marker technologies to develop wheat cultivars with desirable agronomic traits (Norbekov *et al.,* 2024). Study findings contribute to this goal by identifying promising wheat genotypes like Tuyatish and Nodir that can further serve in breeding programs aimed at enhancing heat tolerance.

The presented research underscores the significance of physiological and biochemical traits in determining wheat's adaptability to high-temperature stress conditions. By identifying heat-tolerant genotypes, this study provides a base for future breeding efforts to ensure food security in the face of global climate challenges. These findings also align with the broader literature, reinforcing the need for continued research on molecular mechanisms underlying heat tolerance in wheat to facilitate the development of resilient crop cultivars.

CONCLUSIONS

The study demonstrated significant impact of high-temperature stress on wheat seedlings, highlighting genetic variability for heat tolerance. Heat-tolerant common wheat cultivars Tuyatish and Nodir maintained stable growth under stressed conditions, whereas lines like FAWWON-IRR-D-77 exhibited substantial reduction in growth traits. These findings underscore the importance of identifying resilient genotypes to enhance adaptation to heat stress. Developing heat-tolerant wheat cultivars is critical for ensuring food security amidst rising global temperature.

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