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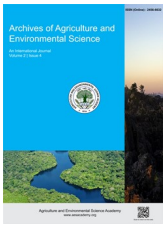

ORIGINAL RESEARCH ARTICLE

Reactions of anthocyanin rich in maize genotypes to low temperature treatments according to photosynthesis, gas exchange properties, and bio-active compounds

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ABSTRACT

Low temperatures during the early growing stages limit the productivity of maize considerably. Investigating responses of different coloured corns (*Zea mays* L.) to chilling may reveal alternative genotypes which can be preferred under early sowing conditions of water-shortage farming areas. The aim of this study is determining whether the color factor affects the tolerance to chilling in maize and which properties are effective on the low temperature tolerance. We exposed corns with different colours (white, yellow, red, purple) to different temperatures [8°C, 12°C, 16°C, 25°C (control)] and analysed the effects of temperature on morphological, physiological, bio-active properties and stress indicators. Using 14-day old seedlings, we noted that purple corn had the highest seedling length, seedling weight, chlorophyll content, stomatal conductance, chlorophyll B and total phenolic content in the 8°C and followed by white (photosynthesis rate, chlorophyll fluorescence, chlorophyll A and carotenoids), yellow (transpiration rate, sub stomatal CO_2, and total antioxidant activity) and red corns (water use efficiency, total anthocyanin content and proline). On the other hand, white corn maintained its superiority in other treatments, receiving the highest values in 9 of 17 characteristics at 12°C, in 8 of 17 at 16°C and in 10 of 17 in the control. Performance of purple corn in the 8°C was the most remarkable one in all genotypes and treatments. Based on our results, it has been concluded that white and purple corns are more chilling tolerant genotypes and may be alternative for early sowing conditions in drought farming areas.

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INTRODUCTION

Global maize productivity and grain quality can be negatively affected by climate changes. In order to maximize yield, it is necessary to maintain optimal temperatures during the growing stages. When temperatures are suboptimal during any critical stage for an extended period, the growth and yield formation processes can be adversely affected (Waqas *et al*., 2021). Plants are significantly influenced by low temperatures as an abiotic stress factor and respond differently that depends on their developmental stages as well as the extent and duration of the temperature decrease. Plants responses to low temperature conditions are certainly mediated by a variety of biochemical, morphological, physiological, cellular, and molecular changes which assist them in tolerating, and surviving under a range of temperature change. During cold acclimation, plant lipid composition is altered, osmolytes are accumulated (sugars and proline), and transcript levels of cold-induced genes are elevated (Ramazan *et al*., 2021).

Zea mays L., a tropical and subtropical plant, has a high temperature threshold for germination, therefore it is inherently sensitive to low temperatures, particularly during germination, and is rarely cultivated in higher latitudes or mountainous regions (Zhang *et al*., 2020). Low temperatures during the early growing season

limit the productivity of maize considerably. In Europe, United States, Russia, Central Asian States and the Indian subcontinent, farmers are confronted with the problem of chilling stress despite the fact that severity, duration, nature and timing vary according to regions (Farooq *et al*., 2009). As low temperature is one of the most critical abiotic stress factors that limits growth, development and distribution of plants (Nguyen *et al*., 2009), it is important to identify the components of maize in response to cold stress to better understand how it adapts to chilling. Farooq *et al*. (2009) reported that the optimum temperatures for maize growth are between 25°C and 28°C whereas temperatures less than 12°C–15°C cause chilling stress and below 12° C can be fatal. Furthermore, Long (1983) noted a correlation between the significant decrease in C_4 productivity and the decrease in mean annual temperatures.

Additionally, plants exposed to low temperature exhibit chilling stress symptoms due to lower water uptake by roots (Nguyen *et al*., 2009). Symptoms include reduction in plant growth rate, leaf elongation (Sowiński *et al*., 2003; Verheul *et al*., 1995), mineral and water uptake, stomatal conductance (Aroca *et al*., 2003) and chlorophyll fluorescence (Aroca *et al*., 2003; Foyer *et al*., 2002) as well as increase in ROS (reactive oxygen species) (Cervilla *et al*., 2012) production (Foyer *et al*., 2002) and antioxidant activities (Aroca *et al*., 2003; Foyer *et al*., 2002). Vital components such as the photosynthetic apparatus are particularly susceptible to the adverse effects of low temperature (Farooq *et al*., 2009), which could possibly result in chlorosis, indicating a decline in chlorophyll synthesis (Farooq *et al*., 2009; Miedema, 1982). Furthermore, low temperature stress also affects the composition of fatty acids, cell membrane fluidity and metabolic rate at the cellular and molecular levels (Nishida & Murata, 1996) as results of previous changes chilling mainly damage the plant by transforming the liquid phase of cellular membrane into gel phase (Wolfe, 1978).

On the other hand, CO_2 assimilation in leaves may decrease due to membrane damage, photo-inhibition, and disturbance on various enzyme activities. One of the major deterrents of plant growth is the macromolecule injuries caused by ROS under chilling stress. Additionally, low-molecular weight osmolytes, such as glycine betaine, proline, and organic acids, are crucial to sustaining the cellular function under chilling stress. Previous studies have reported that polyamines and several enzymes act as antioxidants and also reduce the adverse effects of chilling stress (Farooq *et al*., 2009). Another study has highlighted that low temperature also affects membrane properties and causes a decline in hydraulic conductance of symplastic components. However, little is known about the factors responsible for the decline in hydraulic conductance (Markhart *et al*., 1979).

Plants show resistance to low temperatures for weeks in nature, with lignins rich in phenolics in their cell walls. Such structural differentiations protect cells from ice formation in intercellular spaces or on epidermal surfaces. The accumulation of anthocyanins in the epidermal cells of secondary plant tissues causes the osmotic potential of the cell to decrease, delays the freezing of the plant's surface components and protects the plants from the negative effects of low temperature (Chalker-Scott, 1992; Ishikawa, 1984). Corn is a crop with wide variation in grain color. Colored corns contain high amounts of carotenoids, flavonoids, phenolic compounds and anthocyanins (Khamphasan *et al*., 2018). Anthocyanins are water soluble flavonoids and are in the class of poly-phenolic pigments (Tian *et al*., 2019) which have a wide color spectrum from orange to blue in the plant kingdom (Yang and Zhai, 2010). In many studies conducted to date, anthocyanins detoxifying effects on free radicals and the ability to detoxify ROS that cause cellular damage were highlighted (Tian *et al*., 2019; Urias-Lugo *et al*., 2015). It has been reported that colored corns have higher antioxidant activities than colorless ones in consistent previous knowledge (Kapcum and Uriyapongson, 2018). Tolerance of maize to cold conditions in the tropics requires genetic improvement which implies vigorous seedling growth without suffering from chilling injuries under low temperature conditions (Zaidi *et al*., 2010). The objectives of the current work are, investigating the reactions of different colored corns under low temperature conditions, determining the responses of corn genotypes that already contains anthocyanins to different temperatures and detecting the most ideal genotype for low temperature stress conditions.

MATERIALS AND METHODS

Plant material and growth conditions

White, yellow, red, and purple corn seeds were used from long term self-pollinated populations obtained from the "Republic of Turkey Ministry of Agriculture and Forestry, Maize Research Institute". Soil was obtained from the "Selcuk University Agriculture Faculty Trial Area" to represent the soils of the Middle Anatolian Region of Turkey (see Table 1 for physicochemical properties of the soil). The soil sample was air-dried first, and then sieved using a 0.5 cm sieve. Fourteen seeds were sowed in 2lt pots with 1:1:1 ratio of soil, sand, and peat. This was to make sure that we had at least 10 strong and healthy seedlings. The trial was set up according to "Factorial Experimental Design" with three replications. We had four genotypes (white, yellow, red, and purple) and 4 treatments [(8°C, 12°C, 16°C and 25°C (control)] in this study as factors. The seedlings were cultivated in the climate chamber for 14 days whose climatic conditions were 60% air moisture and 25°C - 20°C light-dark conditions for 16h - 8h (Pietrini *et al*., 2002). After two weeks in the controlled climate chamber, the seedlings were transferred to the chilling cabinets at 8°C, 12°C, 16°C for two days to induce anthocyanin accumulation; seedlings in the control were left under normal conditions (25°C). Performed observations and measurements were as follow.

Growth attributes

Three seedlings were harvested per pot. Their weights (g) and lengths (Anjum *et al*., 2011) were measured and results were recorded as growth attributes (Parveen *et al*., 2019).

Table 1. Physicochemical properties of the soil that represents Middle Anatolian Region of Turkey.

pH	: 7.74	P (mg kg^{-1})	: 22.60	Cu (mg kg^{-1})	: 0.63
EC (µS cm^{-1})	: 70.30	K (mg kg^{-1})	: 253.00	Mn (mg kg^{-1})	: 2.54
OM (%)	: 1.50	B (mg kg^{-1})	: 0.55		

EC : Electrical conductivity

OM : Organic matter

Photosynthetic properties

Photosynthesis rate (A, µmol CO_2 m^{-2} s^{-1}), transpiration rate (E, mmol H_2O m^{-2} s^{-1}) and sub-stomatal CO_2 (Ci, µmol mol^{-1}) were determined using the "LCi Compact Portable Photosynthesis System - ADC Bioscientific" equipment for the three seedlings in three replicates. Water use efficiency (WUE) values were calculated according to the methods of Hasan *et al.* (2017). Similarly, chlorophyll fluorescence (Fv Fm^{-1}) values of three seedlings in each pot were determined using a "Plant Efficiency Analyser (PEA, Hansatech Instruments Ltd.)". Additionally, CC (chlorophyll contents) (Pietrini *et al.*, 2002) in leaves of the three seedlings in each pot were estimated using a chlorophyll meter (SPAD 502) and recorded as Spad. Finally, stomatal conductance (SC) (mmol $m^{-2}s^{-1}$) of the three seedlings per pot was measured using a leaf porometer "Model SC-1 Decagon Devices".

Photosynthetic pigment concentrations

Photosynthetic pigment concentrations were analysed based on the method of Özdemir (2021). First, a 0.5 g fresh weight leaf sample was homogenised using 1 g glass dust and 25 ml acetone (100%). The extract was filtered while 1 g anhydrous sodium sulphate was being added. Then the volume of the filtrate was recorded, and absorbance was measured at 400 - 700 nm with a UV spectrophotometer.

Pigment concentrations were calculated with the formula below:

$$ChlA\ (\mu g\ ml^{-1}) = 11.75A_{662} - 2.35A_{645} \quad \text{(Eq. 1)}$$

$$ChlB\ (\mu g\ ml^{-1}) = 18.61A_{645} - 3.96A_{662} \quad \text{(Eq. 2)}$$

$$Total\ carotenoids\ (\mu g\ ml^{-1}) = \frac{1000A_{470} - 2.27\ ChlA - 81.40\ Chl\ B}{227} \quad \text{(Eq. 3)}$$

Bioactive compounds

Total anthocyanin content (TAC): We followed the procedure of Cervilla *et al.* (2012) to determine anthocyanin content. 0.1 g dry weight sample in 5 ml propanol and HCl solution was homogenised and centrifuged at 5,000 rpm. All samples were kept at room temperature for 24 h and centrifuged in tubes at 6,500 rpm.

Finally, the absorbances were calculated and corrected at 535 - 650 nm using the formula below:

$$A = A535 - A\,650 \quad \text{(Eq. 4)}$$

Total antioxidant activity (TAA): The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of phenolics was evaluated by measuring the capacity of the DPPH radical to bleach a black coloured methanol solution based on previous methods (Khampas *et al.*, 2013). Then a 4.5 ml DPPH solution was added to a 0.5 ml phenolic extract. The mixture was vortexed and left to wait for 30 min in dark. The absorbance of samples was measured at 517 nm against a solvent blank (Özdemir, 2021).

The scavenging rate of DPPH radicals was calculated with the formula below:

$$Scavanging\ rate = \left[\frac{A0 - A1}{A0}\right] \times 100 \quad \text{(Eq. 5)}$$

where A_0 is the absorbance of the control (0.5 ml extraction solvent with 4.5 ml DPPH solution) and A_1 is the absorbance in the presence of phenolic extract solution.

Total phenolic content (TPC): Total phenolic content was determined following the procedures of Konrade and Klava (2017), with some modifications. First, a 0.5 g ground sample was extracted using 5 ml methanol (80%) for 48 h, then 2.5 ml of Folin-Ciocalteu reagent (diluted 10 times) was added. After 3 min, 2.0 ml sodium carbonate (Na_2CO_3) solution [2% (w/v)] was added. The solution was mixed and left in a dark place for minimum 30 - 60 min. afterwards, the absorbance values of the samples were measured at 750 nm against a solvent blank. Lastly, the results were recorded as mg gallic acid equivalent per 100 g of dry weight (mg GAE 100 g^{-1} DW) (Özdemir, 2021).

Stress indicators

Proline: Proline content of maize leaves was determined based on previous methods (Bates *et al.*, 1973). First, 0.1 g fresh leaf sample was extracted using SSA (3%, w/v), then the mixture was centrifuged at 5,000 rpm. Ninhydrin and glacial acetic acid solutions were added to the supernatant and the mixture was heated at 95°C in water bath. Afterwards, toluene was added, and absorbance was measured at 520 nm.

Malondialdehyde (MDA): Lipid peroxidation analysis was performed on maize leaves based on previous methods (Madhava and Sresty, 2000). First, a 0.5 g frozen leaf sample was ground in powder using liquid nitrogen then TCA buffer was added. The extract was centrifuged at 10,000 rpm and TCA and TBA solutions were added to 1 ml supernatant. We then headed the solution and centrifuged it at 10,000 rpm again. Absorbance was determined at 532 - 600 nm.

Statistical analysis

All data shown are the mean values (n=3). Data were statistically analysed using the analysis of variance (One-Way ANOVA) with MINITAB software and Tukey's multiple range test (level of significance, $p < 0.05$).

RESULTS AND DISCUSSION

Growth attributes

Based on statistical analyses a wide variation in seedling length was observed among the genotypes ($p < 0.01$) and treatments ($p < 0.01$). Interaction among the sources of variation was also significant (genotype × treatment; $p < 0.05$). In the 8°C treatment, purple corn seedlings had the highest seedling length, followed by yellow, white, and red corns. The seedling length of yellow corn in the 16°C treatment was 4.50% higher than in the control while the seedling length of purple and white corns had the highest values in the control, followed by yellow and red corns (Tables 2, 3). In terms of seedling weight, significant variations were observed between the variation sources and genotype × treatment ($p < 0.01$) interactions. Results revealed that white and red corns had the lowest seedling weight values in the 8°C treatment; yellow and purple corns had the lowest values in 16°C and all genotypes had the highest seedling weight values in the control. The seedling weight values of red corn in the 8°C treatments were also lower than those in the control, but the difference was not as significant as in other genotypes. The seedling weight of red corn in the 16°C treatment was the same under normal conditions (Tables 2, 3).

On the other hand, white corn had the highest values in the control, followed by purple, yellow and red corns. Results also revealed that white corn (the most striking genotype in terms of seedling weight) could not retain its superiority under lower temperature conditions. Meanwhile, purple corn, which is the second-best genotype, was stronger under unfavourable conditions (8°C - 12°C) than the white corn.

Low temperatures can severely impair the growth and productivity of maize (Farooq *et al.*, 2009) as chilling stress decreases the rate of both cell division and elongation (Ben-Haj-Salah & Tardieu, 1995), severely affecting the development of leaves and shoots (Warrington and Kanemasu, 1983). At low temperatures, plants thicken their mesophyll and cuticle layers, leading to smaller leaf areas (Farooq *et al.*, 2009) and lower development rates, concerning particularly leaves and nodes (Kosová *et al.*, 2005; Sowiński *et al.*, 2003). Similarly, low temperatures affected the seedling growth and development in this study. The seedling length of purple corn in the 8°C treatment was 16% lower than it was in the control, followed by that of yellow (20.75%), red (27.04%) and white (39.37%) corns. Based on seedling length results, purple corn was the least affected genotype by chilling stress. Chilling forced the plants to accumulate antioxidants and anthocyanins to overcome its negative effects (Guerra-Peraza *et al.*, 2012; Pietrini *et al.*, 2002). Purple corn seedlings have already been found to be rich in anthocyanins like their grains. It is thought that this aspect of purple maize may have increased its low temperature tolerance. Another trait adversely affected by chilling is seedling weight whose values in all genotypes in the 8°C treatments were found lower, with the lowest red corn. Additionally, we noted that white corn had the most significant decline in seedling weight compared with the control (73.18%), followed by yellow (63.54%), purple (41.56%) and red (32.82%) corns. Results have also shown that seedlings with coloured grains may have chilling resistance and can grow under cold conditions.

Photosynthetic properties

Since photosynthesis comprises a long chain of reactions and is affected by many physiological processes A, chlorophyll fluorescence, E and WUE features were evaluated together. The lowest A of white corn was from the control, followed by the 8°C, 12°C and 16°C treatments. The results of chlorophyll fluorescence analysis did not correlate with the previous results, revealing the lowest values in the 16°C treatment, followed by the 8°C treatment. On the other hand, chlorophyll fluorescence values in the 12°C and control treatments were close (Tables 2, 3). Seedlings of white corn in the 8°C treatment had higher A values than in the control. However, lower chlorophyll fluorescence levels in the treatments decreased the effectiveness of photosynthesis while higher chlorophyll fluorescence values gained more photosynthetic capacity in the control.

Table 2. Variance analysis results for maize SL (seedling length), SW (Seedling weight), A, chlorophyll fluorescence (Fv Fm^{-1}), E, WUE, Ci, SC, CC, ChlA, ChlB, Carotenoids, TAC, TAA, TPC, proline and MDA properties.

Variation sources	DF	SL	SW	A	Fv Fm^{-1}	E	WUE	Ci	SC	
Genotypes	3	631.944**	43.394**	0.809**	0.002	0.031**	52.058**	193.688**	3331.953**	
Treatments	3	1296.056**	63.341**	0.273	0.028**	0.033**	83.324**	65010.722**	648.371**	
G*T	9	46.313*	0.453**	0.609**	0.002	0.002**	0.950**	4319.815**	490.952**	
Error	32	119.444	13.614	0.145	0.003	0.089	62.551	12997.389	11.975	
Total	47	631.944	43.394	0.809	0.002	0.031	52.058	193.688	3331.953	
CV (%)		10.51	11.97	12.72	5.34	9.29	12.43	5.34	7.64	
	DF	**CC**	**ChlA**	**ChlB**	**Carotenoids**	**TAC**	**TAA**	**TPC**	**Proline**	**MDA**
Genotypes	3	30.987**	18.643**	239.237**	3.510**	2772.277**	70.209	26.579**	0.002**	90.433**
Treatments	3	63.845**	5.582**	569.571**	15.650**	1711.886**	209.204**	19.668**	0.001**	74.829**
G*T	9	28.856**	2.498**	90.803**	0.383**	1072.210**	183.389**	11.587**	0.000**	9.537**
Error	32	4.490	0.750	7.707	6.205	19.400	25.056	2.469	0.000	0.316
Total	47	30.987	18.643	239.237	3.510	2772.277	70.209	26.579	0.002	90.433
CV (%)		5.22	3.54	10.43	13.22	7.80	10.53	10.62	14.90	4.03

*p<0.05; **p<0.01
DF (Degree of freedom)

Table 3. The effects of different temperatures on the SL (seedling length), SW (seedling weight), chlorophyll fluorescence (Fv Fm^{-1}) and Ci, (sub-stomatal CO_2 concentrations), CC (chlorophyll content) and ChlA (chlorophyll A), ChlB (chlorophyll B) and carotenoids traits of white, yellow, red and purple corns.

	SL (cm)				SW (g)			
	White	Yellow	Red	Purple	White	Yellow	Red	Purple
8°C	51.33±4.23ef	56.00±6.70c-f	47.67±6.65f	70.00±3.37a-e	3.37±0.08gh	3.00±0.37h	2.67±0.12h	6.48±0.45de
12°C	45.33±6.45f	61.00±2.55b-f	53.33±5.50def	63.00±5.47b-f	3.54±0.43gh	4.13±0.27fgh	3.73±0.32gh	6.12±0.23ef
16°C	73.00±13.51a-d	74.00±5.29abc	58.33±3.43b-f	78.00 10.93ab	9.47±0.45bc	2.35±0.14h	3.97±0.21gh	5.33±0.28efg
25°C (control)	84.67±2.98a	70.67±9.91a-e	65.33±6.52a-f	84.33±9.14a	12.56±0.34a	8.22±0.57cd	3.97±0.21gh	11.09±0.14ab
	Fv Fm^{-1}				Ci (µmol mol^{-1})			
8°C	0.792±0.01abc	0.710±0.05abc	0.668±0.05c	0.692±0.03bc	176.00±2.49fg	237.00±1.25e	234.33±2.13e	178.33±7.58fg
12°C	0.818±0.01ab	0.814±0.00ab	0.813±0.00ab	0.784±0.01abc	182.33±5.89f	248.67±1.09e	138.33±7.78g	183.33±8.46f
16°C	0.788±0.02abc	0.817±0.00ab	0.808±0.01ab	0.833±0.00a	302.00±8.06cd	365.67±9.02a	270.67±6.61de	370.67±3.54a
25°C (control)	0.819± 0.00ab	0.821±0.01a	0.812±0.00ab	0.814±0.01ab	243.67±6.61e	366.33±6.40a	354.00±1.89ab	319.33±12.66bc
	CC (Spad)				ChlA (µg mL^{-1})			
8°C	38.73±0.74bc	36.70±0.87c	36.17±0.78c	40.33±1.09bc	26.48±0.44ab	24.31±0.04b-e	23.29±0.10def	22.69±0.16ef
12°C	39.88±1.31bc	41.58±1.55bc	39.55±0.92bc	42.38±1.79bc	25.59±0.11abc	24.92±0.03bc	24.01±0.08cde	24.21±0.05b-e
16°C	44.23±0.10b	51.10±0.75a	37.80±1.23c	40.97±0.78bc	24.79±0.08bcd	24.97±0.50bc	24.50±0.02bcd	21.894±1.04f
25°C (control)	41.50±0.57bc	39.20±0.50bc	39.83±0.68bc	39.03±0.99bc	26.87±0.15a	25.53±0.01abc	24.55±0.18bcd	24.40±0.12bcd
	ChlB (µg mL^{-1})				Carotenoids (µg mL^{-1})			
8°C	25.06±3.03c-f	35.08±0.99ab	37.96±0.30a	39.76±0.45a	5.82±0.03a	5.78±0.24abc	4.22±0.51a-e	2.16±0.13de
12°C	32.44±1.73abc	24.94±0.33def	25.89±1.31c-f	18.86±0.45fg	5.97±1.68ab	5.34±0.31abc	3.64±0.25b-e	1.40±0.14e
16°C	37.34±2.56ab	11.31±0.86h	20.40±1.78efg	19.21±1.23efg	5.91±0.19ab	5.35±0.27abc	3.09±0.19cde	4.52±0.19a-d
25°C (control)	30.08±1.97bcd	16.55±0.28h	26.38±0.52cde	21.42±2.22efg	6.60±0.21a	5.87±0.13abc	5.33±0.97abc	2.42±0.66de

The effects of different temperatures [8°C, 12°C and 16°C, 25°C (control)] on the SL (seedling length), SW (seedling weight), chlorophyll fluorescence (Fv Fm^{-1}) and Ci, (sub-stomatal CO_2 concentrations), CC (chlorophyll content) and ChlA (chlorophyll A), ChlB (chlorophyll B) and carotenoids properties of white, yellow, red and purple corns. Samplings were done at 14th days old seedlings. Values are mean ± S.E. (standard error) based three replications (n=3).

Also, some similar changes in E were observed with the increasing temperatures. For example, white corn had the lowest E value in the control, followed by the 8°C, 16°C and 12°C treatments. E value in the 8°C treatment was higher than in the control as well. This is probably due to the disruption of root hydraulic conductance at lower temperatures (Tables 2, 3; Figure 1B). In terms of WUE, the control treatment had the highest values, followed by the 16°C, 8°C and 12°C treatments. Additionally, white corn's low E value gained more WUE under normal conditions, but with lower A and higher chlorophyll fluorescence (Tables 2, 3; Figure 1C).

Additionally, A values of yellow corn seedlings also varied with temperature. The lowest value was observed in 16°C while the highest one was in the 12°C treatment. The second lowest value was obtained from the 8°C treatment. Chlorophyll fluorescence values also varied, with the lowest value in the 8°C treatment. The decrease in A value in the 16°C treatment was offset by increasing chlorophyll fluorescence, which caused an increase in WUE in the 16°C treatment compared to the 8°C treatment. We observed that chilling caused lower photosynthesis in yellow corn and a linear increase between chlorophyll fluorescence and increasing temperatures. Low temperatures caused higher transpiration in yellow corn, with the lowest chlorophyll fluorescence in the 8°C. The highest WUE was obtained from the 12°C treatment, followed by the control and 16°C, while the lowest one was obtained from the 8°C. We noted that the decreasing E and increasing chlorophyll fluorescence levels supported WUE and A levels. Changes in A value of red corn was correlated with temperature. The lowest value was in the 8°C treatment, followed by the 12°C, 16°C and control treatments. Similarly, the lowest chlorophyll fluorescence was in the 8°C treatment, followed by the control, 12°C and 16°C. A linear increase between E values and temperature was observed as well. Although the 12°C treatment had the lowest A values it also had the highest WUE values, possibly due to higher chlorophyll fluorescence values. On the other hand, WUE values in the 8°C and 16°C treatments were close while the lowest value was obtained from the control. In the control treatment, photosynthetic performance of red corn was balanced by higher A and chlorophyll fluorescence levels. The fact that higher E reduced WUE also resulted in higher CO_2 uptake.

Based on these results, purple corn had the highest A value in the 12°C treatment, followed by the 8°C, control, and 16°C treatments. Although A value in the 16°C was lower than the 12°C treatment, chlorophyll fluorescence value in the former was the highest among all groups, resulting in a better overall photosynthesis in plants in this group. This may have been caused by the reduction of E values, resulting in lower CO_2 uptake. On the other hand, E value in the 8°C treatment was the highest, followed by the control, 16°C and 12°C treatments. Water use efficiency in the 12°C treatment was the highest in addition to higher A values, indicating an increasing effect of WUE on A in purple corn in the 12°C treatment. This does not apply to the 8°C treatment because low temperature stress can also cause higher E values due to the instability of root hydraulic conductance.

We observed that chilling caused a decline in stomatal conductance, Ci and chlorophyll content features of white corn. For example, the lowest values were obtained from the 8°C treatment, while the highest ones were obtained from the 16°C treatment. Additionally, the second highest levels of all three traits were from the control. These results may indicate the triggering effects of the 16°C treatment on previous properties of white corn (Tables 2, 3; Figure 1D). On the other hand, the

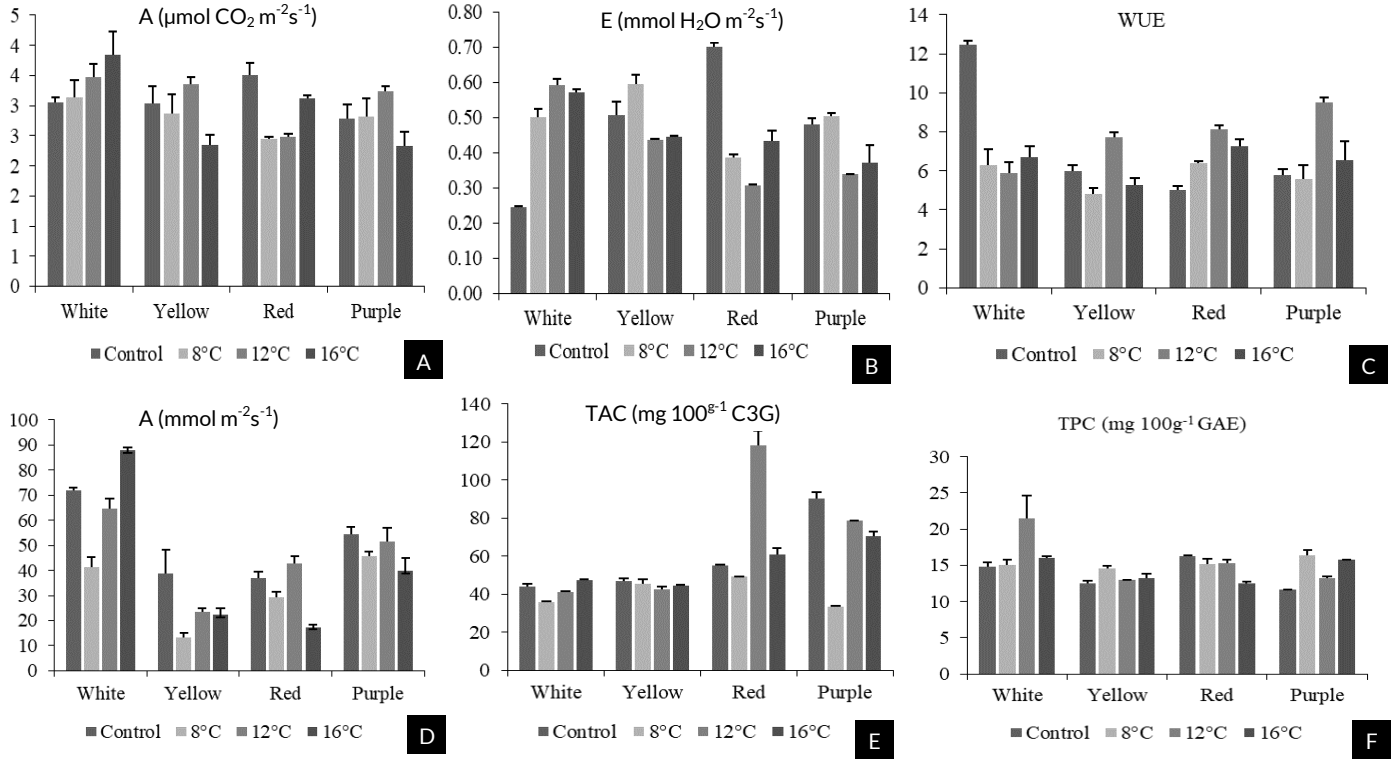

Figure 1. *The effects of different temperatures [control (25°C), 8°C, 12°C and 16°C)] on the A (photosynthesis rate), E (transpiration rate), WUE (water use efficiency), SC (stomatal conductance), TAC (total anthocyanin content) and TPC (total phenolic content) features of white, yellow, red and purple corns. Samplings were done at 14th days old seedlings. Values are mean ± S.E. (standard error) based three replications (n=3).*

lowest stomatal conductance, Ci and chlorophyll content values were in the 8°C treatment of yellow corn, while the highest values of stomatal conductance and Ci were obtained from the control and the highest chlorophyll content values from the 16°C treatment. Inconsistent fluctuations were observed between previous treatments of yellow corn as opposed to white one. In the 12°C treatment, we observed that red corn had the highest stomatal and the second-highest chlorophyll content. Additionally, red corn had the highest Ci values in the 16°C treatment. Genotypes were able to tolerate temperature as low as 12°C; as a result, the reactions became negative as temperatures dropped below 12°C.

Meanwhile, purple corn was observed to have the highest stomatal conductance in the control, the highest Ci in the 16°C treatment and the highest chlorophyll content in the 12°C treatment. The lowest Ci was obtained from 8°C treatment. Also, stomatal conductance had the lowest values in the 16°C treatment and the lowest chlorophyll content in the control. Additionally, purple corn could not tolerate chilling particularly in Ci. The negative effects of low temperature on plants are associated with photosynthetic electron transport, CO_2 reduction cycle and photosynthesis inhibition due to interrupted stomatal conductance (Allen and Ort, 2001). Additionally, a previous study revealed that maize leaf development had a significantly lower photosynthetic capacity, lower quantum efficiency of CO_2 fixation, and lower quantum efficiency of electron transfer at PSII under low temperature conditions than under favourable conditions (Zaidi *et al.*, 2010).

In the current study, A values of yellow and red corns in the 8°C treatment decreased when compared with the control. On the other hand, white corn's chlorophyll fluorescence in the control was 3.29% higher than it was in the 8°C treatment, followed by the chlorophyll fluorescence value of yellow (13.52%), purple (14.98%) and red (17.73%) corns. The chlorophyll fluorescence values of any genotype can increase or decrease its photosynthetic capacity because of the changing maximum quantum effect of CO_2 since chlorophyll fluorescence determines how much carbon the plant absorbs in a day (Farooq *et al.*, 2009; Long *et al.*, 1994). Since photosynthetic activity in maize leaves is interrupted at temperatures lower than 12°C under high light conditions, this causes photo-inhibition and inactivation of the PSII reaction centre, resulting in limited photosynthetic capacity (Melkonian *et al.*, 2004; Ortiz-Lopez *et al.*, 1990). Despite these adverse effects of chilling, white corn particularly had the highest A and chlorophyll fluorescence values in the 8°C treatment. Additionally, red corn retained its photosynthetic capacity with A and chlorophyll fluorescence values that were respectively 29.99% and 17.73% less than the values in the control.

On the other hand, although high chlorophyll fluorescence values seem to support photosynthesis, previous studies have different opinions. Guerra-Peraza *et al.* (2012) stated that plants that adapted to low temperatures had lower chlorophyll fluorescence values, which may be associated with the effects of high light on chlorophyll fluorescence at low temperatures. According to Melkonian *et al.* (2004), photo-inhibition of PSII in maize leaves exposed to chilling results in the inhibition of carbon metabolism since the inhibition of high light impedes the excitation energy needed for CO_2 assimilation. Based on this data, purple corn may be the ideal genotype under chilling conditions with its lower chlorophyll fluorescence and A values in the 8°C treatment. Kosová *et al.* (2005) stated that chlorophyll fluorescence values of maize, as wells as the solar energy

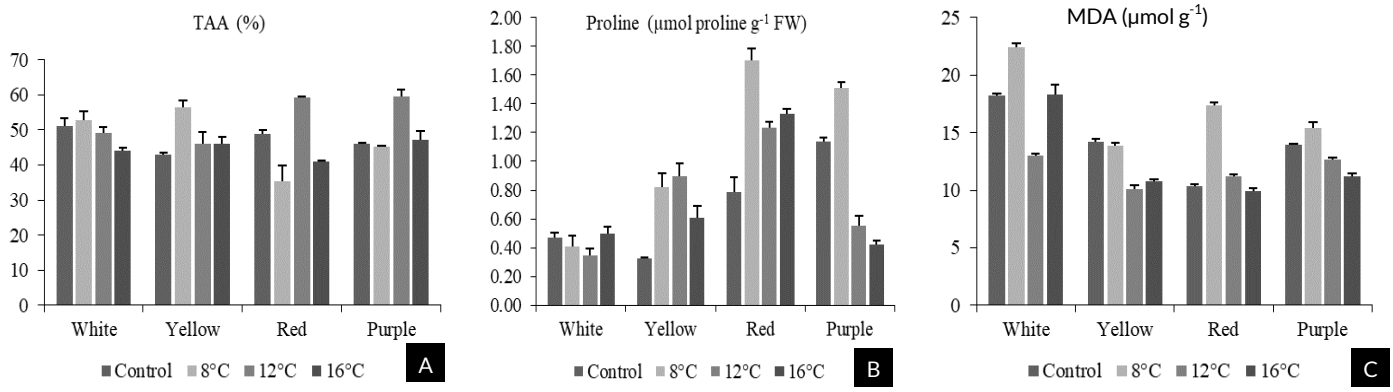

Figure 2. *The effects of different temperatures [control (25°C), 8°C, 12°C and 16°C)] on the TAA (total antioxidant activity), proline and MDA (malondialdehyde) features of white, yellow, red and purple corns. Samplings were done at 14th days old seedlings. Values are mean ± S.E. (standard error) based three replications (n=3).*

absorption of PSII, declined under low temperature conditions. This decline may be a part of the protective mechanisms of plants against the negative effects of high light under low temperature conditions.

Despite the decrease in leaf water and turgor potentials, plants exposed to chilling do not close their stomata for about 24–48 h. This phenomenon causes stomatal movement loss which contributes to transpiration control. As low temperatures cause a decrease in the hydraulic conductance, failure of the stomata to close causes water stress in plants (Lee *et al.*, 1993; Pardossi *et al.*, 1992). Chilling tolerant plants can counteract the negative effects of drought during stages of distress by increasing hydraulic conductance and decreasing stomatal conductance (Vernieri *et al.*, 2001), which can alleviate the symptoms of drought caused by low temperatures (Lee *et al.*, 1993; Vernieri *et al.*, 1991).

The E value of all genotypes, except for red corn, increased in the 8°C treatment, indicating water loss. Furthermore, stomatal conductance of red corn also decreased (20.29%). This could mean that red corn reacts to chilling faster by closing its stomata, preventing water loss through transpiration. Melkonian *et al.* (2004) also reported a 20% reduction in stomatal conductance after 2.5 h in low temperature treatment (5.5°C). Stomatal closure following chilling may be due to the guard cells affected by the low temperature or decreased rubisco activity due to enhanced internal CO_2 concentration in leaves (Farooq *et al.*, 2009). The concordance between E and stomatal conductance properties of red corn in the 8°C treatment was remarkable and no such correlation between E and stomatal conductance traits in the other genotypes was observed. Instead, an unstable situation between E and stomatal conductance was observed in the other genotypes, probably due to the drought caused by chilling. McWilliam *et al.* (1982) noted that loss of stomatal control combined with a decline in root hydraulic conductivity caused wilting under low temperature conditions.

While the Ci of all genotypes was lower in the 8°C treatment compared with the control, Ci levels of seedlings in the 12°C and 16°C treatments were close to their normal values. One of the previous studies has reported that the $NADPH^+$ pool may be limited by a decrease in the CO_2 fixation rate (Cakmak & Marschner, 1992; Hodges *et al.*, 1997) and inhibition in the carbon metabolism, which are the symptoms of low temperature stress (Hodges *et al.*, 1997; Schöner and Krause, 1990). On the other hand, exposure to chilling during leaf development impairs photosynthesis because of the reduction in CC (Farooq *et al.*, 2009). This is a possible indication of chlorosis (Miedema, 1982), which is a sign of chloroplast malfunction observed in corn seedlings under low temperature stress (Guerra-Peraza *et al.*, 2012).

In terms of chlorophyll content, all values in the 8°C treatment, except for those of purple corn, were lower than the values in the control, which may have been greatly influenced by temperature changes. Increasing temperatures increased chlorophyll content directly, with the highest values obtained from yellow corn in the 16°C treatment. According to Zhao *et al.* (2020), chlorophyll level is an important index in determining photosynthetic capacity. Additionally, chlorophyll content decreases under chilling stress, possibly due to the disrupting effects of low temperature on chlorophyll biosynthesis by inhibiting the activities of chlorophyll biosynthetic enzymes. However, previous studies have different opinions on chlorophyll biosynthesis at low temperatures. Aydınoglu and Akgul (2019) reported that chilling regressed growth and development in maize without decreasing photosynthesis. In the current study, the response of each genotype to chilling was different. The most remarkable response was that of purple corn whose chlorophyll content was unaffected in the 8°C treatment.

Photosynthetic pigment concentrations

The highest ChlA level among all genotypes was gathered from the control. Furthermore, yellow, and red corns had the lowest ChlA level in the 8°C treatment while white and purple corns had the lowest levels in the 16°C treatment. In terms of ChlA features, white and purple corns were in the same group. Consequently, ChlA contents of all genotypes were more alike than ChlB, as ChlB results were more variable (Tables 2, 3). In terms of ChlB, the highest values were recorded for the 8°C treatment, except for white corn which had the lowest level. Carotenoid level also varied among the genotypes. The highest level was obtained from the control, except for purple corn. The highest carotenoid level of white corn was in the control, followed by the 8°C, 12°C and 16°C treatment. Under chilling conditions, carotenoids decreased linearly with temperature drop.

Consequently, temperature had prominent effects on carotenoid level of white corn. The same result was recorded for red corn as well. The highest carotenoid level of red corn was detected in the control, followed by the 8°C, 12°C and 16°C treatment (Tables 2, 3). Although mean carotenoid levels of yellow corn were observed in the same group, the highest value was obtained from the control, followed by the 8°C, 16°C and 12°C treatments. Total carotenoid level of purple corn was lower than the other genotypes, with the highest value in the 16°C treatment, followed by the control. The results of the 8°C and 12°C treatments were similar and formatted the other way of the group. Based on these results, we could state that temperature decrease had more significant effects on carotenoid concentrations in purple corn when compared with the other genotypes.

Low temperature also affected ChlA levels. Values obtained from the controls of all genotypes were higher than those in the treated seedlings. ChlB concentration increased, except for white corn, in the 8°C treatment. Only ChlB of white corn was lower than the control, while ChlB values of red, purple, and yellow corns were higher (30.57%, 46.12% and 52%, respectively). More remarkable changes were observed in ChlB compared with ChlA. The down-regulation of PSII by plants to dissipate excitation energy (Guerra-Peraza *et al.*, 2012) may indicate a mechanism to counteract the negative effects of chilling. PSII is known to be rich in ChlB, which means that down-regulating PSII also decreases the amount of ChlB. In this case, decreasing ChlB is associated with dissipating excitation energy. In this study, ChlB decreased only in white corn in the 8°C treatment, while it increased in all other genotypes. Under the same conditions, the highest chlorophyll fluorescence was also recorded from white corn.

On the other hand, Kosová *et al.* (2005) reported that although low temperature stress did not change the total chlorophyll and ChlA concentration, it decreased the photosynthetic capacity of the photosynthetic apparatus, as reflected in the results of our study. Levels of ChlA did not change significantly. Thus, we can assume that the photosynthesis capacity decreased because of lower chlorophyll fluorescence values in seedlings exposed to low temperatures. Results showed that the carotenoid level of white corn was the highest in the 8°C treatment, which was closer to the level in the control than in the other two treatments. Additionally, carotenoid level of yellow corn in the 8°C treatment was also higher than the other treatments. However, carotenoid levels of coloured corns were lower than those of white and yellow corns. The highest carotenoid level of purple corn was recorded in the 16°C treatment. Carotenoids and tocopherols are responsible for detoxifying ROS and scavenging of free radicals in plant tissues. Furthermore, carotenoids also protect the chlorophyll from photo-oxidation (Ma *et al.*, 2013). Consequently, white corn (rich in carotenoids) is also rich in antioxidants. This prevents the negative effects of chilling.

Bioactive compounds

In terms of the TAC trait, genotypes had different reactions to lower temperatures. Although red and purple corns are naturally richer in anthocyanins than the other genotypes, this is not directly related to the reaction to low temperature because TAC of yellow corn is higher than that of purple in the 8°C treatment (Tables 2, 3, Figure 1E).

Additionally, white corn had the lowest TAC in the 8°C treatment, followed by the 12°C, control, and 16°C treatments. However, TPC levels were not consistent with TAC results (Tables 2, 3, Figure 1F). The TPC of white corn obtained from 8°C was lower and this indicates that non-anthocyanin phenolic contents declined at low temperature as well. The antioxidant content of white corn in the 8°C treatment increased although TAC and TPC levels decreased. Additionally, chilling also triggered antioxidant metabolism of white corn (Figure 1A).

In the 8°C treatment, total anthocyanin level of yellow corn was lower than the control, but higher than the other two treatments. The highest TAA and TPC values were in the 8°C treatment. Additionally, TAA levels in the 12°C and 16°C treatments were higher than the control, but lower than the 8°C treatment. Similar results for TPC were found as well. The TAC results in the 12°C and 16°C treatments were higher than the control, but lower than the 8°C treatment. These results showed that yellow corn efficiently synthesised bio-activated compounds to prevent the negative effects of chilling stress. Chilling triggered anthocyanin metabolites in red corn more than in the others. According to the findings the highest TAC (as well as TAA) value in the 8°C treatment was recorded for red corn, followed by yellow, white, and purple corns. Also, a correlation was observed between TAA decrease and temperature increase, indicating the triggering effects of chilling on antioxidant metabolism of red corn. Although the highest TPC was obtained from the control of red corn, all means were in the same group.

The highest TAC was obtained from the control of purple corn, while the lowest one was from the 8°C treatment of the same genotype. The means of TPC of purple corn were in the same group although 8°C of the purple corn had the highest TPC among all genotypes. Both TAC and TAA values of purple corn were lower in the 8°C treatment. However, the highest TPC indicates that purple corn uses phenolics to counteract the effects of chilling when compared to other bio-activated compounds. The anthocyanin content of yellow corn in the 8°C treatment increased, unlike in other three genotypes whose anthocyanin contents decreased at low temperature. This effect on the anthocyanin content of yellow corn was also observed in previous studies (Chalker-Scott, 1999).

Anthocyanins protect plants from low temperatures with help of many mechanisms. One of them is by increasing leaf temperature, as reported by Es' kin (1960) and Smith (1909). Additionally, increasing anthocyanin concentration also reduces the freezing point of the cytoplasm, preventing any possible damage to the leaf tissues (Chalker-Scott, 1999). Anthocyanins also have antioxidant properties against powerful oxidants (Foot *et al.*, 1996), scavenging ROS and preventing lipid peroxidation (Tsuda *et al.*, 1996; Tsuda *et al.*, 1994). Previous studies observed anthocyanin and antioxidant accumulation in the chilling tolerant plants under low temperature conditions (Guerra-Peraza et al., 2012). Hence, we can assume that yellow corn is

more chilling tolerant than the other genotypes based on the TAC results. Since chilling conditions can cause an increase in the amounts of toxic oxygen compounds of plant tissues, plants and other organisms have developed antioxidant systems to protect against these compounds (Hodges *et al.*, 1997). The ability of plants to adjust their antioxidant systems to varying ROS concentrations is vital for all species under stressful conditions (Kocsy *et al.*, 2001).

Based on TAA results of all genotypes, white and yellow corns were observed to have the highest levels of TAA in the 8°C treatment (Table 2; Figure 2A). Values obtained from the 12°C and 16°C treatments of white corn were also less than the values in the control. Many comparative studies on different species have reported that the antioxidant capacity of chilling-resistant genotypes is significantly higher than that of the others (Hodges *et al.*, 1997). Thus, we can assume that white and yellow corns are more chilling tolerant than red and purple corns, based on the TAA features. Low temperatures cause mechanical damages through the crystallisation of water. Plants exposed to long-term chilling accumulate lignin's and phenolics in the cell walls to increase their tolerance, thus, preventing the mechanical damages due to chilling (Chalker-Scott, 1999). Similarly, we observed that low temperature treatments increased TPC of yellow and purple corns. Other genotypes had less in the 8°C treatment, demonstrating the accumulation of phenolics in plant tissues to improve chilling tolerance.

Stress indicators

Chilling had varying effects on the proline content of the genotypes (Tables 2; Figure 2B). For example, proline concentration of white corn was lower in the 8°C treatment than in the control. The lowest value was obtained from the 12°C treatment. However, chilling stress did not activate proline metabolism of white corn as effectively as the other genotypes. We noticed that although white corn had a lower proline concentration, it had the highest MDA value in the 8°C treatment. This indicates that lower proline concentration may be correlated with higher MDA levels (Tables 2; Figure 2C). Also, in the 8°C treatment, proline contents of all genotypes except for white corn were higher than the control, indicating the triggering effects of chilling on proline metabolism in corn. Additionally, MDA levels of all genotypes in the same treatment, except for yellow corn, were also higher than the control. The highest proline concentration was in the 8°C treatment of red corn, followed by purple corn. Proline level of yellow was also higher than the control, while in the 12°C and 16°C treatments the values were in between. Finally, a correlation was observed among lower MDA levels and higher proline concentrations in all genotypes.

Proline concentration in the seedlings increased during the 8°C treatment, except for white corn which had the highest MDA. The proline concentration of purple corn was higher (24.00%, 53.58% and 60.34%, respectively) than the control, indicating that low temperatures triggered proline metabolism in the genotypes. Proline, which is thought to have versatile effects on stress tolerance in plants, is an osmotic regulator that stabilises proteins and membranes, induces osmotic stress-related genes, enhances ROS detoxification and provides readily available nitrogen and carbon (Brugičre *et al.*, 1999).

When exposed to low temperature stress plants produce ROS which bonds with unsaturated fatty acids to form MDA. When MDA concentration increases, polyunsaturated fatty acids also increase in the cytoplasm. These acids are more susceptible to UVB damage; thus, low temperature stress creates UVB sensitivity as well (Chalker-Scott, 1999; Smith, 1909). Results showed that white corn had the highest MDA and lowest proline level in the 8°C treatment. Farooq *et al.* (2009) reported that while intracellular proline concentration increased lipid peroxidation decreased, which resulted in higher chilling tolerance in plants. Considering this, white corn has the lowest chilling tolerance because of its highest MDA and lowest proline concentration. The lowest MDA was obtained from yellow corn in the 8°C treatment (also showing higher proline levels). Levels of MDA of red and purple corns were also higher in the 8°C treatment than the other treatments but lower than the MDA of yellow corn, probably because of their higher proline levels.

CONCLUSION

The aim of this work was to determine alternative maize genotypes which can tolerate chilling that is observed under early sowing conditions. Early sowing is preferred by the producers to benefit from the water potential of seed zone, especially in arid and semi-arid regions. The genotypes were screened morphologically and physiologically according to their reactions to the treatments with different temperatures. According to the results purple and white colored corns had more positive reactions than red and yellow ones under chilling stress. The findings obtained from the experiment showed that colour can be effective in low temperature tolerance, however, it is not possible to claim clear boundaries because similar responses towards chilling were observed both in purple and white corns simultaneously. It has been concluded that low temperature studies which are going to be carried out with different-colored corns, may significantly contribute to the determination of low temperature tolerant genotypes.

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