



Agronomic Characteristics and Cold Tolerance of some Wheat Genotypes under Different Ecological Locations^(§)

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ABSTRACT

This study aimed to evaluate the agronomic characteristics and cold tolerance of 18 wheat genotypes (11 advanced lines and 7 standard cultivars) under different ecological conditions of Eastern Anatolia. Field experiments were conducted during the 2021-2022 growing season in four locations (Erzurum-Aziziye, Erzurum-Pasinler, Erzincan, and Muş) using a randomized complete block design with three replications. Agronomic traits such as grain yield, protein content, thousand grain weight, and hectoliter weight were measured. Cold tolerance was tested under controlled conditions at -15, -17, and -19 °C. The results revealed significant ($P < 0.01$) genotypic differences for all parameters. The average survival rates were 67.07%, 54.74%, and 38.08% at -15, -17, and -19 °C, respectively, indicating a clear decline with decreasing temperature. Among the genotypes, Parla and genotype 10 displayed the highest cold tolerance, while Esperia and Palandöken 97 exhibited superior protein and hectoliter weights. Lines 1 and 5 are thought to be suitable candidates for registration due to their high yield and large grains. PCA separated the genotypes based on yield, quality, and cold response, showing that the traits are largely independent. These results indicate a wide genetic variation for cold tolerance and quality traits among the tested materials, suggesting that genotypes such as Parla and Palandöken 97 could serve as valuable parents in breeding programs targeting cold tolerance and yield stability in harsh winter conditions.

Keywords: Wheat, cold tolerance, grain yield, protein content, thousand grain weight, genotype $\times$ environment interaction, principal component analysis (PCA)

Introduction

The sustainability of global cereal production depends on the adaptation of genotypes to environmental stress conditions for yield and quality. Particularly in major crops such as wheat, not only agronomic traits like grain yield but also quality components such as protein content, hectolitre weight, and thousand-grain weight have gained increasing importance (Han et al., 2014). Different genotypes may variably perform for quality traits under the same growing conditions. Moreover, exposure to low-temperature stresses such as frost and freezing is

known to adversely affect wheat development, survival, and grain formation. For example, based on the LT_{50} criterion used in frost tolerance assessment, genotypic differences were observed, and this trait was found to be associated with thousand-grain weight (Dumlu et al., 2025). In this context, elucidating the relationships between quality, yield characteristics, and cold tolerance is critical for genotype selection, especially in high-altitude or extremely cold environments. In this study, 18 genotypes were evaluated across four different locations (Aziziye, Erzincan, Muş, and Pasinler), and for each genotype, yield, thousand-

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grain weight, hectolitre weight, protein content, and cold tolerance test results were analysed. Thus, the aim was to compare the genotypes not only in terms of yield potential but also regarding their quality and cold adaptation capacities.

Materials and Methods

Plant Material and Experimental Design

In this study, 18 wheat genotypes, 11 advanced lines and 7 standard cultivars (Ayyıldız, Alturna, Alparslan, Doğu 88, Esperia, Palandöken 97, and Parla), were used as genetic material. The trials were conducted in the 2021-2022 growing season at four locations representing different altitudes and climates. Experiments followed a randomised complete block design (RCBD) with three replications to minimise environmental variation. The research was conducted on 7.2 m² plots for two years during the 2021-2022 growing seasons. In both years, wheat was sown as a winter crop using a seed drill on October 15th. Diammonium phosphate and nitro power (26%) were used as fertilizers in the experiments. All of the phosphorus fertilizer was applied with sowing, half of the nitrogen fertilizer immediately after sowing, and the remaining half during the stem elongation period, at a rate of 6 kg N and 6 kg P₂O₅ per decare.

Data Collection

Grain yield per decare (kg/da), protein content (%), hectoliter weight (kg hL⁻¹), and thousand-grain weight (g) were measured. Cold tolerance tests were conducted under controlled environmental conditions. The experiment involved applying three different low temperature treatments to the plants: -15, -17, and -19°C (Dumlu et al., 2025). After completing the cold treatments in a climate chamber and cold room, the pots were transferred to a greenhouse environment. To determine the effects of low temperature treatments on plant viability, the plants were grown in the greenhouse for three weeks. At the end of this period, the number of surviving plants was recorded (Figure 2).

Statistical Analysis

Data were analyzed using analysis of variance (JMP 7). Differences among genotypes averages for the traits were tested by the LSD method at the 1% level. Principal Component Analysis (PCA) was used to identify associations among traits and to group genotypes according to their performance.

Results and Discussion

The results of this study demonstrate that cold stress has a strong impact on survival and yield-related traits. The superior performance of Parla and Alturna under cold conditions suggests the presence of effective

physiological and molecular mechanisms, such as the activation of the CBF/COR gene family, which enhances freezing tolerance (Dhillon et al., 2013; Knox et al., 2010).

The wide variation among genotypes agrees with findings from Fowler and Gusta (1979), who reported LT₅₀ values ranging from -18°C to -24°C among winter wheat cultivars. The PCA results further confirmed that cold tolerance and yield traits are controlled by distinct but complementary mechanisms.

Yield Performance Across Locations

The grain yields of the 18 wheat genotypes across the experimental locations are presented in Table 1. Analysis of variance revealed highly significant differences ($P < 0.01$) among locations and in the combined dataset for grain yield. The mean yield at the Aziziye was 674.4 kg da⁻¹, with the highest yield obtained from line 4 (761.1 kg da⁻¹) and the lowest from the cultivar Palandöken 97 (530.6 kg da⁻¹). These findings indicate clear differences among genotypes and suggest that certain lines are better adapted to the environmental conditions of Aziziye. Similar observations have been reported in previous studies, where genotypes exhibited differential yield responses within the same environment (Öztürk et al., 2021). In Erzincan, the mean yield was 630.8 kg da⁻¹. The highest yield was recorded for genotype 1 (698.4 kg da⁻¹), while the lowest was obtained from the cultivar Doğu 88 (556.7 kg da⁻¹). Compared with Aziziye, yield levels in Erzincan were slightly lower and varied within a narrower range. This pattern reflects a genotype × environment interaction, which is frequently used to explain yield variability among environments (Kara et al., 2018). At the Muş location, the mean yield was 380.6 kg da⁻¹, with the highest yield recorded for Palandöken 97 (474.6 kg da⁻¹) and the lowest for genotype 1 (263.4 kg da⁻¹). The relatively low mean yield indicates that the environmental conditions of Muş were more limiting and less favourable for wheat production. Consistent with this, several studies have reported wider yield gaps among genotypes under environmentally constrained conditions (Yılmaz et al., 2024). In Pasinler, the average yield was 515.3 kg da⁻¹. The highest yield was obtained from the Alturna genotype (652.8 kg da⁻¹), and the lowest from genotype 6 (461.1 kg da⁻¹). This location provided an intermediate yield environment, allowing for a balanced expression of genotypic variation. Similar findings have been reported by Demir (2000), who observed that moderate environments tend to reduce extreme yield differences among genotypes. Across all locations, the overall mean yield was 543.5 kg da⁻¹. Aziziye and Erzincan emerged as high-yielding sites, Muş

represented a low-yielding environment, and Pasinler occupied an intermediate position (Table 1). These results confirm the strong influence of genotype $\times$ environment interaction and demonstrate that genotype performance is environment-dependent. Hence, it is unrealistic to expect high-yielding genotypes to perform uniformly across diverse growing conditions (Kara et al., 2018; Yılmaz et al., 2024).

Protein Content and

Genotype $\times$ Environment Interactions

The grain protein contents of the 18 wheat genotypes determined across four different locations are presented in Table 2. According to the variance analysis results, the effects of locations and the combined analysis were found to be highly significant ($p < 0.01$) for protein content. The mean protein content in the Aziziye location was calculated as 13.17%. The highest protein content was obtained from the genotype Doğu 88 (16.14%), while the lowest was recorded in genotype 8 (9.61%). This indicates clear differences among genotypes, suggesting that certain genotypes adapted more effectively to the environmental conditions of Aziziye. Similarly, Öztürk et al. (2021) reported that different wheat genotypes exhibited variable protein performance within the same location due to genotype $\times$ environment (G $\times$ E) interactions. In Erzincan, the average protein content was found to be 13.33%. The highest value was again observed in Doğu 88 (16.39%), while the lowest was in genotype 8 (9.77%). These results indicate that protein levels in Erzincan were slightly higher than in Aziziye, although the variation among genotypes followed a similar trend. Kara et al. (2018) emphasised that environmental factors such as soil structure and temperature exert a considerable influence on protein synthesis, and that differences among genotypes are shaped by these environmental effects. In the Muş location, the mean protein content was determined as 12.12%. The highest protein percentage was once again obtained from Doğu 88 (14.85%), while the lowest was found in genotype 8 (8.84%). The lower mean protein value in Muş suggests that the region's environmental conditions—particularly temperature fluctuations and limited soil nitrogen availability—restricted protein accumulation. In line with this, Yılmaz et al. (2024) reported that under environments with low yield potential, greater variations in protein content tend to occur among genotypes. In Pasinler, the mean protein content was measured as 12.65%, with the highest value recorded in Doğu 88 (15.49%) and the lowest in genotype 8 (9.23%). This location represented a moderately favourable environment, enabling distinct yet balanced differences among genotypes. Demir, (2000) similarly

noted that in environments with moderate productivity, genotypic differences tend to be more homogeneous and less extreme. When all locations were combined, the overall mean protein content was calculated as 12.83%. The genotype Doğu 88 had the highest mean value (15.74%), while genotype 8 exhibited the lowest (9.37%). These findings clearly demonstrate that the G $\times$ E interaction significantly influenced grain protein content. Aziziye and Erzincan were identified as high-protein environments, Muş as a low-protein site, and Pasinler as an intermediate environment. Generally, the fact that high-protein genotypes (such as Doğu 88 and Esperia) did not show consistent performance across all environments indicates that genetic potential interacts dynamically with environmental conditions. Consistent with the literature, it is well established that protein content in wheat is determined by both genetic and environmental factors. In particular, nitrogen availability, temperature, and rainfall play a major role in determining protein accumulation (Kara et al., 2018; Yılmaz et al., 2024; Demir, 2000). Therefore, in breeding programmes aimed at achieving higher protein content, not only genotype selection but also the specific environmental characteristics of each location must be carefully considered (Table 2). This result confirms the generally negative relationship between protein content and yield, which tends to be strengthened under variable environmental conditions (Triboi et al., 2003; Hernandez et al., 2018).

Hectolitre Weight and

Genotype $\times$ Environment Interaction

The hectolitre weight (HLW) values of the 18 wheat genotypes evaluated across four different locations revealed that the genotypes responded differently to environmental conditions, indicating a significant genotype $\times$ environment (G $\times$ E) interaction effect. According to the variance analysis results, differences among locations and the combined analysis were statistically highly significant ($p < 0.01$) for hectolitre weight. In the Aziziye location, the mean HLW was determined as 79.59 kg hL⁻¹, with the highest value recorded in genotype 1 (85.4 kg hL⁻¹) and the lowest in genotype 11 (74.6 kg hL⁻¹). In Erzincan, the average HLW was slightly higher (80.44 kg hL⁻¹), where the highest value was observed in Esperia (85.72 kg hL⁻¹) and the lowest again in genotype 11 (75.24 kg hL⁻¹). The Pasinler and Muş locations exhibited lower mean HLW values compared with the other environments. The average HLW in Pasinler was 76.41 kg hL⁻¹, whereas in Muş it was 73.25 kg hL⁻¹. These values are considerably lower than those obtained in Aziziye and Erzincan. In Muş, the highest HLW was obtained from genotype 1 (78.57 kg hL⁻¹), while the lowest was

found in genotype 10 (65.5 kg hL^{-1}). In Pasinler, the highest value was again recorded in genotype 1 (81.98 kg hL^{-1}), and the lowest in genotype 10 (68.35 kg hL^{-1}). These findings demonstrate that genotypes, particularly under Muş and Pasinler conditions, experienced notable reductions in grain density and filling capacity. This can largely be attributed to the climatic characteristics of these regions, such as lower temperatures, shorter and cooler vegetation periods, or suboptimal grain filling conditions associated with low radiation and high humidity. Evers and Millar (2002) reported that grain filling and endosperm density are closely related to environmental stress, while Williams et al. (2008) found that hectolitre weight may vary by up to 10% depending on temperature and water stress. Similarly, Hernandez et al. (2018) and Sharma et al. (2020) observed that low temperature, high humidity, and shortened grain-filling duration negatively affected HLW in wheat genotypes grown under different climatic regions. In the combined analysis, the mean HLW across all locations was calculated as 77.47 kg hL^{-1} . The highest average value was obtained from genotype 1 (82.74 kg hL^{-1}), and the lowest from genotype 10 (71.96 kg hL^{-1}). These results indicate that some genotypes exhibit more stable performance under varying environmental conditions. Finlay and Wilkinson (1963) and Yan and Kang (2002) emphasised that genotypes with high mean performance and low environmental sensitivity in multi-environment trials tend to possess broad adaptation ability. In this context, genotype 1 and Esperia can be considered promising materials with broad environmental adaptability and stable performance in terms of hectolitre weight, whereas genotypes 10, 11, and 8, which consistently exhibited lower HLW values, appear to be more susceptible to environmental stress. These findings are consistent with those reported by Peterson et al. (1992), confirming that hectolitre weight is determined by the combined effects of both genetic structure and environmental factors (Table 3).

Thousand Grain Weight and Genotype $\times$ Environment Interaction

The thousand grain weight (TGW) values of the 18 wheat genotypes evaluated across four different locations revealed significant differences both among genotypes and between environments. According to the results of the variance analysis, the effects of location and the combined analysis were found to be highly significant ($p < 0.01$) for thousand grain weight. In the Aziziye location, the mean TGW was determined as 34.98 g , with the highest value recorded in genotype 1 (41.21 g) and the lowest in genotype 4 (29.35 g). In Erzincan, the average TGW was calculated as 35.57 g ;

genotype 5 exhibited the highest value (44.91 g), while genotype 4 showed the lowest (29.64 g). In the Muş and Pasinler locations, mean TGW values were 31.83 g and 33.59 g , respectively. These comparatively lower values, particularly in Muş, can be attributed to the adverse effects of low temperatures and the shorter growing season during the grain-filling stage. Similarly, Liu et al. (2016) and Kumar et al. (2019) reported that thousand grain weight in wheat is negatively affected by environmental stresses, particularly low temperature and drought conditions. Moreover, the variation in genotype performance across locations highlights the importance of genotype $\times$ environment (G $\times$ E) interaction. Finlay and Wilkinson (1963) and Yan and Kang (2002) emphasised that genotypes showing high mean performance combined with low environmental sensitivity in multi-environment trials possess broad adaptation capacity. In line with this, Öztürk et al. (2021) and Kara et al. (2018) observed that different wheat genotypes exhibited variable TGW values within the same environment, resulting from the interaction between genetic structure and environmental factors. Similarly, Liu et al. (2016) and Kumar et al. (2019) reported that grain weight in wheat is adversely affected by low temperatures and water stress. According to the combined analysis, the mean TGW across all locations was calculated as 34.06 g . The highest value was obtained from genotype 5 (40.03 g), while the lowest was recorded in genotype 4 (28.47 g). These findings confirm that both genetic potential and environmental conditions play a determining role in thousand grain weight, and that some genotypes possess broad environmental adaptability (Table 4). This trait is closely associated with grain density and plumpness and is strongly influenced by environmental stress factors (Evers and Millar, 2002; Williams et al., 2008).

Cold Tolerance and LT₅₀ Evaluation under Controlled Conditions

In this controlled study, the winter hardiness levels of advanced breeding lines and standard cultivars were evaluated at -15 , -17 , and -19°C in three replications, and significant differences among genotypes were observed at all temperature levels ($p < 0.01$). According to the results, the mean survival rate was 67.07% at -15°C , 54.74% at -17°C , and 38.08% at -19°C . This indicates that as temperature decreases, the survival rate of the genotypes declines, clearly defining the threshold of cold tolerance. Based on LT₅₀ (the temperature at which 50% of plants are killed), 15 genotypes maintained more than 50% survival at -15°C , while at -17°C , 13 genotypes exhibited survival above 50% and five genotypes showed regeneration between 30-50%. At -19°C , however, the average survival rate decreased to 38.08% .

At this lowest temperature, significant differences were detected among genotypes in terms of survival rate: the highest survival was observed in the cultivar Parla (63.33%) and genotype 10 (53.48%), whereas the lowest survival was recorded in genotype 11 (17.22%). This finding suggests that Parla exhibits superior cold tolerance by maintaining metabolic activity and preserving cellular membrane integrity under low temperatures. Such performance is consistent with mechanisms reported in highly cold-tolerant winter wheat cultivars, which are associated with strong cold acclimation capacity and elevated expression of the *CBF/COR* gene family (Dhillon et al., 2013; Knox et al., 2010). Conversely, the low survival rate of genotype 11 (17.22%) can be attributed to membrane disruption, osmotic imbalance, and insufficient synthesis of protective proteins under cold stress. Similarly, Fowler and Gusta (1979) reported that genotypes with lower survival rates at sub-zero temperatures typically exhibit higher LT_{50} values, indicating weaker cold tolerance. The results obtained at -19°C therefore demonstrate a wide range of variation in winter hardiness among the tested genotypes, highlighting that genotypes such as Parla possess superior adaptation capacity to extremely low temperatures and could serve as valuable genetic material in future breeding programmes for winter-hardy wheat. These findings are in agreement with previously reported LT_{50} values - for instance, -24°C in the cultivar *Norstar* (Fowler and Gusta, 1979) - and confirm that there is broad genetic variation in cold tolerance among wheat genotypes. Similarly, Knox et al. (2010) reported that LT_{50} in winter wheats varies between -18°C and -24°C depending on genotype, acclimation duration, and *CBF/COR* gene expression. Overall, this study demonstrates that most of the tested genotypes possess moderate to high levels of winter hardiness and show strong potential for local adaptation. The significant genotypic differences observed further indicate that these materials represent an important genetic resource for breeding programmes aimed at improving winter survival in wheat (Table 5).

The 3D scatter plot illustrates the survival rates of 18 wheat genotypes measured at -15 , -17 , and -19°C , thereby visualising the differences in their levels of cold tolerance. As observed, the survival rate of the genotypes decreases with declining temperature, clearly demonstrating the physiological impact of cold stress on plant vitality. In particular, the cultivar Parla, with a survival rate of 63.33%, and genotype 10, with 53.48%, exhibited superior performance under low-temperature conditions, indicating a strong capacity for cold acclimation. This observation aligns with previous findings in the literature, where enhanced

cold tolerance in winter wheat has been associated with increased expression of *CBF* (C-repeat Binding Factor) and *COR* (Cold Regulated) gene families (Dhillon et al., 2013; Knox et al., 2010). Conversely, genotype 11, which recorded a low survival rate of 17.22%, likely suffered from an inability to maintain cell membrane integrity, low membrane lipid saturation, and inadequate antifreeze protein synthesis. Fowler and Gusta (1979) similarly reported that genotypes exhibiting low survival rates under freezing stress tend to have higher LT_{50} values, indicating weaker cold tolerance. The findings of this study are consistent with LT_{50} values previously reported in the literature-for example, -24°C in the cultivar *Norstar* (Fowler and Gusta, 1979), thereby confirming the presence of wide genetic variation in cold tolerance among the tested genotypes. Consequently, genotypes such as Parla and genotype 10, which maintain high survival rates under extremely low temperatures, can be considered valuable genetic materials for advanced breeding programmes targeting improved winter hardiness in wheat (Figure 1).

Principal Component Analysis (PCA) of Yield, Quality, and Cold Tolerance Traits

A Principal Component Analysis (PCA) was conducted to examine the multivariate relationships between yield and quality traits (hectolitre weight, thousand grain weight, and protein content) and the cold tolerance values determined at different temperature levels (-15 , -17 , and -19°C). The first two principal components explained the majority of the total variance, indicating that the variables were effectively summarised within a two-dimensional plane (Figure 3). According to the PCA biplot, hectolitre weight and thousand grain weight were positioned in the same region-specifically in the upper-right quadrant-indicating a strong and positive association between these two traits. This finding suggests that genotypes with larger and denser grains tend to have higher hectolitre weights. In contrast, protein content was located in the opposite direction of the first principal component, revealing a negative relationship between this trait and the yield-related variables. This observation aligns with previous studies reporting a general inverse correlation between protein content and grain yield in wheat (Triboi et al., 2003; Hernandez et al., 2018). The cold tolerance variables (-15 , -17 , and -19°C) clustered together in the lower-right quadrant, indicating that they were positively correlated with each other but weakly associated with yield and quality traits. Among these, the -19°C variable most clearly distinguished genotypes in terms of cold tolerance. Similarly, studies by Dhillon et al. (2013) have shown

that, under extreme low-temperature conditions, the physiological mechanisms determining genotype selection are largely independent of yield components. Overall, the PCA results demonstrated that quality-related traits (hectolitre weight and protein content) and stress-tolerance traits (cold tolerance) were clearly separated, suggesting that these two groups of traits are controlled by distinct genetic and physiological mechanisms. This finding emphasises the importance of simultaneously evaluating yield stability and cold adaptation in breeding programmes conducted in high-altitude regions with a high risk of frost.

Cluster Analysis Based on Yield, Quality, and Cold Tolerance Traits

According to the heatmap, the combined analysis results illustrate the clustering of 18 wheat genotypes based on yield, protein content, thousand grain weight, hectolitre weight, and cold tolerance traits under different low-temperature conditions (-15, -17, and -19°C). In the colour scale, red tones represent higher values, while green tones indicate lower ones. Overall, varieties such as Esperia and Palandöken 97 stood out in certain quality parameters-particularly in protein content and hectolitre weight-whereas Alturna and several breeding lines (notably lines 4, 5, and 6) exhibited higher grain yield and thousand grain weight. In cold tolerance tests, the lines that exhibited greener hues at -17 and -19°C (lines 3, 4, 5, and 6) were found to be more sensitive to low temperatures. This observation supports the potential genetic trade-off between cold tolerance and high yield or grain weight reported in the literature (Li et al., 2018). Moreover, a generally negative correlation between protein content and yield-commonly described in wheat (Triboi et al., 2003)-was also evident here, as high-protein genotypes (Esperia and Palandöken 97) tended to show comparatively lower yields. Thus, the clustering pattern in the heatmap indicates that the genotypes can be grouped into genetically distinct subclusters based on their cold tolerance and quality characteristics. These differences could be effectively exploited in breeding programmes aimed at optimising both yield performance and environmental adaptation potential (Figure 4).

Venn diagram illustrating the overlap of wheat genotypes across four key traits

The Venn diagram illustrates the comparative distribution of 11 advanced wheat lines and 7 standard cultivars (Alparslan, Alturna, Ayyıldız, Doğu 88, Esperia, Palandöken 97, and Parla) based on protein content, cold tolerance (-19°C), grain yield, and hectolitre weight, according to the combined analysis results. The analysis revealed that the cultivar Alparslan

was located in the overlapping region of both the protein and cold tolerance categories, indicating a genetic advantage combining high protein potential with tolerance to low temperatures. Similarly, line 1, which intersected with the cultivar Ayyıldız in the same region, exhibited a comparable combination of high protein content and cold tolerance. Esperia was positioned at the intersection of the yield and hectolitre weight groups, together with line 5, suggesting that these two genotypes stand out with their high grain plumpness and yield potential. This indicates that Esperia and line 5 could serve as valuable parents materials in breeding programmes targeting both physiological resilience and agronomic productivity. Likewise, line 8, together with the cultivar Parla, was positioned exclusively within the hectolitre weight region, highlighting their superiority in terms of grain density and quality attributes. The absence of triple or quadruple intersections suggests that these traits are largely independent, being controlled by distinct genetic mechanisms. In agreement with previous reports indicating weak correlations between yield, protein content, and cold tolerance in wheat (Kurt et al., 2022; Tadesse et al., 2019), the present findings demonstrate that some advanced lines share close trait similarities with specific standard cultivars. In particular, the Ayyıldız-Line 1, Esperia-Line 5, and Parla-Line 8 pairings emerge as promising candidates for future breeding programmes aimed at developing cold-tolerant, high-yielding, and high-quality wheat genotypes, and could therefore be prioritised as potential parents genitors in hybridisation efforts (Figure 5).

The results indicated highly significant ($p < 0.01$) genotypic differences for all traits. Parla and Alturna displayed superior cold tolerance and yield stability across environments, while Esperia and Palandöken 97 exhibited high protein and hectoliter values. The mean survival rate declined with decreasing temperature, with 67.07% at -15°C, 54.74% at -17°C, and 38.08% at -19°C.

At -19°C, Parla had the highest survival rate (63.33%), followed by genotype 10 (53.48%), whereas genotype 11 had the lowest (17.22%). This pattern indicates wide variation in cold tolerance among genotypes. The correlation matrix showed that yield and cold tolerance were positively related, while protein content was relatively independent of yield performance.

Conclusions

In this study, 18 wheat genotypes (11 advanced breeding lines and 7 standard cultivars) were evaluated across four different locations (Aziziye,

Erzincan, Muş, and Pasinler) in terms of yield, quality, and cold tolerance characteristics. The findings revealed significant variation among genotypes and demonstrated that environmental conditions exerted a strong influence on these traits. In terms of grain yield, the Aziziye (674.4 kg da⁻¹) and Erzincan (630.8 kg da⁻¹) locations were the most productive. The highest yield was obtained from line 4 (761.1 kg da⁻¹), while the lowest was recorded for the cultivar Palandöken 97 (530.6 kg da⁻¹). Regarding protein content, the cultivars Doğu 88 and Esperia stood out, with an overall mean of 12.83%. The highest protein content (15.74%) was observed in Doğu 88, while the lowest (9.37%) was recorded in line 8. For hectolitre weight, line 1 (82.74 kg hL⁻¹) and the cultivar Esperia (82.66 kg hL⁻¹) exhibited the highest values, whereas genotype 10 recorded the lowest. The thousand grain weight (TGW) was highest in line 5 (40.03 g) and line 1 (39.82 g). These genotypes, with their larger kernels, reflected high yield potential and stable performance under stress conditions. In cold tolerance tests, the mean survival rates were 67.07% at -15°C, 54.74% at -17°C, and 38.08% at -19°C. The highest survival rate was observed in the cultivar Parla (63.33%), whereas the lowest was found in line 11 (17.22%). This demonstrates that Parla possesses a superior cold acclimation capacity.

The PCA biplot analysis revealed a clear structural variation among the examined wheat genotypes. The first two principal components together explained 70.4% of the total variance, indicating that the agronomic and physiological traits analysed effectively captured the genotypic differences. The PC1 axis primarily demonstrated an inverse relationship between yield and the cold tolerance levels (-15°C, -17°C, -19°C). This finding indicates that high-yielding genotypes tended to be more sensitive to low temperatures, whereas lower-yielding genotypes exhibited greater cold tolerance. This is consistent with previous studies reporting increased stress sensitivity in high-yielding genotypes (Poudel et al., 2020; Ding et al., 2020). On the PC2 axis, protein content and hectolitre weight showed positive loadings, forming the main component for distinguishing quality-related traits. The cold tolerance variables were positioned in the same direction, confirming their high correlation and indicating that genotypes responded similarly to low-temperature stress. In the PCA biplot, Parla, line 1, and Palandöken 97 clustered within the cold-tolerant group, whereas lines 2, 4, and 11 were grouped as high-yielding but cold-sensitive genotypes. These results highlight PCA as an effective tool for

evaluating adaptation and stress tolerance across different environments. The findings suggest that the use of cold-tolerant genotypes as parents in breeding programmes could enhance yield stability in winter wheat cultivation. Conversely, high-yielding but less tolerant genotypes could be better utilised in mild-climate production systems. Future integration of PCA results with genomic selection and marker-assisted breeding data is expected to facilitate more accurate and durable genetic gains for stress tolerance. The results obtained from this study provide important insights for advanced wheat breeding programmes: Parla, genotype 10, and Palandöken 97 can be considered as winter-hardy parents materials due to their combination of high cold tolerance and moderate-to-high yield potential. Doğu 88 and Esperia, with their high protein content and hectolitre weights, are promising for quality-oriented breeding. Lines 1 and 5, with high yield and large grain size, are strong candidates for varietal registration. According to the PCA findings, selecting genotypes that balance yield, quality, and cold tolerance will provide more efficient genetic progress. The results offer valuable phenotypic foundations for application in marker-assisted and genomic selection strategies (Figure 6).

This study revealed substantial genetic diversity at both agronomic and physiological levels and identified promising genotypes combining high yield potential with strong winter hardiness and grain quality traits. The findings provide a solid scientific basis for the development of cold-tolerant, high-quality, and stable-yielding wheat cultivars adapted to the Eastern Anatolia region. The combined use of field performance data, cold tolerance tests and PCA analysis enabled a comprehensive evaluation of wheat genotypes under contrasting ecological conditions.

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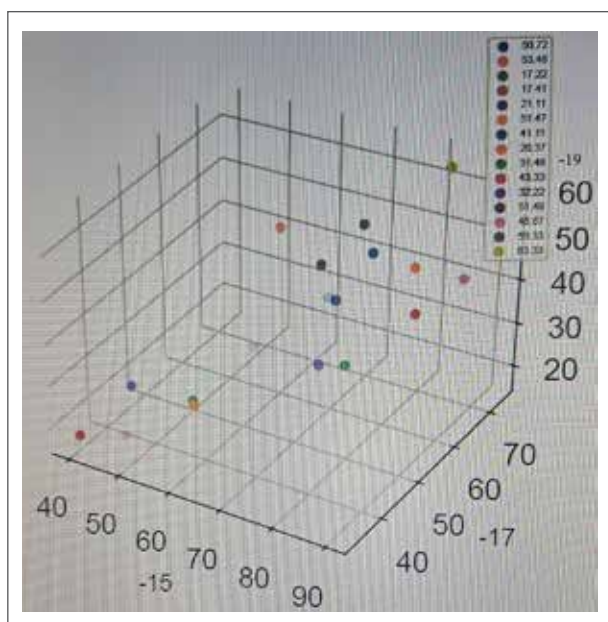


Figure 1. 3D scatter plot of cold tolerance levels.

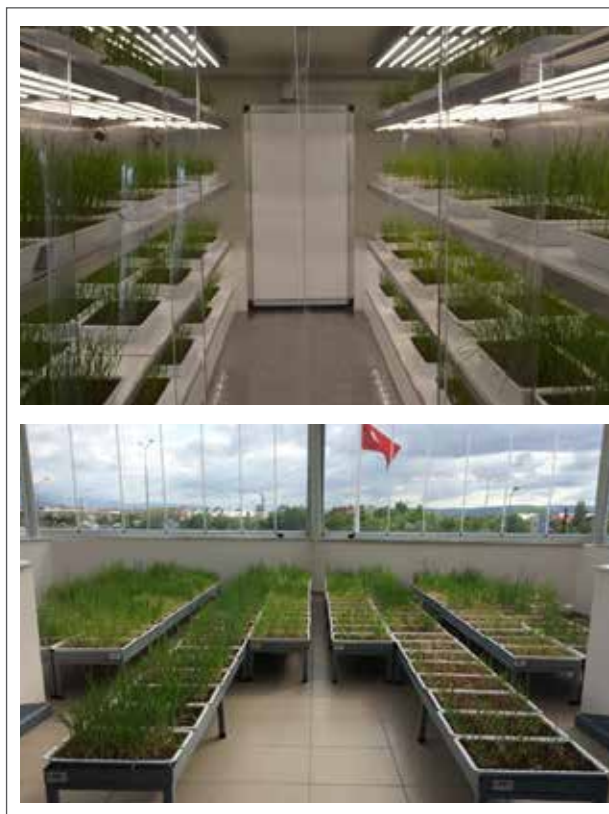


Figure 2. Climate chamber and greenhouse images from cold tolerance studies.

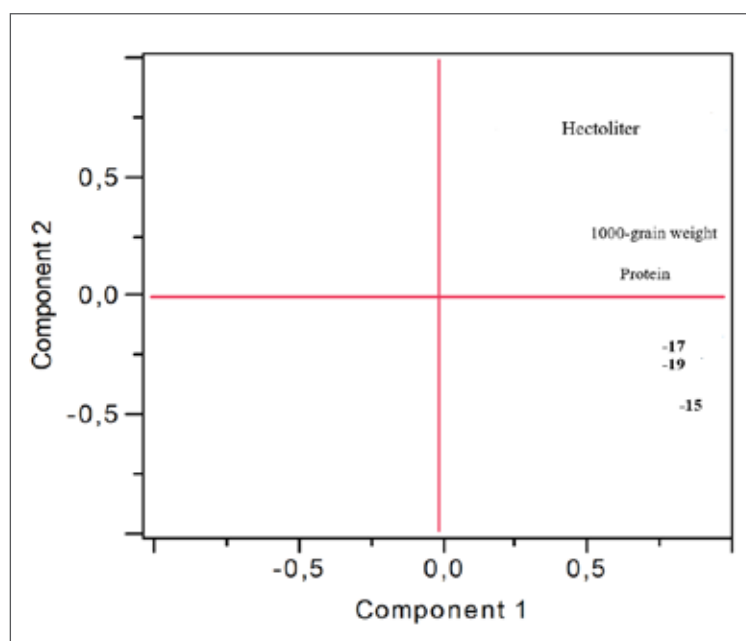


Figure 3. Multivariate Evaluation of Genotypic Relationships (PCA) chart.

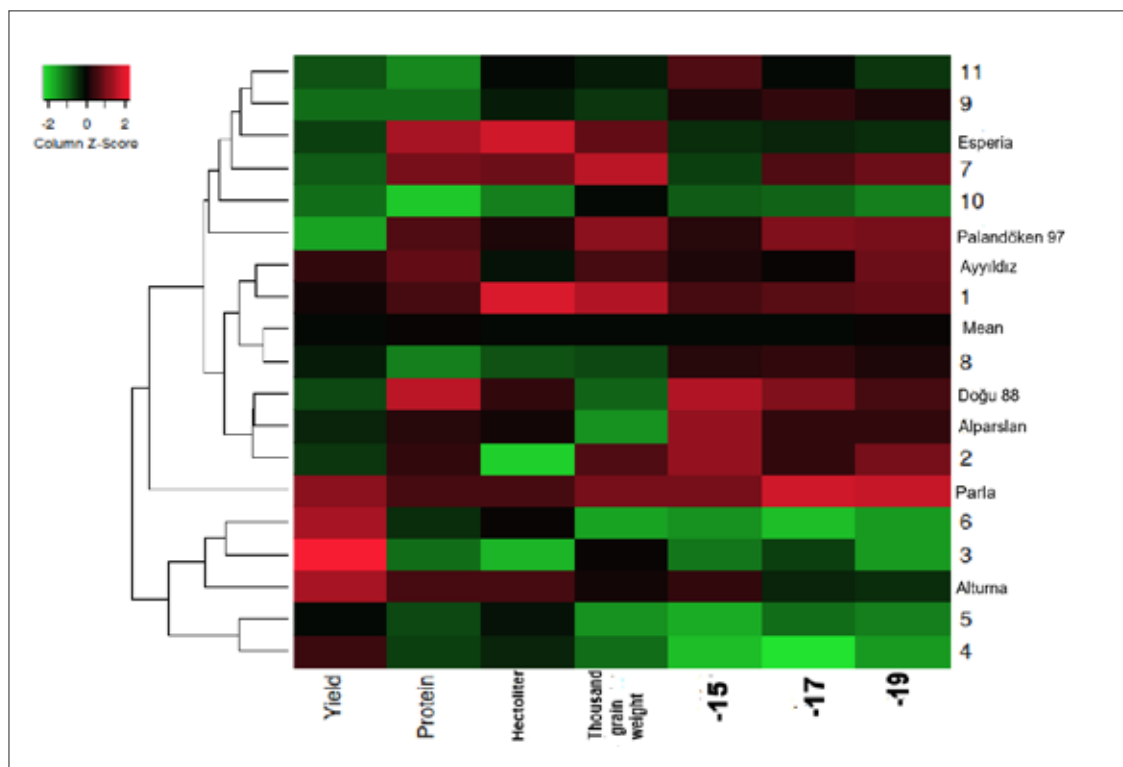


Figure 4. Cluster heatmap analysis of genotypes based on yield, quality, and cold tolerance traits.

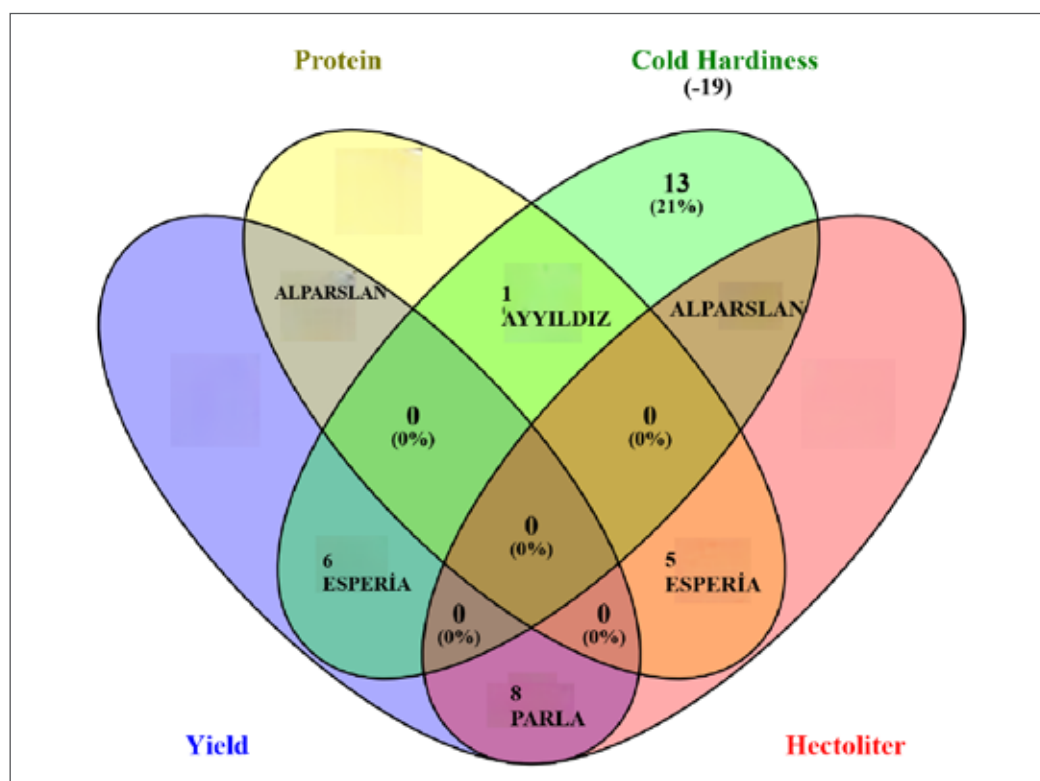


Figure 5. Venn diagram illustrating the overlap of wheat genotypes across four key traits.

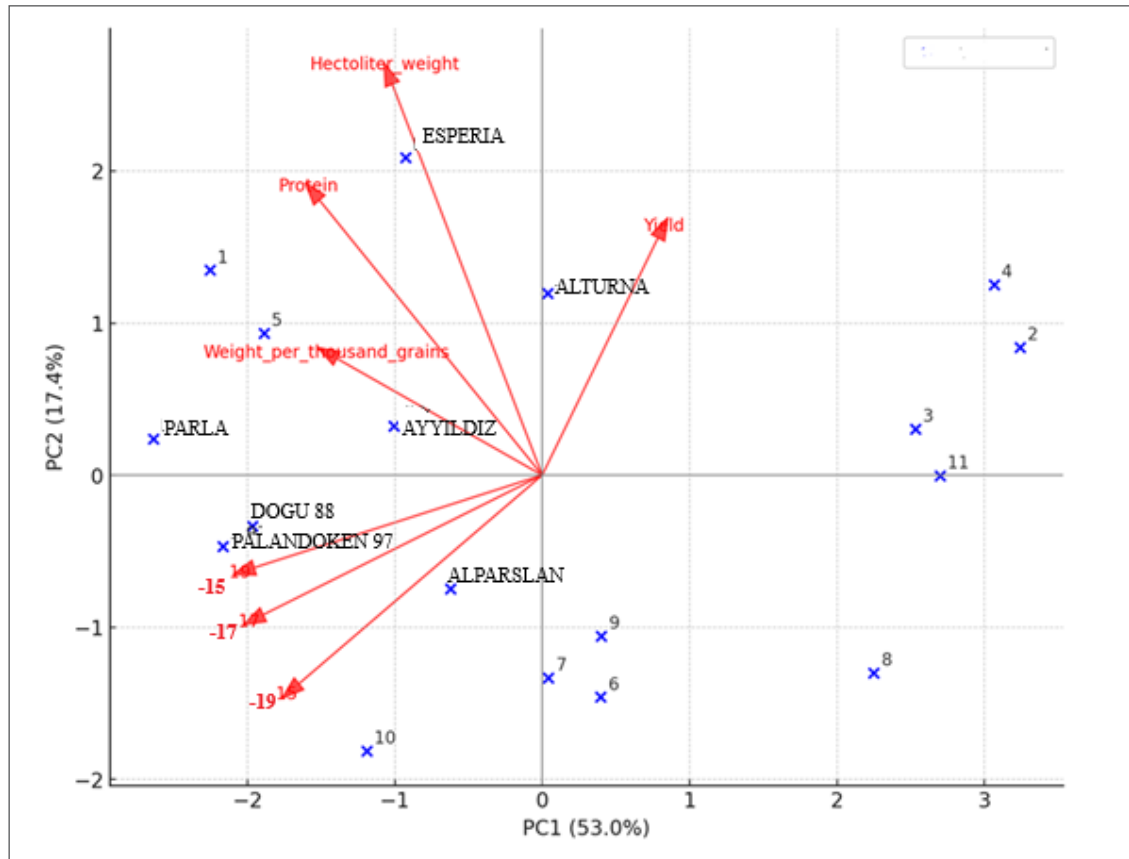


Figure 6. Principal component biplot analysis of the wheat genotypes evaluated.

Table 1. Grain yield performance of 18 wheat genotypes across four different locations.

Genotype	Aziziye	Erzincan	Muş	Pasinler	Combined
11	716,7 a-c	677,8 ab	472,4 a	573,6 bc	610,1 a
4	761,1 a	672,0 a-c	390,9 b-e	529,4 c-e	588,3 ab
Alturna	752,2 ab	638,9 c-e	304,5 fg	652,8 a	587,1 ab
Parla	711,9 a-d	637,7 de	425,0 a-d	540,0 b-d	578,7 bc
2	696,1 b-e	683,1 ab	332,4 e-g	525,0 c-e	559,2 cd
Ayyıldız	645,3 e-g	658,3 b-e	328,8 e-g	594,2 ab	556,6 c-e
1	658,9 d-g	698,4 a	263,4 g	571,7 bc	548,1 d-f
3	632,5 fg	666,4 a-d	324,0 e-g	543,9 b-d	541,7 d-g
6	644,2 e-g	602,3 fg	438,2 a-c	461,1 f-h	536,5 d-h
Alparslan	605,6 g-i	638,0 de	360,7 d-f	529,4 c-e	533,4 d-h
10	558,1 ij	597,2 g	450,7 a-c	514,4 c-f	530,1 e-h
Esperia	687,2 c-f	663,0 b-e	276,6 g	479,7 e-h	526,6 f-h
Doğu 88	572,8 h-j	556,7 h	452,9 ab	508,9 d-f	522,8 f-i
9	631,9 fg	629,9 e-g	380,9 c-e	441,4 gh	521,0 f-i
5	615,8 gh	560,9 h	406,6 a-d	493,9 d-g	519,3 g-i
8	608,9 g-i	633,3 d-f	381,3 c-e	431,7 h	513,8 h-i
7	622,8 gh	599,5 fg	386,4 b-e	440,8 gh	512,4 h-i
Palandöken 97	530,6 j	541,7 h	474,6 a	443,3 gh	497,5 i
Mean	674,4 A	630,8 B	380,6 D	515,3 C	543,5
CV	5,0	4,2	11,0	7,3	6,0
F Value	10,24**	15,14**	7,04**	8,29**	8,67**
LSD	51,3	33,8	70,6	59,9	55,3

Table 2. Protein ratios of genotypes by locations (%).

Genotype	Aziziye	Erzincan	Muş	Pasinler	Combined
Doğu 88	16,14 a	16,39 a	14,85a	15,49a	15,74a
Esperia	15,94 b	16,19 a	14,66b	15,3b	15,54b
5	15,19 c	14,89 bc	13,97c	14,58c	14,61c
Ayyıldız	14,74 d	14,97 b	13,56d	14,15d	14,37d
Palandöken 97	14,32 e	14,55 cd	13,17e	13,75e	13,96e
1	13,98 f	14,89 bc	12,86f	13,42f	13,94ef
Parla	14,29 e	14,52 cd	13,15e	13,72e	13,94ef
Alturna	14,24 e	14,46 d	13,1e	13,67e	13,88f
10	14,21 e	13,48 f	13,07e	13,64e	13,54g
Alparslan	13,78 g	14,00 e	12,68g	13,23g	13,44h
4	12,47 h	12,64 g	11,47h	11,97h	12,16ı
2	12,05 ı	12,22 h	11,09ı	11,57ı	11,75j
3	11,86 j	12,03 h	10,91j	11,39j	11,57k
7	11,27 k	11,44 ı	10,37k	10,82k	10,99l
11	11,25 k	11,44 ı	10,35k	10,8k	10,97l
6	10,98 l	11,15 ıj	10,1l	10,54l	10,71m
9	10,80 m	10,97 j	9,94m	10,37m	10,53n
8	9,61 n	9,77 k	8,84n	9,23n	9,37o
Mean	13,17 B	13,33 A	12,12 D	12,65 C	12,83
CV	6,0	10,2	6,1	5,0	7,0
F Value	1168,2**	214,5**	1214,9**	1210,7**	19,5**
LSD	0,14	0,35	0,14	0,15	0,14

Table 3. Hectolitre weight of genotypes by location.

Genotype	Aziziye	Erzincan	Muş	Pasinler	Combined
1	85,4 a	85,15 a	78,57 a	81,98 a	82,74 a
Esperia	85,0 b	85,72 a	78,20 b	81,60 b	82,66 a
5	83,6 c	81,21 b-d	76,91 c	80,26 c	80,09 b
Alturna	81,2 d	81,89 b	74,70 d	77,95 d	78,97 c
Parla	81,2 d	81,89 b	74,70 d	77,95 d	78,97 c
Doğu 88	80,8 e	81,49 bc	74,34 e	77,57 e	78,58 d
Palandöken 97	80,3 f	80,98 b-e	73,88 f	77,09 f	78,09 e
Alparslan	80,0 g	80,68 b-e	73,60 g	76,80 g	77,80 f
4	79,8 gh	80,33 b-e	73,42 gh	76,61 gh	77,61 g
9	79,6 h	80,13 b-e	73,23 h	76,42 h	77,41 h
3	79,3 ı	79,83 c-f	72,96 ı	76,13 ı	77,12 ı
Ayyıldız	79,2 ı	79,87 b-f	72,86 ı	76,03 ı	77,02 ı
7	78,9 j	79,43 d-f	72,59 j	75,74 j	76,73 j
2	78,7 j	79,24 ef	72,40 j	75,55 j	76,54 k
6	77,5 k	78,04 fg	71,30 k	74,40 k	75,37 l
8	76,3 l	76,84 gh	70,20 l	73,25 l	74,20 m
11	74,6 m	75,24 h	68,63 m	71,62 m	72,55 n
10	71,2 n	79,98 b-e	65,5 n	68,35 n	71,96 o
Mean	79,59 B	80,44 A	73,25 D	76,41 C	77,47
CV	3,0	15,2	3,3	4,0	3,1
F Value	1200,0**	13,5**	1190,2**	1202,8**	130,9**
LSD	0,26	1,89	0,24	0,26	0,33

Table 4. Thousand grain weights of genotypes by location.

Genotype	Aziziye	Erzincan	Muş	Pasinler	Combined
5	38,72 c	44,91 a	35,24 c	37,17 c	40,03 a
1	41,21 a	41,08 b	37,50 a	39,56 a	39,82 b
Palandöken 97	39,58 b	39,92 bc	36,02 b	38,00 b	38,40 c
Parla	38,94 c	39,27 b-d	35,44 c	37,38 c	37,77 d
Esperia	38,32 d	38,65 cd	34,87 d	36,79 d	37,17 e
10	37,82 e	38,00 c-e	34,42 e	36,31 e	36,64 f
Ayyıldız	37,46 f	37,78 de	34,09 f	35,96 f	36,34 g
Altuna	35,73 g	36,04 ef	32,51 g	34,40 g	34,66 h
11	35,18 h	35,48 f	32,01 h	33,77 h	34,12 i
8	35,09 h	35,36 f	31,93 h	33,69 h	34,04 i
9	34,30 i	34,57 fg	31,21 i	32,93 i	33,27 j
7	33,15 j	33,43 gh	30,17 j	31,82 j	32,16 k
6	32,61 k	32,89 g-i	29,68 k	31,31 k	31,64 l
Doğu 88	31,52 l	31,80 h-j	28,68 l	30,26 l	30,58 m
2	31,06 m	31,35 i-k	28,26 m	29,82 m	30,13 n
Alparslan	29,83 n	30,10 jk	27,15 n	28,64 n	28,94 o
3	29,78 n	30,07 jk	27,1 n	28,59 n	28,89 o
4	29,35 o	29,64 k	26,71 o	28,18 o	28,47 p
Mean	34,98 B	35,57 A	31,83 D	33,59 C	34,06
CV	5,0	4,2	5,0	6,4	3,1
F Value	1196,35**	40,21**	1210,30**	1190,10**	101,52**
LSD	0,30	1,96	0,26	0,29	0,33

Table 5. Cold Resistance Degrees of Genotypes (%).

Genotype	-15	-17	-19
1	75,93 d	62,22 c	50,72 c
2	40,00 n	33,33 k	17,41 i
3	41,67 m	44,44 i	21,11 h
4	46,67 l	36,67 j	17,41 i
5	58,89 i	61,55 c	51,47 c
6	71,48 f	58,89 d	41,11 f
7	70,00 g	58,99 d	41,11 f
8	53,70 j	45,19 i	20,37 h
9	76,67 d	54,17 f	31,48 g
10	85,93 b	58,52 d	53,48 b
11	50,53 k	49,05 h	17,22 i
Alparslan	86,30 b	58,52 d	43,33 e
Altuna	73,33 e	51,85 g	32,22 g
Ayyıldız	70,95 fg	55,56 e	51,48 c
Doğu 88	90,00 a	65,93 b	46,67 d
Esperia	61,11 h	51,48 g	32,22 g
Palandöken 97	71,90 f	65,56 b	53,33 b
Parla	82,22 c	73,33 a	63,33 a
Mean	67,07 A	54,74 B	38,08 C
CV	10,0	9,3	14,2
F value	1197,9**	1194,8**	1199,6**
LSD	1,28	0,83	1,22

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