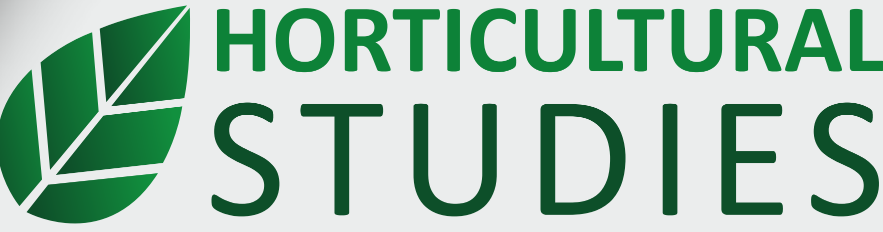

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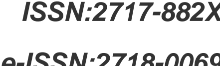

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RESEARCH PAPER

The Use of Controlled Atmosphere Box in Sweet Cherry Storage

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Abstract

The aim of the study was to examine the use of controlled atmosphere (CA) box, a new technology, for sweet cherry storage. In addition, this technology was compared to normal (NA) and modified atmosphere (MAP) storages commonly used in sweet cherry preservation. The '0900 Ziraat' sweet cherry variety, the most popular in Türkiye, was used as the material. Fruit harvested at optimum stage were transported to the laboratory immediately, and pre-cooled at 1°C. After pre-cooling, fruit were stored at 0°C and 90±5% relative humidity (RH) for 5 weeks in NA, MAP conditions, and in CA box at 2°C. During the storage period, weight loss, fruit skin color, stem color, respiration rate, soluble solids content (SSC), titratable acidity (TA), gas composition of box and MAP were determined at weekly intervals. Fruit were also evaluated for sensory attributes during cold storage. Samples taken from cold storage in each week were stored for 2 days at 20°C for shelf life and then fruit were re-evaluated. According to evaluation criteria, the CA boxes gave better results than the other storage conditions at the end of the storage period of 35 days. Sweet cherry cv. '0900 Ziraat' could be stored for 5 weeks in CA box and 4 weeks in MAP, with marketable quality.

1. Introduction

Sweet cherry is one of the most important crops of Türkiye, constituting approximately 28% of world production with production capacity of 724 944 tons. According to the Food and Agriculture Organization (FAO) of Statistical Database the world production of sweet cherry is 2 609 550 tons (FAO, 2020). Sweet cherries are excellent source of minerals, vitamins, antioxidant, fiber, carotenoids and bioactive elements (Gimenez et al., 2016). Consumption of sweet cherries plays an important role in preventing diseases and maintaining healthy life. Sweet cherries are usually consumed as fresh fruit (Wani et al., 2014), and a non-climacteric fruit with a high transpiration rate and susceptibility to fungal rots and physiological disorders (Petriccione et al., 2014). Because of high respiration and rapid softening rates, sweet cherries are highly perishable, with a very short shelf life (Lara et al., 2015). The limited harvest season together with its soft texture limits its availability in the market over longer periods; therefore, it needs to be marketed in a short time (Wani et al., 2014). During the marketing period, fruit prices go down with the increase of commodity supply more than demand. Prolonging of storage life of sweet cherries is crucial to balance commodity supply and demand, and create price stability in this period (Koyuncu and Dilmaçünal, 2008).

Cold storage is a common technique for reduce the rate of many metabolic processes in perishable fruits, to maintain quality, and to prolong the storability of cherries considered to be non-chilling

sensitive (Petriccione et al., 2014). In order to extend the post-harvest life of the fruit, it is necessary to use new technologies. Among a lot of technologies, the use of MAP has been reported to be effective in sweet cherry storage. MAP is used to supplement low temperature management to delay senescence, reduce physiological disorders, and suppress decay in many fresh fruit and vegetable products loss by increasing the level of CO_2 and decreasing the O_2 content (Serrano et al., 2005).

The CA box (Janny MT) is a newly developed storage technology that uses a special membrane that permits the passage of gases through passive diffusion. There is no need to add gas from external resources. The atmosphere of box is stabilized by the respiration of the crops and by passive diffusion through the membrane. The drop in the level of oxygen and the increase in the level of carbon dioxide in the CA module are regulated by the membrane (Kuentz, 2015).

In this study, it was aimed to compare CA box with plastic box and MAP that are used commercially for sweet cherry storage. In addition, this research was carried out to answer the question: can CA box be a promising storage technology for sweet cherry?

2. Material and Methods

The fruit of sweet cherry cv. '0900 Ziraat' were harvested at optimum stage, and transported to Fruit Research Institute (Eğirdir/Isparta) postharvest laboratory, immediately. Sweet cherry fruit at uniform size, free from visual symptoms of disease or blemishes, were harvested at commercial maturity (harvest parameters; fruit firmness, changes in color, SSC and TA). Fruit were precooled (the internal temperature of fruits reduced to 2-3°C) by forced air at 1°C temperature. After pre-cooling, fruit were divided into three groups. Cherries (first and second groups) packaged in plastic boxes and modified atmosphere bags (MAP/Life Pack®) were stored at 0°C and 90±5% RH in NA. Third group fruit placed in CA modules (Janny MT) were stored at 2°C and same RH. During the storage period of 5 weeks, the following analyses were performed at weekly intervals. Samples taken from cold storage in each week were stored for 2 days at 20°C for shelf life and then fruits were re-evaluated.

2.1. Weight loss

Weight loss of cherries was measured over 5 kg fruit in each replicate and expressed as the percentage of loss of weight with respect to the initial weight. Weight loss was determined by the formula;

$$Weight\ loss = \frac{First\ weight - Last\ weight}{First\ weight} \times 100$$

2.2. Respiration rate

The respiration rate of fruit was measured with a gas chromatography (Agilent 6840) Chemstation A.09.03 [1417]. Measurements were made in split/splitless (S/SL) of inlet in split mode with gas sampling valve with 1 ml gas sample by using fused silica capilar column (GS-GASPRO, 30 m × 0.32 mm I.D., U.S.A), with thermal conductivity detector (TCD) Approximately 200 g from each replicated was enclosed for 1 h in a 0.3 L glass jar. A gas sample was withdrawn from the headspace for determination of respiration rate. The temperature of the oven and TCD detector were 40°C (isothermal), and 250°C respectively. Respiration rate (measured as CO_2 production) was expressed as mL CO_2 $kg^{-1}h^{-1}$.

2.3. Changes in color

Fruit skin and stem color was determined using a colorimeter (CR400, Minolta Co., Japan) over 15 fruit in each replicate. Minolta color measurement apparatus was calibrated according to the standard white calibration plate (Y = 92.3, x = 0.3136 and y = 0.3194). The values were expressed by the CIE L^* (brightness-darkness), a^* (+ a^*: red, − a^*: green) and b^* (+ b^*: yellow, − b^*: blue), C* (color saturation), h° (hue angle).

2.4. Fruit firmness

Fruit firmness was determined using a texture analyzer (Guss FTA Type GS14, Strand, South Africa). It was defined as the maximum load required to penetrate the probe (5 mm diameter) into the fruit flesh (6 mm). The results were expressed in Newton (N).

2.5. Soluble solids content and titratable acidity

Soluble solids content was measured using a digital refractometer (HI 96801, Hanna, UK) and expressed as percentage (%). Titratable acidity was measured via titration of fruit juice with 0.1 N NaOH using automatic titrator (Mettler Toledo, T50 model) and expressed as malic acid content (g 100 ml^{-1}).

2.6. Gas composition

Gas concentration (O_2 and CO_2) in the packages and boxes were measured by Tiempo Test Silver (Janny MT CA, France). The instrument is capable of reading between 0-100% oxygen and carbon dioxide. The carbon dioxide analysis is accomplished by an infrared sensor.

2.7. Sensory analysis

External appearance was rated on a hedonic scale of 0-2 (0: good commercial quality, 1: some damage but still commercially salable, 2: not

commercially salable) described by Feng et al. (2004) Pitting was evaluated on scale of 0-4 (0: none, 1: very low, 2: low, 3: medium, 4: high). All analysis were performed at initially and 7+2 days intervals during storage.

2.8. Statistical analysis

Data were subjected to analysis of variance (ANOVA, JMP7), means were separated by means of LSD test (P<0.05, 0.01, 0.001).

3. Results and Discussion

Weight loss of fruit increased during cold storage and shelf life trials (Figure1). At the end of cold storage, the weight loss of cherries was between 0.60% (CA) and 6.94% (NA). During five weeks of cold storage, and shelf life the highest average weight loss values were (4.38%, 9.13%) obtained from the NA conditions. MAP and CA box conditions gave statistically similar results in terms of weight loss. The water vapor permeability properties of the MAP and box materials were effective in reducing weight loss compared to NA conditions. Fresh fruit continue respiration activity after harvest and produce excessive water vapor due to respiration. If the products are packaged and stored in the cold, the produced water builds up in the package (Ayhan, 2010). Therefore, storage of the products in suitable water vapor permeability materials is important in preventing weight loss. The results found in this research agree with those of previous studies (Koyuncu et al., 2005a; Goliáš et al., 2007).

Changes in fruit color during cold storage and shelf life are given in Table 1. L* values, which shows fruit brightness-darkness, fluctuated throughout cold storage. While the highest mean L*value was obtained from the CA boxes (25.33), the greatest decrease (24.74) occurred in NA condition. The effect of storage conditions on fruit color during shelf life period was not significant. As the storage time progressed, the fruit skin color C* values (represent saturation) fluctuated according to harvest time. C* values were lower at 3th and 4th weeks of storage than those of initial values, but increased again at 5th week indicating change of red color of skin. Likewise, a* values (+a* = redness, -a* = greenness) decreased from 12.75 to 10.47, and b* value (+b* = yellowness, -b* = blueness) increased from 4.77 to 5.75 during this period (data not shown). Similar results were found by Koyuncu et al. (2005a) in same cultivar in MAP. However, some researchers (Koyuncu et al., 2005b; Gimenez et al., 2016) stated that C*values of sweet cherry skin increased as storage period progressed. On the other hand, Padilla-Zakour et al. (2004) found that chroma values changed depending on varieties and MAP conditions under cold storage. The average h° values increased regularly during storage period, and reached 23.35 compared with harvest time value of 20.61. The increase of hue angle (h°) in sweet cherries indicates a decrease in red color intensity (Drake and Elfving, 2002). The results found in this research are in accordance with the studies carried out by Drake and Elfving (2002) and Koyuncu et al. (2005a). Other previous researchers who found opposite results (Koyuncu et al., 2005b; Petriccione et al., 2014; Lara et al., 2015). Belović et al. (2014) stated that different trends in color changes refer to different storage periods and different storage conditions and thus cannot be always compared directly.

Storage conditions significantly affected stem color L*, a* and b* values (Table 2). Stem color L* values decreased with increasing storage periods. While the lowest L * values (35.02 in cold storage and 30.44 at shelf condition) were obtained from cherries stored at NA conditions, the highest values were determined as 41.29 and 34.93, respectively, in CA boxes. The value of –a*, which expresses green color of fruit stem, increased due to chlorophyll breakdown as senescence progresses. Chlorophyll is broken down in green part of fruit during storage (Botondi et al., 2003) depending on the surrounding atmosphere of the commodities. Controlled atmosphere box maintained green stem color of cherries better than other conditions since these boxes could regulate low O_2 and high CO_2

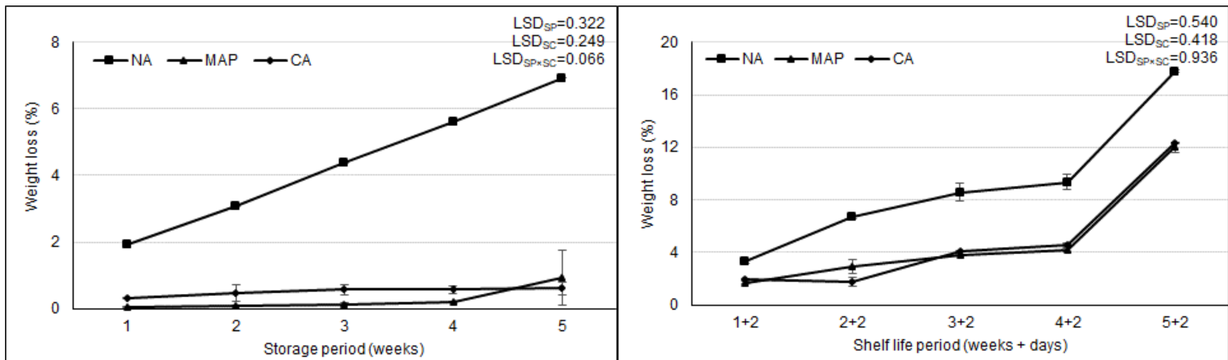

Figure 1. Changes in weight loss of ‘0900 Ziraat’ sweet cherry during cold storage and shelf life in NA, MAP and CA box (SP: Storage period, SC: Storage conditions, CA: Controlled atmosphere, NA: Normal atmosphere, and MAP: Modified atmosphere).

Table 1. Changes in skin color (L*, C*, h°) of '0900 Ziraat' sweet cherry during cold storage and shelf life in NA, MAP and CA box.

Storage conditions		Storage trials							Shelf life trials					
		Storage period (weeks)							Storage period (weeks+days)					
		0	1	2	3	4	5	Means	1+2	2+2	3+2	4+2	5+2	Means
L*	NA	24.85	24.84	25.24	26.03	22.24	25.21	24.74	27.86	27.81	27.02	27.08	27.27	27.41
	MAP	24.85	25.04	25.50	27.21	24.06	23.20	24.98	27.51	27.59	27.57	27.29	27.18	27.43
	CA	24.85	25.43	26.03	27.57	23.25	24.85	25.33	27.11	27.41	27.49	27.56	27.58	27.43
	Means	24.85	25.10	25.59	26.94	23.18	24.42		24.49	27.60	27.36	27.31	27.34	
	SC p<0.01, SP p<0.0001, SC × SP p<0.0001								SC Nonsignificiant, SP p<0.05, SC × SP p<0.0001					
C*	NA	13.61	16.55	14.81	11.03	11.75	16.13	13.98	11.79	12.50	10.19	12.20	12.98	11.93
	MAP	13.61	17.75	14.93	11.72	12.53	13.15	13.95	11.96	10.74	11.55	12.59	12.08	11.79
	CA	13.61	16.49	15.80	13.59	13.61	14.68	14.63	9.61	12.33	12.10	11.16	12.31	11.50
	Means	13.61	16.93	15.18	12.11	12.63	14.65		11.12	11.86	11.28	11.98	12.46	
	SC Nonsignificiant, SP p<0.0001, SC × SP p<0.05								SC Nonsignificiant, SP p<0.01, SC × SP p<0.01					
h⁰	NA	20.61	20.78	22.63	24.95	24.66	23.95	22.93	23.96	23.64	25.88	25.41	25.87	25.22
	MAP	20.61	21.71	22.80	26.48	23.69	23.20	23.08	24.67	25.08	27.68	25.72	27.23	26.11
	CA	20.61	21.95	22.25	23.49	22.82	22.90	22.34	26.14	24.22	24.96	27.29	27.42	25.70
	Means	20.61	21.48	22.56	24.98	23.72	23.35		24.92	24.31	26.17	26.14	26.84	
	SC p<0.05, SP p<0.0001, SC × SP p<0.01								SC Nonsignificiant, SP p<0.01, SC × SP p<0.05					

SP: Storage period, SC: Storage conditions, CA: Controlled atmosphere, NA: Normal atmosphere, and MAP: Modified atmosphere

Table 2. Changes in stem color (L*, C*, h°) of '0900 Ziraat' sweet cherry during cold storage and shelf life in NA, MAP and CA box.

Storage conditions		Storage trials							Shelf life trials					
		Storage period (weeks)							Storage period (weeks+days)					
		0	1	2	3	4	5	Means	1+2	2+2	3+2	4+2	5+2	Means
L*	NA	42.05	31.24	34.29	35.83	34.29	32.43	35.02	27.94	30.91	29.44	30.17	33.72	30.44
	MAP	42.05	34.10	42.92	36.99	41.27	39.25	39.43	33.38	32.76	30.74	33.78	35.18	33.17
	CA	42.05	39.01	42.08	44.03	40.31	40.29	41.30	32.99	39.70	33.51	34.41	34.02	34.93
	Means	42.05	34.78	39.76	38.95	38.62	37.32		31.44	34.46	31.23	32.79	34.31	
	SC p<0.0001 SP p<0.0001 SC × SP p<0.0001								SC p<0.0001SP p<0.001 SC × SP p<0.01					
C*	NA	-15.18	-8.38	-2.76	-2.00	-1.39	-0.32	-5.02	-4.39	-1.42	0.73	0.99	0.85	-0.65
	MAP	-15.18	-12.43	-11.38	-10.05	-7.92	-5.80	-10.46	-7.35	-4.08	-3.84	-1.31	-1.90	-3.70
	CA	-15.18	-14.16	-13.63	-12.20	-11.28	-8.82	-12.55	-9.80	-9.26	-8.01	-8.28	-4.37	-7.94
	Means	-15.18	-11.66	-9.26	-8.08	-6.86	-4.98		-7.18	-4.92	-3.71	-2.87	-1.81	
	SC p<0.0001, SP p<0.0001, SC × SP p<0.0001								SC p<0.0001, SP p<0.0001, SC × SP p<0.0001					
h⁰	NA	28.01	24.46	20.98	19.73	17.33	15.57	21.01	20.05	19.38	18.81	18.94	17.50	18.94
	MAP	28.01	24.38	24.07	23.75	21.39	20.71	23.72	23.39	22.37	22.49	23.05	21.73	22.61
	CA	28.01	27.26	28.38	28.76	25.44	25.89	27.29	25.11	24.52	24.04	23.67	22.78	24.02
	Means	28.01	25.36	24.48	24.08	21.38	20.72		22.85	22.09	21.78	21.89	20.67	
	SC p<0.0001, SP p<0.0001, SC × SP p<0.0001								SC p<0.0001, SP p<0.0001, SC × SP p<0.0001					

SP: Storage period, SC: Storage conditions, CA: Controlled atmosphere, NA: Normal atmosphere, and MAP: Modified atmosphere

inside package. Stem color b* values, represent yellowness, decreased as the storage period progressed in all conditions as expected. The highest average b * values (27.29 and 24.02) were obtained from fruit stems stored in CA box. As can be seen in Table 2, sweet cherries stored in NA had the lowest b * values (15.57 and 17.50) showing darker stem color at the end of storage. Changes in color intensity and quality are important indicators of maturity and quality for fresh cherries (Sharma et al., 2010). Better preservation of fruit skin and stem color in MAP and CA box conditions can be explained by the change of medium gas composition towards oxygen reduction and carbon dioxide increase resulting in slow respiration rate and other metabolic activities. By creating higher CO_2 and lower oxygen O_2 concentrations in the surrounding atmosphere of the commodities, decay, respiration rate, and enzymatic activity can be controlled, resulting in an increase in postharvest quality (Erkan and Eski, 2012).

Changes in fruit firmness during cold storage and shelf life are presented in Figure 2. Throughout the storage period and shelf life a gradual decrease in firmness was observed in all conditions. The fruit firmness values of cherries at harvest time (11.24 N) decreased to 7.43 N (NA) and 9.50 N (CA) at the end of storage period. The effects of storage periods and conditions on fruit firmness were statistically significant (p<0.0001) in all conditions. Fruit firmness is an important quality attribute in sweet cherries that affects consumer acceptance, fruit storage potential and resistance to mechanical damage (Wang and Long, 2014). The results demonstrated that MAP and CA box were more effective than NA to maintain fruit firmness. This can be due to less water loss and low respiration rate of fruit stored under MAP and CA box. It is known that

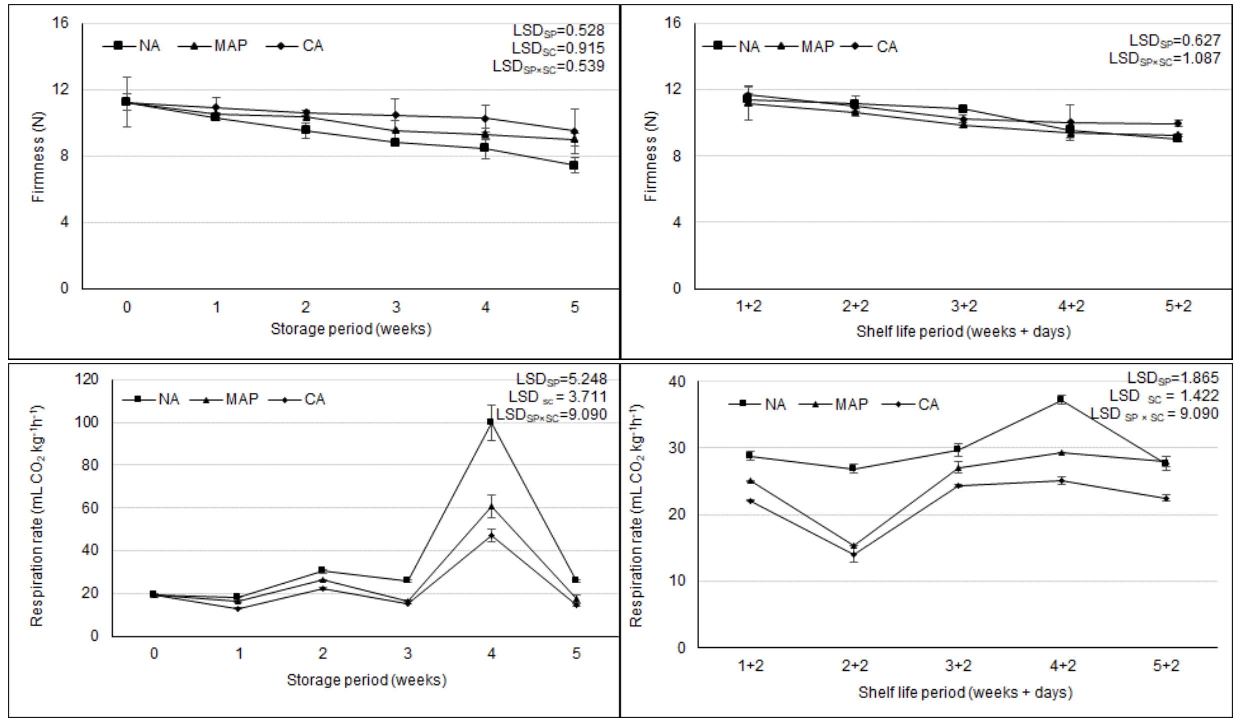

Figure 2. Changes in fruit firmness and respiration rate of ‘0900 Ziraat’ sweet cherry during cold storage and shelf life in NA, MAP and CA box (SP: Storage period, SC: Storage conditions, CA: Controlled atmosphere, NA: Normal atmosphere, and MAP: Modified atmosphere).

weight loss of sweet cherries might increase with increasing respiration rate during storage. Goliáš et al. (2007) indicated that slow ripening of the sweet cherry in the very low oxygen treatments was reflected by higher firmness after cold storage. Our results are in agreement with Bahar and Dündar (2001) and Goliáš et al. (2007) who found that fruit firmness of sweet cherries stored in MAP decreased during storage. However, in some previous researches, an increase in cherry fruit firmness in cold storage has been reported for different varieties (Remon et al., 2000; Kappel et al., 2002; Koyuncu et al., 2005a; Wang and Long, 2014).

Fluctuations in respiratory rates were observed during storage and shelf life periods (Figure 2). Fluctuation has progressed first, followed by a decreasing curve. The effects of storage condition and periods on respiration rates were significant. The lowest average respiratory rate (21.87 mL CO_2 $kg^{-1}h^{-1}$) was obtained from fruit stored at controlled atmosphere condition. Similarly, suppressing effect of CA boxes on respiration rate (21.59 mL CO_2 $kg^{-1}h^{-1}$) also continued throughout shelf life period. The highest respiration rate values were 36.44 mL CO_2 $kg^{-1}h^{-1}$ and 30.03 mL CO_2 $kg^{-1}h^{-1}$ at cold storage and shelf life conditions, respectively. These lower respiration rate of fruit in CA box compared to other package materials (MAP and plastic box) resulted from lower O_2 and higher CO_2 concentrations in CA boxes. As can be seen in Figure 2, the lowest O_2 was measured in CA box with an equilibrium O_2 concentration of 1.42- 3.20% after one week. The next lowest respiration rate was in MAP, since it had lower O_2 concentration (between 5.12- 9.01%) than NA (21.0%). Wang and Long (2014) reported that the lowest respiration rate of sweet cherries was in MAP with an equilibrium O_2 concentration of~2.0%, followed by other MAP with ~7.7%. The concentration of O_2 to preserve the quality of sweet cherries under CA was between 3-10% (Mitcham et al., 2003). Similarly, Wang and Long (2014) found that respiration rates of sweet cherries were affected very little by O_2 concentration from 21% to ~10%, but declined in a logarithmic manner from ~10% to ~1%.

Changes of TA during cold storage and shelf life are given in Table 3. One of the main factors of taste formation in fruit is TA (Karaçalı, 2009). Storage condition and periods significantly affected TA contents of cherries. TA of fruit decreased with increasing storage time in both cold room and shelf life. During the 5-week cold storage, the lowest average TA value was obtained from NA as 0.430 g 100 ml^{-1}, and the highest in CA box (0.494 g 100 ml^{-1}). This trend continued in shelf- life studies, and the fruit packaged in CA box gave slightly higher TA (0.445 g 100 ml^{-1}) content than those of others. It was reported that lower O_2 and elevated CO_2 inhibited the respiration rate of cherries during low temperature storage, and the inhibition persisted even after 36 h at room temperature (Wang and Long, 2014). Sweet cherries, a non–climacteric fruit, do not have starch or other carbohydrates to provide the energy for respiration; they use sugars and acids (Khorshidi et al., 2014). The fact that the fruit stored in NA (21.0% O_2) have a higher TA value than those of lower O_2 conditions, is the faster metabolic activity of the fruit in NA (Erbaş and

Table 3. Changes in SSC and TA of '0900 Ziraat' sweet cherry during cold storage and shelf life in NA, MAP and CA box.

Storage conditions		Storage trials: Storage period (weeks) 0	1	2	3	4	5	Means	Shelf life trials: Storage period (weeks+days) 1+2	2+2	3+2	4+2	5+2	Means
SSC	NA	15.4	16.1	15.5	17.0	14.9	14.7	15.6	15.5	16.2	14.4	14.7	16.4	15.4
	MAP	15.4	15.2	15.4	15.2	14.7	14.5	15.1	15.3	16.5	15.3	14.7	15.8	15.5
	CA	15.4	14.7	15.1	16.3	14.6	14.5	15.1	15.4	15.6	13.7	16.1	15.1	15.2
	Means	15.4	15.3	15.3	16.2	14.8	14.6		15.4	16.1	14.5	15.1	15.8	
	SC Nonsignificiant, SP p<0.01, SC × SP Nonsignificiant								SC Nonsignificiant, SP p<0.0001, SC × SP p<0.001					
TA	NA	0.592	0.548	0.428	0.407	0.301	0.304	0.430	0.549	0.516	0.425	0.389	0.305	0.437
	MAP	0.592	0.515	0.488	0.440	0.378	0.380	0.466	0.519	0.498	0.451	0.358	0.338	0.433
	CA	0.592	0.532	0.497	0.464	0.440	0.438	0.494	0.559	0.486	0.444	0.384	0.355	0.445
	Means	0.592	0.532	0.471	0.437	0.373	0.374		0.542	0.500	0.440	0.377	0.333	
	SC p<0.05, SP p<0.0001, SC × SP p<0.05								SC p<0.01, SP p<0.0001, SC × SP Nonsignificiant					

SP: Storage period, SC: Storage conditions, CA: Controlled atmosphere, NA: Normal atmosphere, MAP: Modified atmosphere, SSC: Soluble solids content (%), and TA: Titratable acidity (g 100 ml^{-1}).

Koyuncu, 2016). Wang and Long (2014), reported that the decreased respiration rate in response to lower O_2 concentration implies that the gas permeability of the commercial MAP should ideally equilibrate at an O_2 concentration lower than ~10% to efficiently reduce sweet cherry catabolic activity during storage. Similar observations were recorded by Padilla-Zakour et al. (2004), where MA stored sweet cherries had lower TA levels than air- stored fruit.

The average SSC of fruit (14.6%) decreased at the end of 35 days compared to initial value (15.4%), with fluctuation during cold storage. By contrast, there was minor increase in SSC during shelf life, which can be attributed to higher weight loss by evaporation compared to cold storage. Generally, SSC tended to increase until 21 day of storage and then decreased. However, SSC at harvest time (15.4%) remained almost constant (with mean values of 15.6, 15.1, 15.1%) during cold storage. No significant differences existed among the three conditions during storage, but differences between storage periods for SSC values were significant (Table 3). Previous researchers reported constant soluble solid levels throughout storage time when sweet cherries stored in MAP conditions (Meheriuk et al., 1997; Remon et al., 2000).

The O_2 and CO_2 gases in the MAP and CA box during storage are given in Figure 3. The oxygen concentrations in the first week were 9.01% in MAP and 3.12% in controlled atmosphere box, while these values found as 5.12% and 1.43%, respectively, at the end of storage period.CO_2 values increased in both MAP and CA box during storage. The O_2 gas in the CA box went down to 3.12 % within one week and remained fairly constant (1.42-3.12 %) in the rest of storage period. However, the O_2 concentration of MAP did not go down at these levels, and fluctuated between 3.83 % and 9.01%. Another difference between MAP and CA box was the final concentration of O_2. As cited above, O_2 concentration of MAP dramatically higher than that of CA box in final week. It was thought that the well regulation of gases in Janny MT box lead to preserve fruit quality better than other conditions. Similarly, Padilla-Zakour et al. (2004), reported that sweet cherries stored in MAP with lower O_2 had higher fruit quality according to bags with higher O_2. CA box had higher CO_2 concentration than MAP during 5 weeks, with similar curve.

The results of the sensory tests are presented in Figure 3. The overall acceptability of cherries decreased during storage and shelf life trials. The good commercial quality was obtained from the CA box during 5 weeks, while sweet cherries stored in NA had non-marketable quality after third week of storage. Fruit in MAP maintained their quality until fifth week of cold storage, but overall acceptability scores of these fruit reached to non-marketable limit with plus 2 days shelf life. With respect to pitting, cherries stored in all three conditions had no physiological disorders for the first 2 week in storage (data not shown). While cherries in NA had low level pitting at the 3th week of storage, fruits stored in MAP and CA box showed pitting (very low) in the 4th and 5th week, respectively. But overall acceptability scores of fruit were still well within the range of acceptability (value< 2.0) in these periods. Drake and Elfving (2002) indicated that longer storage time was associated with increased pitting, showing little relationship to the year of harvest. They also found that the poorest appearance score after 21 days of storage (1.58) was still less than 2.0 (limit value), similarly to our results.

4. Conclusion

MAP and CA box had different atmospheric compositions during 5 weeks storage period. CA box had lower O_2 (1.42-3.12%) and higher CO_2 (5.10-7.27%) than MAP, resulting in better quality after 5 weeks. Sweet cherries stored in CA box had more vivid green stem than those of MAP and NA at the end of storage. Cherries packaged in plastic boxes and stored in NA lost their commercial properties after three weeks. At the end of 35 days of cold storage and shelf life, CA module was more effective in maintaining fruit quality compared with other conditions. Sweet cherry cv. '0900 Ziraat'

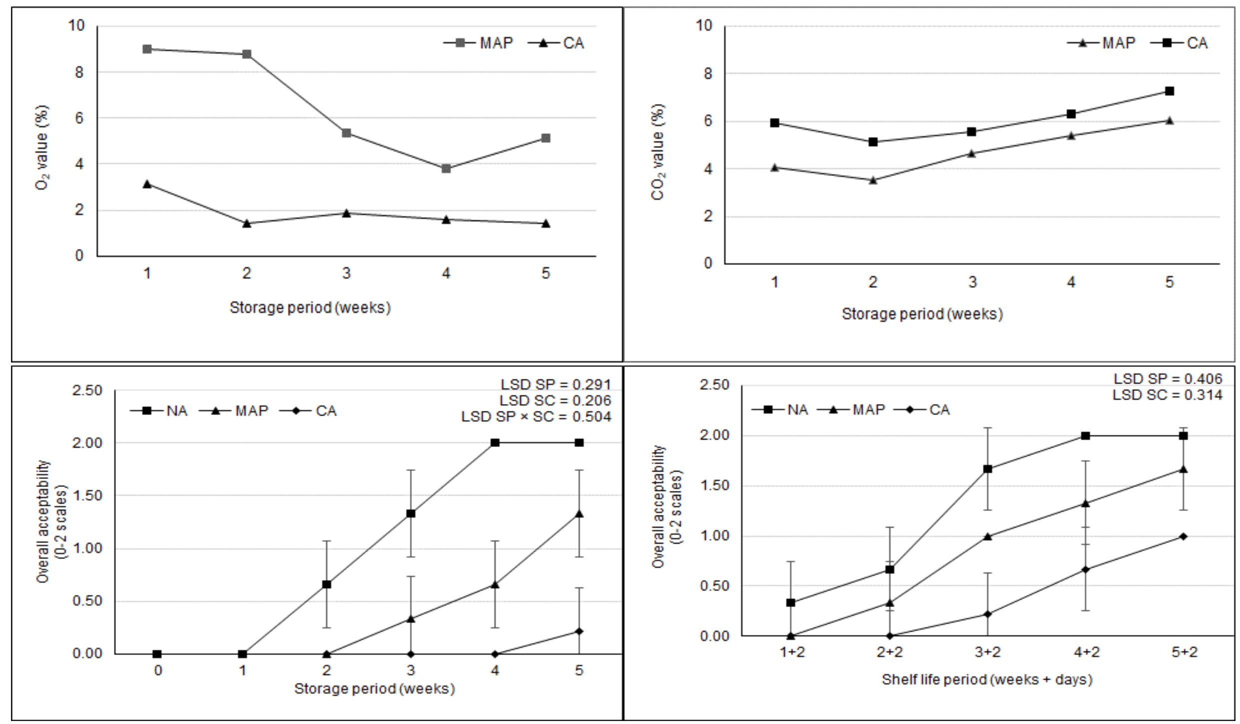

Figure 3. Changes in O_2 and CO_2 composition within MAP and CA box, and fruit overall acceptability of '0900 Ziraat' sweet cherry during cold storage and shelf life in NA, MAP and CA box (SP: Storage period, SC: Storage conditions, CA: Controlled atmosphere, NA: Normal atmosphere, and MAP: Modified atmosphere).

could be stored for 5 weeks in CA box and 4 weeks in MAP, with marketable quality. The module (Janny MT) can be used as an alternative to MAP, which is commonly used in storage in cherry. However, further detailed research on this subject is needed to investigate.

References

Ayhan, Z. (2010). Application of modified atmosphere packaging with new concepts for respiring foods. Proceedings of the 10th International Controlled and Modified Atmosphere Research Conference, 137-142.

Bahar, A., & Dündar, Ö. (2001). The effect of hydrocooling and modified atmosphere packaging system on storage period and quality criteria of sweet cherry cv. Akşehir Napolyonu. *Acta Horticulturae,* 553: 615-616.

Belović, M., Mastilović, J.S., & Kevrešan, T.S. (2014). Change of surface color parameters during storage of paprika (*Capsicum annuum* L.), *Food and Feed Research,* 41:85 – 92.

Botondi, R., Desantis, D., Bellincontro, A., Vizovitis, K., & Mencarelli, F. (2003). Influence of ethylene inhibition by 1-Methylcyclopropene on apricot quality, volatile production, and glycosidase activity of low- and high-aroma varieties of apricots. *Journal Agricultural Food Chemistry,* 51:1189-1200.

Drake, S.R., & Elfving, D.C. (2002). Indicators of maturity and storage quality of 'Lapins' sweet cherry. *HortTechnology,* 12:687-690.

Erbaş, D., & Koyuncu, M.A. (2016). Effects of 1-methylcyclopropene treatment on the storage life and quality of angeleno plum. *Journal of Agriculture Faculty of Ege University*, 3:43-50.

Erkan, M., & Eski, H. (2012). Combined treatment of modified atmosphere packaging and 1-methylcyclopropene improves postharvest quality of Japanese plums. *Turkish Journal of Agriculture and Forestry*, 36:563-575.

FAO, 2020. http://www.fao.org/home/en/ Date of access: 05.04.2022

Feng, X., Hansen, J., Biasi, B., Tang, J., & Mitcham, E.J. (2004). Use of hot water treatment to control codling moths in harvested California "Bing" sweet cherries. *Postharvest Biology and Technology,* 31:41-49.

Gimenez, M.J., Valverde, J.M., Valero, D., & Zapata P.J. (2016). Postharvest methyl salicylate treatments delay ripening and maintain quality attributes and antioxidant compounds of 'Early Lory' sweet cherry. *Postharvest Biology and Technology*,117:102-109.

Goliáš, J., Němcová, A., Čaněk, A., & Kolenčíková, D. (2007). Storage of sweet cherries in low oxygen and high carbon dioxide atmospheres. *Horticultural Science*, 34:26–34.

Kappel, F., Toivonen, P., Mackenzie, D.L., & Stam, S. (2002). Storage characteristics of new sweet cherry cultivars. *Hort Science,* 37:139-143.

Karaçalı, İ. (2009). Bahçe ürünlerinin muhafaza ve pazarlanması. Ege University, Faculty of Agriculture, Publication No:494, P:486, İzmir / Türkiye (in Turkish).

Khorshidi, S., Davarynejad, G., Tehranifar, A., & Fallahi, E. (2011). Effect of modified atmosphere packaging on chemical composition, antioxidant activity, anthocyanin, and total phenolic content of cherry fruits. *Horticulture, Environment, and Biotechnology,* 52:471-481.

Koyuncu, M.A., Cagatay, Ö., Savran, H.E., & Dilmaçünal, T. (2005a). Changes in quality of '0900 Ziraat' cherry fruit in different packages. *Acta Horticulturae,* 795:819-823.

Koyuncu, M.A., Dilmaçünal, T., Savran, H.E., & Yıldırım, A. (2005b). Shelf life quality of Bing sweet cherry following preharvest treatment with gibberellic acid. *Acta Horticulturae,* 795: 825-830.

Koyuncu, M.A., & Dilmaçünal, T. (2008). The effect of packages constitutedifferent modified atmosphere (MA) on the cold storage of '0900 Ziraat' sweet cherry cv. Bahçe Ürünlerinde IV. Muhafaza ve Pazarlama Sempozyumu, 08-11 Ekim 2008, Antalya, 33-41.

Kuentz, C. (2015). Janny MT equipment. http://www.berrycongress.com/resources/documents/ 1427722592 Celine_Kuentz.pdf. Date of Access: 23.11.2016.

Lara, I., Camats, J.A., Comabella, E., & Ortiz, A. (2015). Eating quality and health-promoting properties of two sweet cherry (*Prunus avium* L.) cultivars stored in passive modified atmosphere. *Food Science and Technology International*, 21:133-144.

Meheriuk, M., McKenzie, D.L., Girard, B., Moyls, A.L., Weintraub, S., Hocking, R., & Kopp, T. (1997). Storage of sweetheart cherries in sealed plastic film. *Journal of Food Quality,* 20:189-198.

Mitcham, E.J., Crisosto, C.H., & Kader, A.A. (2003). Sweet cherry recommendations for maintaining postharvest quality. *Postharvest Technology Research and Information Ctr*, *29*.

Padilla-Zakour, O.I., Tandon, K.S., & Wargo, J.M. (2004). Quality of modified atmosphere packaged 'Hedelfingen' and 'Lapins' sweet cherries. *HortTechnology,* 14:3.

Petriccione, M., Sanctis, F., Pasquariello, M.S., Mastrobuoni, F., Rega, P., Scortichini, M., & Mencarelli, F. (2014). The effect of chitosan coating on the quality and nutraceutical traits of Sweet cherry during Postharvest Life. *Food and Bioprocess Technology*, 8:394–408

Remon, S., Ferrer, A., Marquina, P., Burgos, J., & Oria, R. (2000). Use of modified atmosphere to prolong the postharvest life of Burlat cherries at two different degree of ripeness. *Journal of the Science of Food and Agriculture,* 80:1445-1552.

Serrano, M., Romero, D.M., Castillo, S., Guillen, F., & Valero, D. (2005). The use of natural antifungal compounds improves the beneficial effect of MAP in sweet cherry storage. *Innovative Food Science & Emerging Technologies,* 6:115-123.

Sharma, M., Jacob, J.K., Subramanian, J., & Paliyath, G. (2010). Hexanal and 1-MCP treatments for enhancing the shelf life and quality of sweet cherry (*Prunus avium* L.). *Scientia Horticulturae,* 125:239-247.

Wang, Y., & Long, L.E. (2014). Respiration and quality responses of sweet cherry to different atmospheres during cold storage and shipping. *Postharvest Biology and Technology*, 92: 62-69.

Wani, A.A., Sing, P., Gul, K., Wani, M.H., & Langowski, H.C. (2014). Sweet cherry (*Prunus avium* L): critical factors affecting the composition and shelf life. *Food Packaging and Shelf Life,* I: 86-99.

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RESEARCH PAPER

Genotype Effect as one of the Affecting Factors on the Success of Anther Culture in Eggplant (*Solanum melongena* L.)

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Abstract

Anther culture technique is one of the commonly used method in order to obtain haploid plants in eggplant. It is known that androgenic response in eggplant is variable and one of the most important factors determining success is the genotype effect. Low or insufficient embryogenic response limits the use of doubled haploid technology in breeding programs. Therefore, determining the embryogenic responses of genotypes is a step for the use of this technology in breeding studies. In this study, 61 eggplant genotypes at various breeding stages (F1, F2, and F3) in the gene pool of United Genetics Vegetable Seeds Company (Mustafakemalpaşa, Bursa, Turkey) were evaluated in terms of androgenic responses. Significant differences were determined among eggplant genotypes in terms about embryo induction and conversion rates of embryos to full plantlets. In the study, responsiveness to anther culture based on genotype ranged from 0.0%-21.58%. The conversion rate of induced embryos to plants varied between 0.0% and 100%.

1. Introduction

Haploid plants originate from gametes. To obtain haploid plant, androgenesis or gynogenesis or parthenogenesis could be used depending on the response of gamete cells of different species. Therefore, haploids contain only the set of chromosomes found in microspore or egg cells. The chromosome set of a haploid plant is doubled, either spontaneously or artificially, resulting in homozygous doubled haploid (DH) plants. Doubled haploid technology involves both the production of haploid plants and the chromosome doubling process (Ellialtıoğlu et al., 2001).

Despite its disadvantages such as, the high cost of establishing a facility for double haploidization studies, the presence of aneuploid and/or mixoploid types in the plants obtained by this method, the significantly different responses in DH induction both among species and among genotypes of the same species, and the reduction of genetic diversity in the germplasm as a result of intensive use of this method, double haploidization is routinely used in many plant species breeding studies (Seeja and Sreekumar, 2020). However, it is seen that the interest in DH technology in plant breeding is increasing due to its important advantages. Haploids contain only one set of alleles at each locus. Producing 100% homozygous pure lines in a single generation by doubling the chromosome numbers of haploid plants facilitates plant breeding studies and saves time. The fact that haploid plants contain only one set of homologous chromosomes enables recessive mutations to be revealed. Due to the high homogeneity, there is no masking effect and homozygous or even aggregation of specifically targeted genes is facilitated. The use of traditional breeding methods in perennial plant species with long life cycles, self-incompatible species, dioecious and inbred depression is inhibited. DH technology provides new alternatives to develop pure lines in these types. Haploids has a special

importance in hybrid cultivar breeding, since F1 hybrid cultivars are developed using the method of identifying those that give superior combination ability among homozygous lines. Pure lines obtained from DH plants are used as parents in F1 hybrid cultivar breeding (Ellialtıoğlu et al., 2001).

Androgenesis-based anther culture is the most widely used haploid technique in eggplant. It is known that the androgenic response in eggplant is variable and one of the most important factors determining success is the genotype effect (Khatun et al., 2006; Başay and Ellialtıoğlu, 2013; Rivas-Sendra et al., 2017, 2019; Bhattacharya et al., 2019; Vural et al., 2019; Vural and Ari, 2020; Mir et al., 2021; Hale et al., 2022). In addition, many factors such as nutrient media content, types and concentrations of plant growth regulators, age and growth conditions of donor plants, culturing microspores at the appropriate developmental stage (or anthers containing them), different pretreatments applied to anthers, and incubation conditions affect the success of androgenesis. Low or insufficient embryogenic response limits the use of DH technology in breeding programs. Therefore, identifying the embryogenic responses of genotypes is a step forward for the use of this technology in breeding studies. Bhattacharya et al. (2019) found an androgenic response varying from 0.34% to 9.27% from the two media in their anther culture study with a combination of 6 genotypes and 6 media in eggplant. On the basis of genotype, androgenesis rate varying from 2.29% to 7.40% was determined in five of the six genotypes. It has been reported that genotype recalcitrance may hinder the universal acceptance of DH technology and limit its acceptance by plant breeders. Karakullukçu (1991) reported that only 4 genotypes showed androgenic response in anther culture of 13 eggplant genotypes while they only formed embryoids in 2 genotypes, both embryos and haploid plants were obtained from only 2 genotypes, and embryos could not be obtained from the remain 9 genotypes. Vural and Ari (2020) found that although the genotypic effect was not significant in the spring season on embryo and *in vitro* plantlet yield, the effect of the genotype was significant in the autumn. Khatun et al. (2006) reported that the highest callus induction (30%) was recorded in 6 eggplant genotypes, but no shoots developed, only root formation was observed. Double haploid population was developed by microspore culture from a commercial F1 hybrid eggplant variety and the androgenic response of the population was evaluated. The DH population and subsequent generation showed significant variation with lack, low and high androgenic responsive lines. A highly androgenic DH line (DH36) was determined from this population that produced 4 times more callus than the donor plant (Rivas-Sendra et al., 2017). Callus production of the DH36 line was found to be higher (from 10 to 85 times) in the microspore culture made with two hybrids and one line (DH36). Contrary to their effect on hybrid genotypes, modifications to the environments adversely affected the callus growth of DH36. In contrast, the androgenetic productivity of the hybrid cultivar from which DH36 was derived was increased by changing the media composition (Rivas-Sendra et al., 2020).

The aims of this study were to determine the androgenic capacity of eggplant using 61 genotypes and to specify the genotype effect on the potential to induce haploid embryos and conversion to *in vitro* full plantlets.

2. Materials and Methods

This study was carried out in the greenhouse and laboratory of United Genetics Vegetable Seeds Company (Mustafakemalpaşa, Bursa, Turkey). The study conducted in 2019 (from genotype 1 to genotpe 45) and 2020 (from genotype 46 to 61) growing seasons. As the donor plant, 61 eggplant genotypes of the company were used at various breeding level (F1, F2, and F3 ofspring). The seeds of the donor genotypes were sown and the seedlings at the planting stage were grown in the greenhouse. Flower buds harvesting from donor plants started 4-5 weeks after planting.

Staining technique with DAPI (DNA-specific fluorochrome) was used to morphologically determine the buds with anthers containing mononuclear microspores suitable for anther culture. According to Kim and Jang (2000), 1-2 drops of a solution consisting of a mixture of 1µl stock DAPI + 1ml Buffer (Buffer + Triton) was dropped on the anthers. The anthers were lightly crushed with the scalpel tip, allowing the microspores inside to become free. After the preparations covered with a coverslip were kept in the dark for 10-15 minutes, they were observed under a fluorescent light microscope and the morphology of the buds with anthers containing mononuclear microspores was determined.

The buds with the appropriate development period were collected from the donor plants and brought to the laboratory and surface sterilization was performed. The flower buds were rinsed first with water and then in 70% ethyl alcohol. Then buds kept in 10% commercial bleach (containing 5% sodium hypochlorite) for 15 minutes. Rinsed 3-4 times with sterile distilled water. All tissue culture applications were made under aseptic conditions, and a laminar flow sterile cabinet was used for this.

The protocol of Dumas de Vaulx and Chambonnet (1982) was used for culturing the anthers. Accordingly, anthers were cultured in C medium containing 5 mg L^{-1} 2,4-D, 5 mg L^{-1} kinetin and 12% sucrose. Anthers were incubated at 35 °C and dark conditions for the first 8 days of culture. Then anthers were incubated at 25 °C and 16/8 h photoperiod conditions for 4 days and then transferred to R medium containing 0.1 mg L^{-1}

kinetin and 3% sucrose. A few days after the embryos were seen, they were transferred to hormone-free MS medium. Developing plantlets were planted in pots containing sterile peat and their adaptation to the outside environment was ensured under greenhouse conditions.

At least 100 anthers of each genotype were planted in the medium. Anther numbers cultured according to genotypes are given in Table 1. The data of embryo induction and conversion to plantlet rate were subjected to cluster analysis to determine the relationship between genotypes using SPSS software using between group linkages (Nielsen, 2016).

3. Results and Discussion

In Table 1, cultured anther numbers, induced embryo ratios and conversion from embryo to full plantlet ratios according to genotypes are presented. Even though all the growing conditions of donor plants, nutrient medium, and incubation conditions were the same, significant differences were determined in embryo formation and conversion to *in vitro* full plantlets among genotypes. In the study, responsiveness to anther culture (embryo induction) based on genotype ranged from 0.0% to 21.58%. The conversion rate varied from 0.0% to 100%. To understand the androgenic response of the 61 eggplant genotypes, the data were subjected to cluster analysis in which both embryo yield and conversion rate were evaluated together, and the groups are presented in Figure 1.

Eggplant genotypes were divided into 2 main groups. The first main group (1) was included genotypes which did not produce embryos at all or had low averages of embryo formation and plant conversion. The second main group (2) was included genotypes that produce medium or high averages of embryos and plant conversion. No embryo was obtained in 9 (14.7% of 61 genotypes tested) of 19 genotypes in the first main group. In 10 genotypes (16.39%) in the same group, 0.11-6.2% embryo induction rates were obtained. Embryos consisting of 6 genotypes did not convert into plants, while some of the embryos in 4 genotypes developed into plants. In the first main group, 31.14% of the studied genotypes were included.

The second main group, on the other hand, was divided into 2 subgroups depending on both embryo productivity and rate of embryos conversion to plant (2.1 and 2.2). In the first subgroup (2.1) 16 genotypes were included. In this group the embryo induction rates of the genotypes were varied from 0.5% to 16.92%, and the conversion rates from 31.91% to 50.0%. The 26.23% of the studied genotypes were in this group (2.1). The second subgroup (2.2) was again divided into two subgroups (2.2.1 and 2.2.2). In the 2.2.1 group, there were 17 genotypes with embryo ratio from 0.68% to 17.76% and conversion ratio between 53.73-68.75%. Genotypes in this group constituted 27.86% of the studied genotypes. The 2.2.2 group had 6 genotypes (9.83% of all) with the highest percentage of embryos and *in vitro* plantlets. The genotype number-54 had the highest rate of embryo (21.58%) and had a 100% conversion rate. Among all genotypes in cluster analysis, genotype No 54 had the highest androgenic efficiency followed by genotypes No 4, 3, 36, 34, 61 and 55 while the next genotypes number 6, 7, 9, 11, 12, 13, 14, 15, 16, 17, 18, 20, 21, 23, 25, 38, 50, 51 and 58 had the lowest one (Fig. 1).

The number of embryos obtained from different genotypes in anther culture of the *Solanaceae* family and their conversion rates to full plants show significant differences. An important disadvantage in anther culture is the low percentage of embryos development into cotyledons and rooted stage (convert to a complete plantlet). Each embryo possesses two distinct poles, one to form root and the other shoot. Under *in vitro* conditions embryo development could shows varying degrees of abnormality. The abnormal shapes of embryos (root from one pole of the embryo and callus formation from the other end) could formed by the androgenesis. Benelli et al. (2010) reported that abnormalities in embryo development, probably caused by the response to the different culture conditions, are genotype-dependent. Abnormalities in the shoot pole or arrest of development of the embryo can be related to lack of conversion or low conversion rate of the embryos.

The rate of haploid embryo formation by anther culture of 61 genotypes studied varied among 0.00% to 21.58%. It has been determined that the conversion rate to *in vitro* plantlet varies between 0.00-100.00%. Figure 2 presents the androgenic embryos and *in vitro* plantlets obtained in the experiment.

Genetic studies on different plants have showed that *in vitro* haploid plant induction ability is under genetic control and certain genes are found that initiate haploid formation in some species (Ellialtıoğlu et al., 2001). It has been shown by various studies in previous years that the androgenetic response feature is hereditary, and it has been determined that the androgenic capacity in peppers is under a strong genetic control. It was determined that the additive gene effect was 21% and the dominance gene effect was 79%. It was concluded that the predisposition to androgenesis in pepper was recessive epistasis with the presence of homozygous recessive genes and some modifying genes (Denli, 2019). Başay and Ellialtıoğlu (2013) reported that androgenesis potential in eggplant is largely dependent on genotypes, and that it is a useful system to increase androgenesis efficiency from hybrids obtained by crossing genotypes with low androgenesis capacity with genotypes with high androgenesis response. Genotype-related differences in internal amino acid

Table 1. Cultured anther numbers according to genotypes, embryo ratio (number of embryos 100 anther^{-1}) and *in vitro* plantlet ratio (conversion rate-number of plants 100 embryos^{-1}).

Genotype no	Breeding stage	Cultured anther numbers	Embryo no 100 anther $^{-1}$	Plantlet no 100 embryos^{-1}
1	F2	730	12.74	36.56
2	F3	840	9.88	42.17
3	F2	850	0.94	75.00
4	F3	990	1.52	80.00
5	F3	1000	0.50	40.00
6	F3	780	0.64	0.00
7	F1	880	0.11	0.00
8	F2	650	1.23	62.5
9	F3	920	0.00	0.00
10	F3	790	2.03	68.75
11	F3	420	0.00	0.00
12	F3	520	0.00	0.00
13	F3	500	0.00	0.00
14	F3	800	0.75	0.00
15	F2	180	0.56	0.00
16	F2	370	0.00	0.00
17	F2	510	1.76	0.00
18	F2	530	0.00	0.00
19	F2	780	0.38	66.67
20	F1	400	0.00	0.00
21	F1	770	0.00	0.00
22	F1	740	0.68	60.00
23	F1	620	0.00	0.00
24	F1	230	1.74	50.00
25	F3	630	3.49	18.18
26	F3	980	4.80	55.32
27	F3	330	1.52	40.00
28	F3	1240	8.31	55.34
29	F3	790	5.82	63.04
30	F3	740	9.05	53.73
31	F3	750	6.13	50.00
32	F3	550	2.18	58.33
33	F3	910	3.41	58.06
34	F3	960	5.10	75.51
35	F3	550	9.09	48.00
36	F3	620	3.55	72.73
37	F3	1210	3.88	31.91
38	F3	710	6.20	20.45
39	F3	590	3.39	65.00
40	F3	450	11.56	38.46
41	F3	500	5.80	55.17
42	F3	700	7.43	57.69
43	F2	1090	6.79	56.76
44	F3	680	5.00	47.06
45	F3	6620	2.79	33.51
46	F3	3690	0.71	57.69
47	F3	5970	2.91	41.38
48	F3	4640	5.13	39.08
49	F3	3760	4.28	39.13
50	F3	5360	1.51	24.69
51	F2	160	0.63	0.00
52	F2	1070	16.92	45.3
53	F2	1050	6.19	43.08
54	F2	190	21.58	100.00
55	F2	320	10.94	82.86
56	F2	670	17.76	60.50
57	F2	620	7.90	48.98
58	F2	490	4.69	17.39
59	F1	130	14.62	68.42
60	F1	100	6.00	66.67
61	F1	190	7.89	80.00

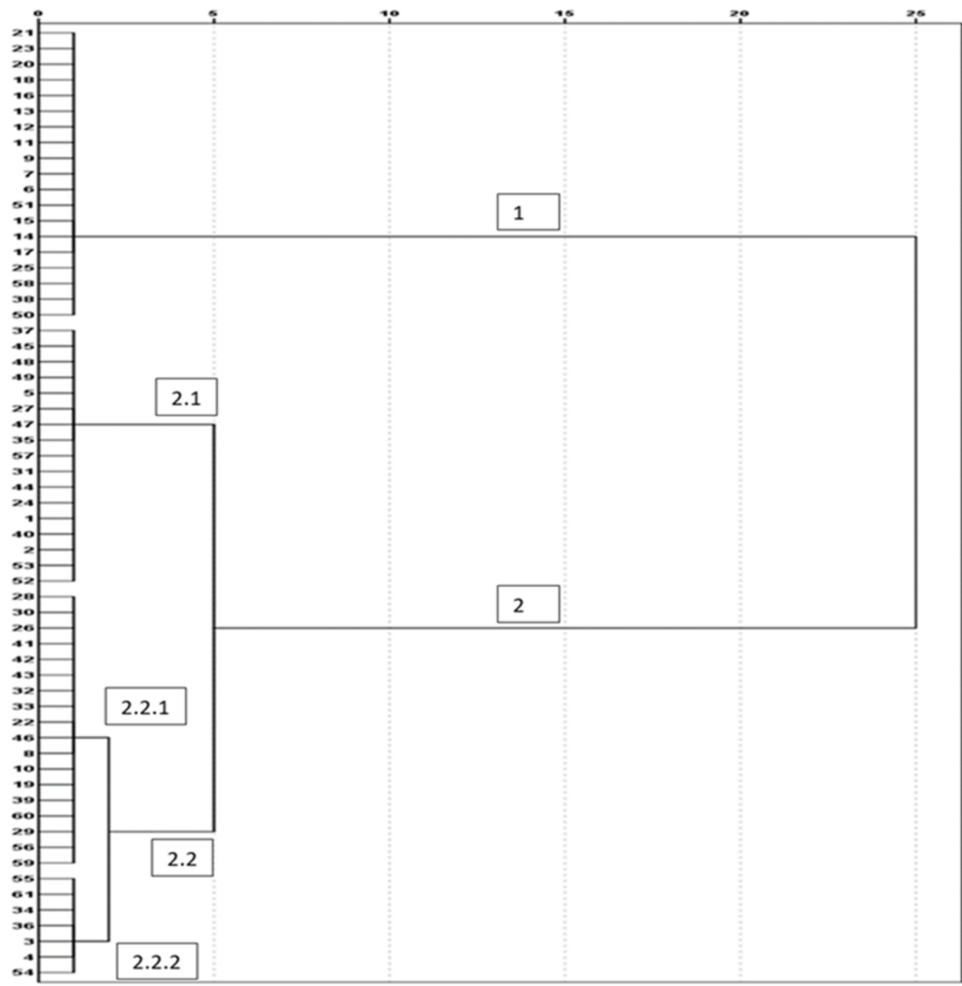

Figure 1. Grouping of 61 eggplant genotypes for androgenetic efficiency in cluster analysis.

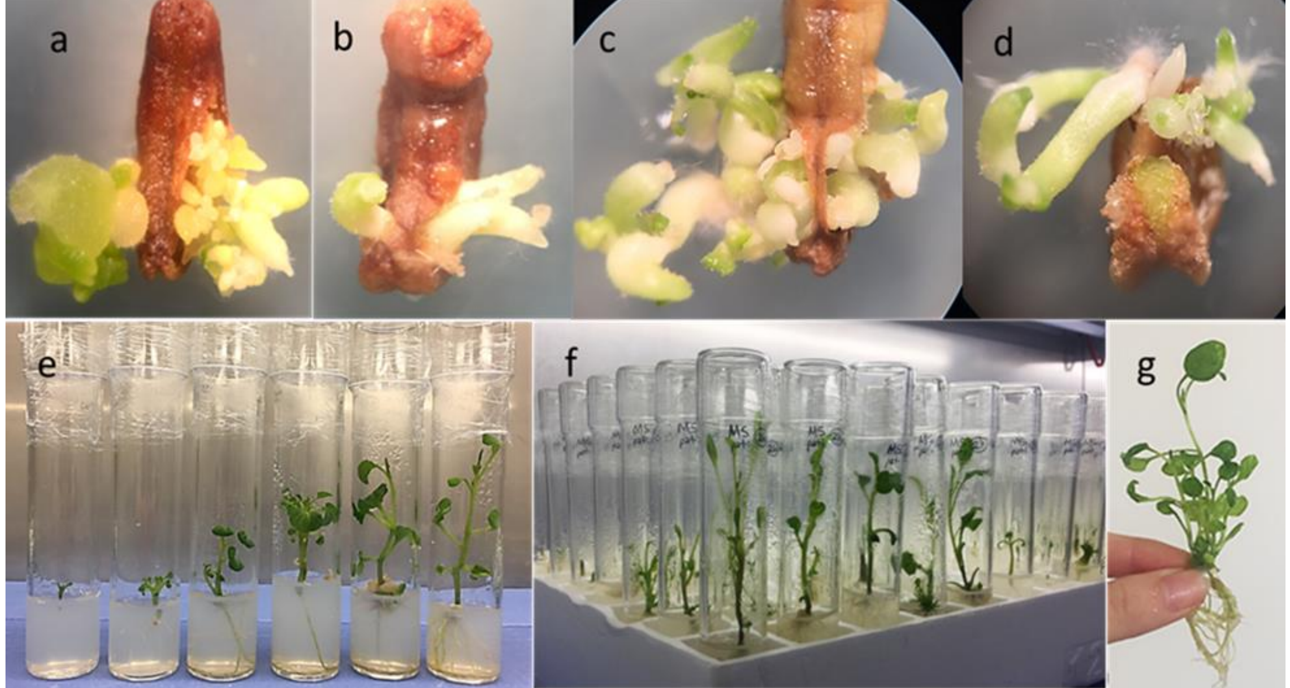

Figure 2. Embryos emerging from eggplant anthers (a, b, c, d), Androgenic plantlets (e, f), A haploid eggplant plantlet that has reached the acclimatization stage (g).

contents (Dunwell, 1976) have been associated with the presence of certain genes that initiate haploid formation in some species (ig gene in maize, pill gene in maize and barley Kermicle, 1969; Hagberg and Hargberg, 1980; Foroughi-Wehr et al., 1982). Muñoz-Amatriaín et al. (2009) reported that genes that play a role mainly in changes in the structure and function of membranes, efficient use of available energy resources, and cell fate are associated with the ability to form embryos from microspores. Genes involved in the stress response, regulation of transcription and translation, and disruption of microspore-specific proteins have been associated with green plant production.

4. Conclusion

Androgenesis potential in different breeding levels of the same eggplant genotype were not studied. Therefore, the androgenic response has not been determined by the breeding level of a genotype. The androgenic response of different genotypes at different breeding levels was investigated. In the cluster grouping of the 61 eggplant genotypes for androgenic efficiency according to the embryo induction and the conversion to plantlet of these embryos, genotypes were included in each group at each breeding level (F1, F2, and F3). Accordingly, it was determined that the genotype effect was more important than the breeding level in terms of androgenic response.

The fact that the genotype effect is so important highlights the importance of continuously improving the DH method and improving existing protocols. It remains valid that appropriate protocols for each genotype should be determined experimentally. While developing androgenesis protocols to overcome genotype effect and recalcitrancy, it is important to develop genotype-independent methods or to study genomic and gene editing technologies.

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References

Başay, S., & Ellialtıoğlu, Ş.Ş. (2013). Effect of genotypical factors on the effectiveness of anther culture in eggplant (*Solanum melongena* L.). *Turkish Journal of Biology*. 37:499-505.

Benelli, C., Germana, M.A., Ganino, T., Beghe, D., & Fabbri, A. (2010). Morphological and anatomical observations of abnormal somatic embryos from anther cultures of *Citrus reticulate. Biologia Plantarum*, 54:224-230.

Bhattacharya, A., Sonone, Y., & Char, B. (2019). Improvement in tissue culture-assisted induction of double haploidy in brinjal (*Solanum melongena* L.) *Journal of Applied Horticulture,* 21:178-181.

Denli, N. (2019). Expansion of genetic basis via interspecies crossing of pepper (*Capsicum annuum*) and determination the inheritance of androgenesis. PhD Thesis, Çukurova University, Adana, Türkiye.

Dumas de Vaulx, R., & Chambonnet, D. (1982). Culture in vitro antheres aubergine (*Solanum melongena* L.): stimulation de la production de plantes au moyen de traitements a +35 degres C associes a de faibles teneurs en substances de croissance. *Agronomie*, EDP Sciences, 2:983-988.

Dunwell, J.M. (1976). A comparative study of environmental and developmental factors which influence embryo induction and growth in cultured anthers of Nicotiana tabacum. *Environmental and Experimental Botany*, 16:109-118.

Ellialtıoğlu, Ş., Sarı, N., & Abak, K. (2001). In: Babaoğlu M, Gurel E, Ozcan, S. editors. Plant Biotechnology-I Tissue Culture and its Applications. Haploid Plant Production, pp137-189 (in Turkish).

Foroughi-Wehr, B., Friedt, W., & Wenzel, G. (1982). On the genetic improvement of androgenetic haploid formation in *Hordeum vulgare* L. *Theoretical and Applied Genetic,* 62:233-239.

Hagberg, A., & Hagberg, G. (1980). High frequency of spontaneous haploids in the progeny of an induced mutation in barley. *Hereditas,* 93:341-343.

Hale, B., Ferrie, A.M.R., Chellamma, S., Samuel, J.P., & Phillips, G.C. (2022). Androgenesis-based doubled haploidy: Past, present, and future perspectives. *Plant Science*, 12:751230.

Karakullukçu, Ş. (1991). Investigation of some factors inducing in vitro androgenesis and haploid plant development in different eggplant genotypes. PhD Thesis, Ankara University, Türkiye.

Kermicle, J.L. (1969). Androgenesis conditioned by a mutation in maize. *Science,*166:1422-1424.

Khatun, F., Meah, M.B., & Nasiruddin, K.M. (2006). Regeneration of eggplant through anther culture. *Pakistan Journal of Biological Sciences,* 9:48-53.

Kim, M.Z., & Jang I.C. (2000). Rapid assesment of microspore development stage in pepper using DAPI and ferric cloride. Journal of *Plant Biotechnology*, 2:129-134.

Mir, R., Calabuig-Serna, A., & Segui-Simarro, J.M. (2021). Doubled haploids in eggplant. *Biology,* 10:685.

Muñoz-Amatriaín, M., Svensson, J.T., Castillo, A.M., Close, T.J., & Vallés, M.P. (2009). Microspore embryogenesis: assignment of genes to embryo formation and green vs. albino plant production. *Functional & integrative Genomics*, 9:311-323.

Nielsen, F. (2016). Hierarchical clustering. In: Introduction to HPC with MPI for data Science.Vol.8. ISBN: 978-3-319-21902-8. Springer. p. 282.

Rivas-Sendra, A., Campos-Vega, M., Calabuig-Serna, A., & Segui-Simarro, J.M. (2017). Development and characterization of an eggplant (*Solanum melongena*) doubled haploid population and a doubled haploid line with high androgenic response. *Euphytica,* 213:89.

Rivas-Sendra, A., Corral-Martinez, P., Porcel, R., Camacho-Fernandez C., Calabuig-Serna, A. & Segui-Simarro, J.M. (2019). Embryogenic competence of microspores is associated with their ability to form a callosic, osmoprotective subintinal layer. *Journal of Experimental Botany*, 70:1267–1281.

Rivas-Sendra, A., Corral Martínez, P., Camacho-Fernández, C., Porcel, R., & Seguí-Simarro, J.M. (2020). Effects of growth conditions of donor plants and in vitro culture environment in the viability and the

embryogenic response of microspores of different eggplant genotypes. *Euphytica*, 216:1-15.
Seeja, G., & Sreekumar, S. (2020). Doubled haploids in genetic improvement: A review. *International Journal of Recent Scientific Research*, 11:36941-36949.
Vural, G.E., Ari, E., Zengin, S., & Ellialtıoğlu, S. (2019). Development of androgenesis studies on eggplant (*Solanum melongena* L.) in Turkey from past to present. In M. Hasanuzzaman, M.C.M.T. Filho, M. Fujita, & T.A.R. Nogueira (Eds.), Sustainable crop production. IntechOpen.
Vural, G.E., & Ari, E. (2020). Triple synergistic effect of maltose, silver nitrate and activated charcoal on high embryo yield of eggplant (*Solanum melongena* L.) anther cultures. *Scientia Horticulturae*, 272:109472.

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RESEARCH PAPER

Phenolic Composition and Antioxidant Activity of Myrtle Fruits and Leaves Grown in Antalya (Türkiye)

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Abstract

Myrtle (*Myrtus communis* L.) is an evergreen shrub belonging to the family of Myrtaceae that grows spontaneously throughout the Mediterranean area. In Türkiye, myrtle tree is grown in pine forests and riversides, particularly in the Taurus mountains, from sea level to 500–600 m. Their antioxidant activity has been attributed to the presence of phenolic compounds and essential oils. The purpose of this work is to characterize myrtle plants through its physical and chemical characteristics such as phenolic and flavonoid content, antioxidant activity for selecting the promising genotypes in Antalya coastal region of Turkey. Phenolic compounds were extracted from leaves and berries. Phenolic composition was determined by LC-MS-MS. Antioxidant activity was measured with 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. Phenolic substance was higher in the leaf samples collected from Kumluca (BM15 and BM16) and in the fruit samples collected from Kemer (BM13) and Kaş (BM19) when compared to other regions. High antioxidant activity was detected in the leaf sample BM16 collected from Kumluca (0.13 µl) and the fruit sample (BM19) collected from Kaş (0.45 µl). It was determined that significantly differences in the phenolic compositions and antioxidant activities of myrtle leaves and fruits were to be found among genotypes grown in different locations.

1. Introduction

Myrtle (*Myrtus communis* L.) is a medicinal and aromatic plant which belongs to the Myrtaceae family has a perennial, evergreen, shrub-shaped and sized up to 1-3 m plants. Wild forms of the myrtle plant grown in the coastal sides of Tunisia, Morocco, Turkey and France while it is cultured in Iran, Spain, Italy and Corsica (Jamoussi et al., 2005). Myrtle, which is one of the typical natural plants of the Mediterranean Basin, is found in natural forms in Adana, Antalya, İçel, Çanakkale, İstanbul, Zonguldak, Sinop, Ordu, Trabzon, İzmir, Samsun, Muğla and Hatay provinces in Turkey. *Myrtus communis* is generally called as mersin in Turkey but also known as “murt”, “hambeles” and “adi mersin” in the Mediterranean coastal sides In addition, its leaves are named "bahar" in some places (Oğur, 1994). In Turkey, myrtle plant grows between the coastal strip and pine forests, particularly in heights of up to 500-600 meters from the sea in the Taurus Mountains (Kaya and Aladağ, 2009).

Because of its medicinal and aromatic properties, the myrtle plant has been used as a medicinal herb in the folk medicine for many years in Turkey. Myrtle plants with white colored fruits were started to cultivate prior to the black ones because their larger fruits but they have very short shelf-life and low antioxidant capacity. White myrtle plants are cultivate by grafting on the wild plants grown in the forests or in the border of orchards.

Recently, It is getting more interest to dark colored and organically grown fruits in the world. It is reported that there is a high correlation between antioxidant activity and the amount of phenolic substance in the fruits and leaves. It is stated that the basic phenolics in myrtle are flavonoids and anthocyanins. The amount of myricetin-3-*O*-galactoside, myricetin-3-*O*-rhamnoside and quercetin-3-*O*-glucoside were found to be high among flavonoids (Montoro et al., 2006). Therefore, recently the demand for black myrtle has increased, as in other black colored fruits, due to its high antioxidant content (Martin et al., 1999). In addition to being consumed fresh, the fruits can also be consumed dried fruit or used in liquor production.

It was found that myrtle leaves contain a modest amount of phenolic acid (caffeic, ellagic acid and gallic acid) and quercetin derivatives (quercetin-3-*O*-galactoside and quercetin-3-*O*-rhamnoside) while the amount of catechin derivatives (Epigallocatechin, Epigallocatechin-3-*O*-gallate) and myricetin derivatives (myricetin-3-*O* galactoside, myricetin-3-*O*-rhamnoside) were found to be high (Reynertson et al., 2008). In addition, it is reported that the leaves have strong antioxidant activity due to their chemical structure (Romani et al., 2004).The leaves and fruits are used due to their constipating, disinfectant, appetizing and hemostatic features (İlçim et al., 1998). It is stated that the myricetin extracted from the leaves contributes to the treatment of a wide range of health problems such as rheumatism, cardiovascular diseases, bronchitis and colds. It was found that myricetin extracted from the leaves was effective in the correction of renal dysfunction in experimental animals (Özcan, 2009). A number of studies have been conducted to examine various therapeutic effects and anti-oxidant, anti-carcinogenic and antiaggregant properties of myricetin (Tzeng et al., 1991; Ong and Khoo, 1997).

Myrtle has a wide range of usage due to high therepautic phenolic content of leaves and fruits. Identification of the substances constituting the content of leaves and fruits is important to get the highest benefit from the plant. Research shows that the collection time, origin and extraction method of the material significantly affect the content of the plant, and certain substances that are considered an indispensable compound in some places can not be detected by other researchers (Akgül and Bayrak, 1989). In this respect, identification of the content of myrtle plants that grow naturally in Antalya conditions, selection of the promising types and cultivation hereof is important in terms of providing materials for future research.

2. Material and Method

2.1. Plant material and locations

The leaves and fruits of the black myrtle plant grown in the natural flora of Antalya were used as material. Leaves and fruits 19 genotypes were collected from 10 different districts of Antalya and the location information of the collected samples is given in Table 1. Myrtle fruit and leaves were collected from elevations between 50 and 470 m. Myrtle plant is widely available up to 400-500 m altitudes. As a result of the surveys conducted, myrtle plants were also found at altitudes above 500 m (up to 850 m). However, the plant density was observed to be quite low. Fruit size was taken as the major selection criteria. Fruits and leaves are coded as BM (Black Myrtle) and numbered from 1 to 19 (Table 1).

Table 1. Location information.

Genotype	Location	GPS record
BM1*	Gazipaşa	160 m, 36° 21.600K, 032° 22.766D
BM2	Gazipaşa	283 m, 40° 28.203K, 36° 44.7365D
BM3	Alanya	455 m, 36° 34.902K, 031° 59.604D
BM4	Alanya	370 m, 36° 30.162K, 032° 09.090D
BM5	Manavgat	67 m, 36° 56.756K, 031° 13.067D
BM6	Serik	50 m, 37° 07.184K, 030° 54.693D
BM7	Serik	50 m, 37° 07.184K, 030° 54.693D
BM8	Serik	290 m, 37° 13.222K, 030° 57.435D
BM9	Serik	330 m, 37° 14.156K, 030° 58.422D
BM10	Merkez	230 m, 36° 53.943K, 030° 30.697D
BM11	Merkez	470 m, 36° 54.003K, 030° 29.907D
BM12	Merkez	220 m, 36° 52.694K, 030° 30.749D
BM13	Kemer	205 m, 36° 32.399K, 029° 31.701D
BM14	Kemer	424 m, 36° 29.298K, 030° 27.151D
BM15	Kumluca	200 m, 36° 19.447K, 030° 25.021D
BM16	Kumluca	288 m, 36° 24.207K, 029° 53.456D
BM17	Finike	343 m, 36° 24.197K, 029° 53.425D
BM18	Demre	166 m, 36° 15.047K, 029° 57.121D
BM19	Kaş	254 m, 36° 23.263K, 029° 52.030D

*BM: Black Myrtle

2.2. Methods

In order to determine the quality criteria in the samples, 10 piece of fruit weight (g), 10 piece of seed weight (g), fruit flesh / seed ratio (%), fruit and leaf size (cm), number of seeds in the fruit (pieces), pH and titratable acidity in fruits (malic acid %), brix (%), total phenolic and flavonoid substance, antioxidant activity and phenolic component analyzes were analysed.

2.2.1. Extraction

In the extraction of phenolic substances from the samples, the method suggested by Cai et al. (2004) was used. Leaves and berries were collected in each area at the time of industrial ripeness, when the berries were fully dark-violet pigmented. After the harvest, the samples were carried to the laboratory, cleaned from impurities and dried at room temperature. After, the leaves and berries were separated and ground in a grinder. 2 g of dried fruit and leaf samples were extracted with 20 ml of 80% methanol for 3 times at room temperature, centrifuged at 4500 rpm (1585 g) for 10 min, and then supernatant passed through a 0.45 µm syringe filter. The extracts were stored at -18°C until analysis.

2.2.2. Total phenolic content

In the colorimetric determination of total phenolic content, the spectrophotometric method defined by Spanos and Wrolstad (1990) was used. For this purpose, 100 µl of extract were taken into a tube and 900 µl of distilled water was added on it. Then, 5 ml of 0.2 N Folin-Ciocalteu solution and 4 ml of saturated sodium carbonate solution (75 g l^{-1}) were added, the tubes were thoroughly stirred by vortex and left in the dark for 2 hours. The total amount of phenolic compound was calculated by utilizing the absorbance value read on the spectrophotometer at the 765 nm wavelength and the curve prepared with gallic acid (GA). Results were expressed as g of gallic acid equivalent (GAE) g^{-1} dry matter of plant material (g GAE g^{-1} DM)

2.2.3. Total flavonoid content

The method described by Karadeniz et al. (2005) was used in the colorimetric determination of the total amount of flavonoids by aluminum chloride. Four ml of distilled water and 0.3 ml of 5% $NaNO_2$ was added on 1 ml of sample and stirred. 0.6 ml of 10% $AlCl_3.6H_2O$ was added after 5 minutes, and 2 ml of 1 mol l^{-1} NaOH was added 5 minutes after that, and the total volume was filled with 10 ml of distilled water. After stirred thoroughly, the total amount of flavonoid was calculated as the equivalent to mg catechin grams of fruit by using the absorbance value read at 510 nm on spectrophotometer and the catechin curve prepared. The results were expressed as g of catechin equivalent (CTE) g^{-1} dry matter (g CTE g^{-1} DM).

2.2.4. DPPH radical scavenging activity

Stable radical DPPH solution was used in the measurements. The volume was completed to 6 ml by adding various amounts of (10, 20, 40, 60, 80, 100 µl) extracts on 600 µl of DPPH solution prepared with methanol. The tubes were stirred with vortex and kept at room temperature in the dark for 15 minutes. 600 µl of DPPH was added with 5.4 ml of methanol to be used as witness, and allowed to stand for 15 minutes under the same conditions as the samples. After incubation, the absorbance value of the tube contents at 515 nm was read using the spectrophotometer. The % inhibition values of the sample extracts were calculated using the following formula.

$$Inhibition\ (\%) = \left[\frac{A_{DPPH} - A_{Sample}}{A_{DPPH}}\right] \times 100$$

A_{DPPH}: Absorbance value of DPPH witness sample
A_{sample}: Absorbance value of the sample extract

The % inhibition values obtained from different amounts of sample extracts and the concentration values were graphed and the concentration (EC_{50}) reducing the effect of DPPH by 50% for each sample was calculated (Cemeroğlu, 2010).

2.2.5. Analyses of phenolic compounds through LC-MS-MS

The method described by Fischer et al. (2011) was used with some modification. Phenolic compounds of myrtle samples were identified through LC-MS / MS (High-performance Liquid Chromatography Coupled With Tandem Mass Spectrometry). Agilent 6430 Triple Quadrupole (Agilent Technologies, Santa Clara, CA, USA) brand electrospray ion-derived mass spectrometry and Agilent-1290 Infinity (Agilent Technologies, Waldbronn, Germany) brand liquid chromatography were used in the analyses. The study was conducted in positive and negative ion mode. The study was carried out at a flow rate of 0.25 ml min^{-1} in the Zorbax SB-C18 (150x2.1 mm, 1.8 CAm) (Agilent Technologies, Palo Alto, CA) column. Solutions at different concentrations from the standards were prepared and calibration curves were formed for quantitation. Myrtle samples were injected by passing through a 0.45 µm diameter PVDF (polyvinylidene fluoride) filter. The injection volume was 10 µl. The mobile phase used in the study was Solvent A = (5/95) Methanol: Water (containing 0.01% formic acid and 5 mM ammonium formate) and Solvent B = Methanol (containing 0.01% formic acid and 5 mM ammonium formate). The elution profile used is as follows: 0-1 min 5% solvent B (constant flow), 1-3 min 30% solvent B, 3-

4 min 60% solvent B, 4-5 min 60% solvent B (constant flow), 5-6 min 70% solvent B, 6-8 min 80% solvent B, 8.01 min 5% solvent B, 8.01-10 min 5% solvent B (constant flow).

2.2.6. Statistical analysis

All analyses were performed with 3 replications and the mean and standard deviation (± SD) values were calculated (Düzgüneş et al., 1987).

3. Results and Discussion

3.1. General characteristics of fruits, leaves and seeds

Some physical and biochemical properties of the myrtle samples collected are given in Tables 2 and 3. Fruit weight was between 0.538-0.993 g, fruit width between 0.90 -1.24 cm, fruit length between 1.03-1.43 cm, pH between 5.04-5.94, titratable acidity between 0.23-0.97%, and brix between 11.5-21.5%. The width, length and stem length of the myrtle leaves vary between 1.12-1.68 cm, 3.05-4.16 cm and 0.20-0.32 cm, respectively.

The average number of seeds in the fruits ranged from 8 to 26 pieces/fruit and the weight of seeds ranged from 0.06 to 0.17 g in all tested genotypes. Seed / fruit ratio was found to be between 10.45 and 23.66% (Table 3).

Our findings show similarities with the literature. Indeed, Traveset et al. (2001) found that the fruit weight of the wild black myrtle growing in Italy was 0.54 g, seed weight was 7.16 mg, fruit length was 10.87 mm. fruit width was 10.21 mm and the number of seeds per fruit was 12.06. Tuberoso et al. (2007) reported the size of the berries grains to be between 0.19-0.41 g in their study conducted in Italy. The number of seeds per berry varied between 4 and 16. Fadda and Mulas (2010) found that the weight of fresh fruit was about 400 mg after 150 days from blooming in the Barbara cultivar and about 800 mg after 180 days from blooming in the Daniela cultivar, in a study they conducted in Corsica.

3.2. Total phenolic and flavonoid substance and antioxidant activity of myrtle

The total amount of phenolic and flavonoid substance and antioxidant activities of myrtle fruits and leaves are given in Table 4. The total amount of phenolic substance in the leaves was found to be 69.05 mg GAE g^{-1} on average. The maximum amount of phenolic substance was found in the BM16 and BM15 genotypes collected from Kumluca with 92.12 and 90.14 mg GAE g^{-1} while the least amount was found in the leaves of the BM3 genotype collected from Alanya with 50.33 mg GAE g^{-1}.

The total amount of flavonoid in the leaves was found to be 3.82 mg CTE g^{-1} on average, where the highest amount was found in the BM15 genotype with 6.38 mg CTE g^{-1} while the lowest amount was found in the leaves of the BM3 genotype with 2.53 mg CTE g^{-1}.

The antioxidant activity was detected to be EC_{50}. A low value indicates high antioxidant activity. The average antioxidant activity of the extracts obtained from the leaves was found to be 0.32 µl. While the highest activity was seen in the leaves of the BM16 genotype with a total phenolic substance of 0.13 µl, the lowest activity was observed in the leaves of the BM11 genotype with 0.46 µl. The correlation between antioxidant activity and the total amount of phenolic substance (r = -0.74526) and flavonoid

Table 2. Some physical and biochemical properties of myrtle fruit (±SD).

Genotype	10 piece of fruit weight (g)	Fruit width (cm)	Fruit lenght (cm)	pH	Titratable acidity (malic acid %)	Brix (%)
BM1*	9.93±0.75	1.24±0.05	1.32±0.08	5.44±0.05	0.23±0.06	15.50±0.71
BM2	6.66±0.34	0.97±0.07	1.40±0.04	5.78±0.06	0.40±0.05	14.30±0.42
BM3	6.66±0.50	1.15±0.06	1.12±0.04	5.33±0.06	0.67±0.09	16.00±1.41
BM4	7.05±0.17	1.06±0.06	1.29±0.07	5.40±0.11	0.37±0.05	16.50±0.71
BM5	8.59±0.22	1.16±0.06	1.36±0.07	5.11±0.01	0.69±0.02	11.50±0.71
BM6	7.28±0.81	1.08±0.06	1.29±0.05	5.86±0.05	0.62±0.07	20.90±0.14
BM7	6.72±0.32	1.13±0.07	1.20±0.03	5.91±0.07	0.42±0.02	18.50±0.71
BM8	7.48±0.37	1.12±0.05	1.43±0.08	5.68±0.09	0.47±0.09	13.60±0.57
BM9	7.20±0.59	1.11±0.06	1.32±0.06	5.75±0.04	0.39±0.02	16.90±0.14
BM10	5.97±0.67	1.05±0.07	1.16±0.06	5.42±0.21	0.57±0.09	18.40±0.85
BM11	5.79±0.65	1.03±0.06	1.22±0.04	5.64±0.09	1.22±0.12	21.50±0.71
BM12	7.06±0.46	1.05±0.05	1.36±0.09	5.69±0.19	0.95±0.07	16.40±0.85
BM13	5.53±0.23	1.00±0.05	1.36±0.05	5.62±0.01	0.42±0.02	18.75±0.35
BM14	6.26±0.68	1.08±0.05	1.15±0.05	5.56±0.02	0.39±0.02	16.85±0.21
BM15	5.51±0.09	1.03±0.06	1.14±0.07	5.54±0.12	0.45±0.02	15.50±0.71
BM16	8.65±0.92	1.19±0.04	1.47±0.10	5.62±0.02	0.35±0.02	14.75±0.35
BM17	5.65±0.31	0.95±0.07	1.13±0.04	5.50±0.05	0.42±0.07	11.50±0.71
BM18	5.38±0.25	0.90±0.04	1.03±0.04	5.04±0.06	0.97±0.19	19.50±0.71
BM19	6.17±0.84	1.07±0.08	1.23±0.06	5.45±0.08	0.54±0.05	19.60±0.57

*BM: Black Myrtle

Table 3. Some physical properties of leaf and seed of myrtle (±SD).

Genotype	Leaf width (cm)	Leaf length (cm)	Petiole lenght (cm)	Number of seeds in the fruit (piece)	10 piece of seed weight (g)	Seed/fruit ratio (%)
BM1*	1.65±0.28	4.07±0.23	0.27±0.05	26±4	0.08±0.01	18.59±2.70
BM2	1.22±0.16	3.41±0.28	0.29±0.02	11±3	0.06±0.01	11.07±0.54
BM3	1.19±0.07	3.34±0.19	0.20±0.05	25±3	0.06±0.01	20.15±0.53
BM4	1.60±0.14	4.07±0.36	0.22±0.01	21±3	0.06±0.01	18.45±2.78
BM5	1.50±0.25	2.87±0.20	0.25±0.04	8±3	0.17±0.01	15.62±4.26
BM6	1.49±0.28	3.38±0.38	0.28±0.04	16±2	0.09±0.01	18.97±0.78
BM7	1.64±0.23	4.09±0.46	0.29±0.04	9±2	0.11±0.01	13.20±2.59
BM8	1.19±0.12	3.38±0.29	0.30±0.02	13±3	0.13±0.01	20.81±1.97
BM9	1.18±0.11	3.02±0.32	0.24±0.02	10±2	0.09±0.01	14.44±3.96
BM10	1.53±0.47	3.49±0.60	0.25±0.06	9±2	0.17±0.01	23.66±2.40
BM11	1.31±0.07	3.55±0.16	0.24±0.03	9±2	0.10±0.01	12.30±0.68
BM12	1.45±0.09	3.67±0.22	0.28±0.04	19±2	0.08±0.01	21.53±2.71
BM13	1.68±0.10	4.16±0.35	0.32±0.05	9±1	0.13±0.03	18.68±0.01
BM14	1.31±0.06	3.37±0.60	0.25±0.02	17±3	0.10±0.01	23.64±3.06
BM15	1.22±0.11	3.05±0.24	0.25±0.02	8±1	0.06±0.01	10.45±3.28
BM16	1.16±0.08	4.16±0.12	0.24±0.02	16±4	0.09±0.01	14.47±2.37
BM17	1.42±0.10	3.11±0.24	0.27±0.02	14±1	0.07±0.01	22.78±2.82
BM18	1.56±0.14	3.63±0.17	0.25±0.05	12±1	0.11±0.02	14.35±0.60
BM19	1.12±0.18	3.27±0.35	0.24±0.02	15±1	0.07±0.01	19.07±0.18

*BM: Black Myrtle

Table 4. Total phenolic (TPC), flavonoid (TFC) content and antioxidant activitiy (AA) of fruit and leaf (±SD).

Genotype	TPC of leaf (mg GAE.g^{-1})	TFC of leaf (mg CTE.g^{-1})	AA of leaf extract (EC50, µl)	TPC of fruit (mg GAE.g^{-1})	TFC of fruit (mg CTE.g^{-1})	AA of fruit extract (EC50, µl)
BM1*	65.03±2.32	3.25±0.11	0.30±0.02	21.60±0.23	1.30±0.01	0.96±0.08
BM2	72.84±6.67	3.79±0.26	0.32±0.03	13.17±1.01	1.04±0.04	1.12±0.14
BM3	50.33±3.33	2.53±0.08	0.44±0.02	23.78±3.59	1.31±0.06	0.83±0.02
BM4	74.94±3.20	3.80±0.05	0.39±0.01	18.81±2.48	0.71±0.10	1.05±0.11
BM5	60.96±8.62	3.33±0.23	0.29±0.01	17.96±1.43	1.19±0.07	1.28±0.11
BM6	54.72±0.60	2.97±0.15	0.39±0.01	22.62±0.24	1.39±0.08	0.94±0.11
BM7	62.59±3.95	3.47±0.12	0.33±0.01	27.64±1.43	1.72±0.03	0.86±0.18
BM8	74.87±2.07	4.11±0.03	0.24±0.04	30.08±1.40	2.57±0.02	0.69±0.02
BM9	77.09±3.79	4.34±0.09	0.33±0.07	30.58±2.37	2.59±0.06	0.80±0.17
BM10	71.79±1.41	3.70±0.08	0.33±0.03	23.39±0.76	1.62±0.05	1.10±0.05
BM11	61.80±6.48	3.20±0.67	0.46±0.02	21.23±1.95	1.25±0.06	1.47±0.01
BM12	68.94±1.53	3.87±0.04	0.35±0.06	22.94±1.62	1.69±0.06	1.23±0.06
BM13	63.34±7.27	3.66±0.07	0.39±0.08	35.01±1.77	2.12±0.06	0.68±0.13
BM14	64.80±6.28	3.49±0.26	0.37±0.01	23.98±1.04	1.22±0.04	1.21±0.03
BM15	90.14±3.03	6.38±0.01	0.19±0.02	27.01±0.37	1.69±0.02	0.66±0.03
BM16	92.12±2.68	5.53±0.32	0.13±0.03	21.42±2.42	1.15±0.07	0.95±0.04
BM17	76.62±5.96	4.16±0.05	0.14±0.03	32.69±2.68	2.06±0.05	0.60±0.08
BM18	64.24±6.18	2.88±0.60	0.35±0.10	25.47±1.40	1.74±0.05	0.91±0.04
BM19	64.70±0.96	4.07±0.08	0.26±0.01	39.16±0.92	3.42±0.13	0.45±0.03
Average	69.05	3.82	0.32	25.19	1.67	0.71

*BM: Black Myrtle

substance (r = -0.73817) in the leaves were found to be significant.

A total of 25.19 mg GAE g^{-1} of phenolic compounds were found in myrtle fruits. The highest total phenolic content was found in the fruits of BM19 genotype collected from Kaş with 39.16 mg GAE g^{-1}, while the lowest amount was found in the fruits of the BM2 genotype from Gazipaşa with 13.17 mg GAE g^{-1}.

The total amount of flavonoids in the fruits of myrtle was found to be 1.67 mg CTE g^{-1} on average. The highest amount of flavonoid was found in BM19 fruits with 3.42 mg CTE g^{-1} and the lowest amount was found in BM4 genotype with 0.71 mg CTE g^{-1}.

The average antioxidant activity value of the fruits was found to be 0.71 µl. The highest activity was found in the BM19 genotype (0.45 µl) which has the highest amount of phenolic and flavonoid substances, while the lowest amount was found in BM11 fruits (1.47). Barboni et al. (2010) found that the total phenolic content of the fruits of myrtle collected from different ecologies in the Corsica island ranged between 22.20 and 67.43 mg GAE g^{-1}. Amensour et al. (2010) prepared extracts using different solvents from the leaves and fruits of the myrtles they collected from Morocco and found the total amount of phenolic substance in the methanol extract to be 31.25 and 14.68 mg GAE g^{-1} for leaves and fruit respectively. Reynertson et al. (2008) reported that the total phenolic content of the leaves of the myrtle fruit ranged between 3.57 and 101 mg g-^{1}. According to our findings, it is seen that the total phenolic substance in myrtle leaves range between 50.33 and 92.12 mg GAE g^{-1} and that in the fruits

between 13.17 and 39.16 mg GAE g^{-1}. Kanoun et al. (2014) found that the total amount of phenol and flavonoids in the leaves of the myrtle they collected from Algeria were 119.23 mg GAE g^{-1} and 6.56 mg CTE g^{-1} respectively, while the amounts in the fruits were 70.26 mg GAE g^{-1} and 3.87 mg CTE g^{-1} respectively. The amounts of phenolic and flavonoid substances in the myrtle fruits and leaves are seen to be highly variable. Given the fact that the genotype, the climate and soil conditions of the sampled region and year affects the amount of phenolic substance (Revilla et al., 1997; Ribereau-Gayon et al., 2000; Montealegre et al., 2006). This variation can be considered normal.

Myrtle leaves were found to contain more phenolic and flavonoid substances, and higher antioxidant activity as a consequence of this, when compared with the fruits. In this regard, our findings comply with those of Kanoun et al. (2014) and Amensour et al. (2010).

3.3. Phenolic compounds of myrtle fruits and leaves

The majority of the phenolic compounds found in the fruits of myrtle are composed of flavonol group compounds. The amounts of the flavonol-group components examined are given in Table 5 and 6 for the fruits and leaves respectively. When Table 5 is examined, average amounts of myricetin, myricetin-3-glucoside, myricitrin, quercetin-3-B-D glucoside and quercitrin in the fruits were found to be 13.22, 134.91, 478.79, 829.48 and 50.38 mg kg^{-1} respectively. No myricetin was detected in the BM13 genotype. The highest amount of myricetin (32.85 mg kg^{-1}) was found in fruits of the SM16 genotype. The lowest amount of myricetin-3-glucoside was found in SM4 (34.87 mg kg^{-1}) whereas the highest was found in BM9 fruits with 233.17 mg kg^{-1}. The lowest amount of myricitrin was found in the fruits of SM4 with 133.22 mg kg^{-1} whereas the highest was found in the fruits of BM17 with 664.38 mg kg^{-1}. The amount of quercetin-3-B-D glucoside in the fruits is rather high and the highest amount of the substance was found in the BM17 genotype with 1117.79 mg kg^{-1}. The amount of quercitrin was found to be higher in the fruits of BM13 with 83.62 mg kg^{-1}. Our findings comply with those of Montoro et al. (2016). The researchers reported that the main polyphenols in the myrtle fruits and extracts were flavonoids and anthocyanins and found that amounts of myricetin-3-*O*-galactoside, myricetin-3-*O*-rhamnoside (myricitrin) and quercetin-3-*O*-glucoside among the flavonoids were higher.

When Table 6 is examined, the average amount of myricetin in the leaf was found to be 3.75 mg kg^{-1}, myricetin-3-glucoside to be 220.79 mg kg^{-1}, myricitrin to be 763.93 mg kg^{-1}, quercetin-3-B-D glucoside to be 1177.12 mg kg^{-1}, and quercitrin to be 31.07 mg kg^{-1}. While the highest amount of myricetin was mostly found in the leaves of the SM1 genotype (12.08 mg kg^{-1}), no myricetin could be detected in the leaves of BM8. The lowest amount of myricetin-3-glucoside was found in the leaves of BM13 with 142.10 mg kg^{-1} whereas the highest amount was found in BM9 with 338.29 mg kg^{-1}. The highest amount of myricitrin in the leaves was found in BM9 with 965.70 mg kg-1. As in the fruit, the most common compound in the leaf was quercetin-3-B-D glucoside, and the highest amount was detected in the leaves of the BM9 genotype with 1662.81 mg kg^{-1}. The highest amount of quercitrin was found in the BM19 genotype with 49.30 mg kg^{-1}.

Romani et al. (1999) found that myrtle leaves contain low amounts of quercetin derivatives

Table 5. Phenolic compounds of myrtle fruits (mg kg^{-1}) (±SD).

Genotype	Myricetin	Myricetin-3-glucoside	Myricitrin	Quercetin-3-B-D glucoside	Quercitrin	Total
BM1*	4.94±0.04	176.85±10.73	471.61±7.92	763.75±55.13	51.69±1.57	1468.84
BM2	3.55±0.39	69.95±1.18	506.32±9.59	908.01±4.58	79.92±3.91	1567.75
BM3	15.77±2.02	117.53±4.24	405.08±22.08	692.96±53.80	41.38±3.51	1273.17
BM4	1.47±0.25	34.87±3.77	133.22±14.28	231.39±25.31	24.98±1.61	425.92
BM5	28.27±1.00	98.88±3.53	618.31±19.22	1041.40±46.63	54.05±3.79	1840.91
BM6	6.20±0.15	130.06±3.15	523.13±4.10	915.36±4.35	44.21±2.01	1618.96
BM7	26.28±4.88	218.04±5.42	436.86±6.00	770.23±38.31	34.33±3.88	1485.74
BM8	3.25±0.54	182.12±5.09	422.96±12.71	771.93±28.45	56.84±1.23	1437.09
BM9	22.12±2.92	233.17±1.35	500.12±4.04	922.04±15.58	45.05±2.40	1722.49
BM10	6.75±0.15	119.29±13.10	540.43±5.14	921.42±33.94	49.40±2.16	1637.30
BM11	20.40±2.74	194.90±2.04	362.95±6.64	645.96±36.42	26.17±1.24	1250.38
BM12	12.11±0.65	127.51±5.22	581.63±1.96	1017.57±10.24	41.14±1.44	1779.96
BM13	nd‡	100.62±5.09	615.97±35.10	949.14±53.39	83.62±3.93	1749.35
BM14	14.67±0.33	66.98±1.26	474.08±28.40	829.15±34.93	47.43±0.02	1432.32
BM15	8.99±0.69	155.22±7.19	358.11±7.04	672.24±1.84	51.44±1.73	1246.00
BM16	32.85±2.32	119.65±7.26	415.50±5.37	724.51±13.41	42.09±1.38	1334.60
BM17	30.60±1.51	224.58±10.89	664.38±25.46	1163.79±23.74	37.74±5.26	2122.09
BM18	5.82±0.54	95.19±4.19	534.29±27.61	1090.56±40.32	72.40±2.21	1798.26
BM19	7.13±0.18	97.97±2.41	532.07±31.04	727.64±18.82	72.97±3.75	1437.79
Average	13.22	134.91	478.79	829.48	50.38	

*BM: Black Myrtle, nd: not determined

Table 6. Phenolic compounds of myrtle leaves (mg kg^{-1}) (±SD).

Genotype	Myricetin	Myricetin-3-glucoside	Myricitrin	Quercetin-3-B-D glucoside	Quercitrin	Total
BM1*	12.08±0.71	225.28±4.22	756.56±17.66	1109.11±57.26	32.46±2.59	2135.49
BM2	3.53±0.04	146.99±9.72	838.10±62.01	1368.34±5.97	48.75±0.76	2405.71
BM3	3.39±0.55	167.07±2.76	657.95±13.93	1080.52±19.10	21.39±0.86	1930.32
BM4	2.96±0.02	222.42±1.04	667.09±9.11	1093.89±35.28	19.30±0.26	2005.66
BM5	2.43±0.68	223.86±0.79	882.92±35.03	942.05±20.14	41.87±2.14	2093.14
BM6	2.14±0.20	206.69±32.04	624.18±19.73	1084.46±31.63	29.28±2.14	1946.76
BM7	3.38±0.54	193.70±0.48	679.93±4.59	1132.69±6.30	19.39±1.98	2029.09
BM8	nd‡	273.11±5.22	772.44±16.63	1126.97±138.11	20.38±0.41	2192.90
BM9	5.75±0.36	338.29±12.56	965.70±15.96	1662.81±24.80	28.68±1.20	3001.23
BM10	2.26±0.37	155.46±13.55	683.14±10.89	1168.34±8.62	31.05±1.49	2040.27
BM11	6.01±0.48	269.45±4.01	661.38±4.68	1173.14±24.81	22.25±1.11	2132.24
BM12	0.88±0.02	237.70±3.10	895.03±14.27	1098.14±1.32	24.41±0.17	2256.16
BM13	2.10±0.22	142.10±0.41	762.13±53.37	1298.73±32.18	29.88±1.91	2234.93
BM14	0.93±0.10	161.53±2.47	661.90±7.82	1145.35±76.89	32.44±0.95	2002.14
BM15	0.45±0.07	243.18±0.59	826.23±27.05	1049.82±58.22	33.50±3.28	2153.17
BM16	8.05±0.58	231.13±2.13	843.65±8.30	1349.03±28.67	29.32±2.04	2461.17
BM17	5.31±0.33	255.38±5.14	774.81±8.97	1394.11±8.84	39.68±1.27	2469.29
BM18	1.90±0.14	197.22±15.88	639.59±32.81	1231.22±73.15	37.04±2.03	2106.96
BM19	7.59±0.58	304.43±24.06	922.00±50.71	856.61±70.14	49.30±1.57	2139.93
Average	3.75	220.79	763.93	1177.12	31.07	

*BM: Black Myrtle, nd: not determined

(quercetin-3-*O*-galactoside and quercetin-3-*O*-rhamnoside (quercitrin)) and high amounts of myricetin derivatives (myricetin-3-*O*-rhamnoside (myricitrin)). In our study, the amount of quercetin derivatives was found to be higher. In this study, the amounts of myricetin and quercetin glucoside derivatives were detected. This difference may have occurred because the amount of galactoside derivatives could not be detected.

4. Conclusions

• Phenolic substance was found to be high in the leaves of the samples collected from Kumluca (BM15 and BM16) and in the fruits of those collected from Kemer (BM13) and Kaş (BM19).
• High antioxidant activity was detected in the leaf sample BM16 collected from Kumluca (0.13 µl) and the fruit sample (BM19) collected from Kaş (0.45 µl).
• The seed / fruit ratio ranged between 10.45% and 23.66%. This rate was 19.07% in BM19 genotype while it was 10.45% and 18.68% in BM15 and BM13 genotypes. Although the number of seeds was less in these two genotypes in comparison to the BM19 genotype, their antioxidant activity values (0.66 and 0.68 µl) were found to be higher.
• The correlations between total phenolic substance and antioxidant activity (r = -0.74526 for leaves, r = -0.78952 for fruit) and between total amount of flavonoid substance and antioxidant activity (r = -0.73817 for leaves, r = -0.71061 for fruit) were found to be significant.
• Considerable differences between genotypes were observed in terms of amounts of phenolic compounds in both leaves and fruits. The highest amount of flavonol-group compounds examined in myrtle fruits and leaves were found to be quercetin-3-B-D glucoside, followed by myricitrin, myricetin-3-glucoside, quercitrin, and myricitrin.
• The phenolic flavonoid substance contents and antioxidant capacities of the fruits and leaves of the myrtle plant growing in the natural Antalya flora has been revealed and the promising genotypes have been identified. These genotypes are important in providing material for future research.
• The amount of phenol and tannin in the fruit is largely due to the seeds and is directly related to the antioxidant activity. However, the selection of dark-colored and large types with fruit flesh that contain high amounts of phenol will allow the emergence of new genotypes with little acerbity without reducing the antioxidant effect in black myrtle.
• It can be recommended that the genotypes with high values in terms of the measured parameters are utilized as natural antioxidant sources in the food and pharmaceutical industry because they contain substances that are both beneficial for human health and are natural antioxidants. There is a transition from synthetic products to natural products for a healthier life in world societies. Therefore, utilization of the fruit species known as the most important natural antioxidant sources is gaining importance.

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References

Akgül, A. & Bayrak, A. (1989). Essential oil content and composition of myrtle (*Myrtus communis* L.) leaves. *Turkish Journal of Agricultural and Natural Sciences*, 13:143-147.
Amensour, M., Sendra, E., Abrini, J., Perez-Alvarez, J.A., & Fernandez-Lopez, J. (2010). Antioxidant activity

and total phenolic compounds of myrtle extracts. *Journal of Food,* 8:95-101.

Barboni, T., Cannac, M., Massi, L., Perez-Ramirez, Y., & Chiaramonti, N. (2010). Variability of polyphenol compounds in *Myrtus communis* L. (Myrtaceae) berries from Corsica. *Molecules*, 15:7849-7860.

Cai, Y., Luo, Q., Sun, M., & Corke, H. (2004). Antioxidant activity and phenolic compounds of 112 traditional chinese medicinal plants associated with anticancer. *Life Sciences*, 74:2157–2184.

Cemeroğlu, B. (2010). Gıda analizleri. *Gıda Teknolojisi Derneği Yayınları*, No: 34, 657 s, Ankara (in Turkish).

Düzgüneş, O., Kesici, T., Kavuncu, O., & Gürbüz, F. (1987). Araştırma ve deneme metotları (İstatistik Metotları II). *Ankara Üniversitesi Ziraat Fakültesi Yayınları*, Yayın No:1021, 381 ss. Ankara (in Turkish).

Fadda, A., & Mulas, M. (2010). Chemical changes during myrtle (*Myrtus communis* L.) fruit development and ripening. *Scientia Horticulturae*, 125:477-485.

Fischer, U.A., Carle, R., & Kammerer, D.R. (2011). Identification and quantification of phenolic compounds from pomegranate (*Punica granatum* L.) peel, mesocarp, aril and differently produced juices by HPLC-DAD-ESI/MS (n). *Food Chemistry*, 127:807-821.

İlçim, A., Dığrak, M., & Bağcı, E. (1998). The investigation of antimicrobial effect of some plant extract. *Turkish Journal of Biology*, 22:119-125.

Jamoussi, B., Romdhane, M., Abderraba, A., Ben Hassine, B., & El Gadri, A. (2005). Effect of harvest time on the yield and composition of Tunisian myrtle oils. *Flavour and Fragrance Journal*, 20:274–277,

Kanoun, K., Belyagoubi-Benhammou, N., Ghembaza, N., & Atik Bekkara, F. (2014). Comparative studies on antioxidant activities of extracts from the leaf, stem and berry of *Myrtus communis* L. *International Food Research Journal,* 21 1957-1962.

Karadeniz, F., Burdurlu, H.S., Koca, N., & Soyer, Y. (2005). Antioxidant activity of selected fruits and vegetables grown in Turkey. *Turkish Journal of Agriculture and Forestry,* 29:297-303.

Kaya, B. & Aladağ, C. (2009). Analyse of distribution areas in Turkey and ecologic characteristics of maquis and garriques communities. *Selçuk University Social Sciences Institute Journal*, 22:67-80.

Martin, T., Rubio, B., Villaescusa, L., Fernández, L., & Díaz, A.M. (1999). Polyphenolic compounds from pericarps of *Myrtus communis*. *Pharmaceutical Biology*, 37:28–31.

Montealegre, R.R., Peces, R.R., Vozmediano, J.L.C., Gascuena, J.M., & Romero, E.G. (2006). Phenolic compounds in skins and seeds of ten grape vitis vinifera varieties grown in a warm climate. *Journal of Food Composition and Analysis*, 19:687-693.

Montoro, P., Tuberoso, C.I.G., Piacente, S., Perrone, A., De Feo, V., Cabras, P., & Pizza, C. (2006). Stability and antioxidant activity of polyphenols in extracts of Myrtus communis L. berries used for the preparation of myrtle liqueur. *Journal of Pharmaceutical and Biomedical Analysis*, 41:1614-1619.

Oğur, R. (1994). Mersin bitkisi (*Myrtus communis* L.) hakkında bir inceleme. *Çevre Dergisi*, 10:21-25 (in Turkish).

Ong, K.C. & Khoo, H.E. (1997). Biological effects of mirisetin. *Gen Pharmacology,* 29:121-126.

Özcan, F. (2009). Diyabete bağlı olarak geliştirilmiş nefropatili rat modelinde mirisetin ve mersin bitkisi ekstraktlarının böbrek dokusu üzerine etkisi. Msc Thesis, Akdeniz University, Antalya (in Turkish).

Revilla, E., Alonso, E., & Kovac, V. (1997). The content of catechins and procyanidins in grapes and wines as affected by agroecological factors and technological practices, *American Chemical Society*, 69–80, Washington, DC.

Reynertson, K.A., Yang, H. Jiang, B. Basile, J.M., & KennellY, E.J. (2008). Quantitative analysis of antiradical phenolic constituents from fourteen edible Myrtaceae fruits. *Food Chemistry*, 109:883–890.

Ribereau-Gayon, P., Glories, Y., Maujean, A., & Dubourdieau, D. (2000). Handbook of enology, Volume 2: The chemistry of wine and stabilization and treatments. John Wiley and Sons Ltd., England.

Romani, A., Pinelli, P., Mulinacci, N., Vincieri, F.F., & Tattini, M. (1999). Identification and quantitation of polyphenols in leaves of *Myrtus communis* L. *Chromatographia*, 49:17-20.

Romani, A., Coinu, R., Carta, S., Pinelli, P., Galardi, C., Vincieri, F.F., & Franconi, F. (2004). Evaluation of antioxidant effect of different extracts of *Myrtus communis* L. *Free Radical Research*, 38:97–103.

Spanos, G., & Wrolstad, R.E. (1990). Influence of processing and storage on the phenolic composition of Thompson seedless grape juice. *Journal of Agricultural and Food Chemistry*, 38:1565-1571.

Traveset, A., Riera, N., & Mas, R. (2001). Ecology of fruit-colour polymorphism in *Myrtus communis* and differential effects of birds and mammals on seed germination and seedling growth. *Journal of Ecology*, 89:749-760.

Tuberoso, C.I.G., Melis, M., Angioni, A., Pala, M., & Cabras, P. (2007). Myrtle hydroalcoholic extracts obtained from different selections of *Myrtus communis* L. *Food Chemistry*, 101:806-811.

Tzeng, S.H., Ko, W.C., Ko, F.N., & Teng, C.M. (1991). Inhibition of platelet aggregation by some flavonoids. *Thrombosis Research,* 64:91-100.

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RESEARCH PAPER

The Performance of Some Tomato Pure Lines under Cold Stress in the Vegetative and Generative Stage

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Abstract

Low temperature stress decreases yield and quality of tomato in greenhouse conditions. For successful tomato cultivation under the cold stress, cultivars performances are extremely important both vegetative and reproductive growth stage. In this study, 20 tomato pure lines and 3 commercial cultivars (Cigdem F_1, Anit F_1 and Bestona F_1) and also *Solanum hirsutum* (LA 1777) known as tolerant genotypes were evaluated at vegetative and reproductive stage. The studies were conducted under both the cold stress in growth chamber and the optimal temperature condition (control) in the greenhouse. They were evaluated by measuring malondialdehyde (MDA), electrolyte leakage (EL) and dry matter yield (DM) at vegetative stage. The results showed that EL rate and MDA content increased while DM decreased under the cold stress when compared with leaves of plants grown at optimal temperature. In reproductive stage, pollen viability and pollen germination were evaluated under both cold stress and control conditions for all genotypes. All the sensitive genotypes exhibited low pollen viability and pollen germination. Consequently, three pure lines were identified with low-temperature tolerant in vegetative and reproductive growth stage.

1. Introduction

Tomato is one of the most economically important vegetable crops after potatoes (Ronga et al., 2018). Türkiye ranks fourth in world tomato production after China, India and the USA. World tomato production is 180.7 million tons, 7% of which is produced in Türkiye. (FAO, 2021).

Both chilling (<20°C) stress and freezing (<0°C) stress are called low temperature stress (Ma et al., 2018; Ражаметов et al., 2020). Low temperature stress is one of the most abiotic stress factors that reduce the productivity of crops (Duan et al., 2012), affected about 24.6% of the entire area of the world's land (Peel et al., 2007). Cultivated tomato is a cold sensitive crop; crop growth and development are severely damaged below 12°C (Elizondo and Oyanedel, 2010; Ronga et al., 2018). Duration of exposure is also important as well as temperature for the severity of damage (Elizondo and Oyanedel, 2010; Barrero-Gil et al., 2016). Both of them adversely affect growth and productivity in tomato plants depending on the severity of the low temperature stress (Gökmen, 2006; Atayee and Noori, 2020; Ражаметов et al., 2020). When the tomato plants are exposed to low temperatures, injury symptoms begins initially at the vegetative growth stage. The most noticeable injury could be observed in the vegetative stage such as stunted seedlings, leaf-hypocotyl wilting, leaf chlorosis and local necrosis (death of tissue) (Cao et al., 2015; Atayee and Noori, 2020). On the other hand, low temperature stress at the reproductive stage of plants causes poor pollen viability, weak fruit set,

poor fruit quality, which result in loss of crop yield. Further, dry matter production (DM) is also widely used parameter to select tolerant plants in the cold stress studies (Foolad et al., 2000; Foolad and Lin, 2001; Gökmen, 2006). Likewise, cold stress gives rise to membrane damage and increases electrolyte leakage. Electrolyte leakage (EL) and lipid peroxidation (LPO) are an important indicator of plant membrane damage level under the cold stress (Duan et al., 2012; Malekzadeh et al., 2014). Most of the previous investigations focused only on individual stages. A few studies that included more than one stage, however, evaluated only a few genotypes, thereby there is no specific conclusions could be guide about the cold stress at different stages. Therefore, the aim of this study is to determine the tolerant pure lines in tomato during vegetative and reproductive growth under the cold stress conditions.

2. Materials and Methods

2.1. Plant materials, growing and stress conditions

In this current study, cold stress studies were conducted with 20 tomato genotypes belonging to Batı Akdeniz Agricultural Research Institute (BATEM) tomato gene pool (over F_6 generation) and three commercial varieties (Cigdem, Anit and Bestona F_1) wild type LA 1777 (*S. hirsutum)* were used as control genotypes. The experiment was governed five replication and each replication consisted of five seedlings per replicate at the early seedling stage, in the greenhouse. Seedlings at 2-3 true leaf stage were grown at the optimal temperature and then transferred to plastic pot. After transplanting the seedlings, they were irrigated with Hoagland nutrient solution and grown up to 3-4 true leaf stage at the optimal temperature. Seedlings of genotypes were exposed to chilling treatment at 5±1°C with a 12 h photoperiod (day/night) and light intesity (200 µmol m^{-2} sec^{-1}) for 5 days in growth chamber and grown at the optimal temperature (control) in the greenhouse. For analysis, samples were collected from the third-fourth leaf in the seedling at both cold stress (T) and control condition (CC) in the study.

2.2. Vegetative growth stage

2.2.1. Measurement of malondialdehyde (MDA)

The MDA content was determined by the reaction of thiobarbituric acid (TBA), as described by Sayyari (2012). 1 g of leaf samples were taken and added 10 ml of 0.1% trichloroacetic acid (TCA), and then centrifuged at 15000 rpm for 5 minutes, further 4 ml of 0.5% thiobarbituric acid (TBA) was added. The mixture obtained kept in the bath at 95°C hot water for 30 minutes and then quickly cooled in an ice bath. Afterwards, absorbance values were read at A532 and A600 nm in the spectrophotometer. The values obtained were calculated with Lipid peroxidation = (A532-A600) x extract volume (ml) / (155mM / cm x sample amount (mg).

2.2.2. Measurement of electrolyte leakage (EL)

The structure and function of cell membranes are damaged under the cold stress. Thus, EL increase in chilling stress. EL was used to evaluate membrane permeability. EL was measured using an electrical conductivity meter, according to the methods by Lutts et al. (1996). In the laboratory, 10 discs of 1 cm diameter taken from tomato leaf samples were washed with pure water and placed in brown glass bottles. After adding 20 ml of pure water to the samples, they were shaken for 24 hours and the EC_1 values were read by pouring the solutions into tubes. The same samples were autoclaved at 120°C for 20 minutes and their EC_2 values were read after the samples reached room temperature. The EL calculated as $EC_1 / EC_2 \times 100$ formula was used to calculate the cell membrane damage of the samples.

2.2.3. Measurement of dry matter yield (DM)

Seedling of the genotypes were individually harvested for shoot (leaf+stem) both control and chilling treatments. Genotype's shoot were dried in an oven at 65°C for 72 h and weighed (± 0.1 g) and dry weight (DM) of individual plants determined (Gökmen, 2006). For each genotype, vegetative growth was defined as ratio of dry weight (DW) under cold stress to under control condition.

2.3. Reproductive stage

After sowing the seeds of the materials, the seedlings that reached the stage of 2-3 true leaves were transplanted to be used in reproductive testing with 5 replications, one plant per pot. Plants were grown at in the greenhouse until the flowering stage and then they were taken to growth chamber 24 h before anthesis after 72 h exposed to cold stress in growth chamber, samples of pollen were collected from each replication of all genotypes. Pollen viability and germination percentages were determined both under cold stress and under control condition in genotypes flowering in pots.

2.3.1. Pollen viability

Viability levels of flower powders were tested with 2,3,5 Triphenyl Tetrazolium Chlorid (TTC), as described by Boyaci et al. (2009). In plants grown in the control greenhouse and growth chamber, the pollens of the flowers that bloom in the first cluster were taken in 3 replications 3 readings were made in each repetition.

2.3.2. Pollen germination ability

The method was used to determine the pollen germination ability (Boyacı et al., 2009). The pollens derived from tomato genotypes flowering in the cold room and control greenhouse were planted in 1% agar + 12% sugar + 300 ppm H_3BO_3 + 300 ppm Ca $(NO_3)_2$ germination medium and kept at 25°C for 20 hours then counted under the light microscope. In the pollen germination test, two petri dishes were prepared for each genotype, and four randomly selected areas were counted and pollen germination percentages were determined.

2.3.3. Cold tolerance index

Cold tolerance index (%) for all traits was calculated as under cold stress (T) and as the percentage of the control condition (CC) for all traits. To determine the cold tolerance genotypes, cold tolerance index (TI) was calculated according to Funatsuki et al. (2005) as follows:

$$TI = \left[\frac{T}{CC}\right] \times 100$$

where; TI: Cold tolerance index; T: Trait value in cold stress condition; CC: Trait value in control condition.

Genotypes whose TI values were close to 100 or 1 were considered tolerant in all traits.

2.4. Statistical analysis

The genotypes means and standart deviations were analyzed for samples within each replicate for all parameters. All statistical analyses were performed with JUMP (version 8.0). Cold tolerance index (TI) among the genotypes in each parameter were subjected to the analyses of variance (ANOVA) and compared among genotypes using LSD multiple range tests at the P<0.05 level. The data shown are means values ± TI for all parameters. Levels of significance are represented by at P<0.05 (*), P<0.01 (**) and P<0.001 (**).

3. Results and Discussion

3.1. Vegetative stage

3.1.1. Measurement of electrolyte leakage (EL)

The amount of EL in tomato seedlings increased under cold stress that we looked for lower EL ratio among the genotypes. (Table 1). To determine the cold tolerance genotypes, we used tolerance index (TI_{EL}) with the electrolyte leakage (EL) ratio. The data statistical anaysis showed that the ratio of EL was significantly increased (Table 1; P<0.001) treated genotypes under the cold stress.

Genotypes were selected as tolerant with the least diffrence of TI_{EL} values in the applied genotypes compared to the control group TI_{EL} values. Moreover, the value of TI_{EL} measured in G5, G8 and G11 were much lower than in all other cultivars. For TI_{EL} can be showed that G5, G8 and G11 were highly tolerant genotypes.

3.1.2. Measurement of dry matter yield (DM)

While the growth of all genotypes decreased in response to cold stress, there was significant genotypic variations. TI_{DM} showed significant (p<0.01) differences between genotypes. Genotypes No.8 (G8) grew as fast as commercial hybrid-1 (CV-1) and commercial hybrid-2 (CV-2) under cold stress conditions. Besides, the value of TI G8 (0.88) and G1 (0.87) showed greater plant vigour as high as commercial varieties among the genotypes (Table 1).

3.1.3. Measurement of malondialdehyde (MDA)

The concentration of MDA was used an indicator of lipid peroxidation (LPO) in plant cells and increases in chilling stress. Therefore, we observed for tolerant genotypes which had lowest MDA content. Furthermore, there were significant (P<0.001) differences among the genotypes MDA tolerance index (TI_{MDA}). TI_{MDA} of the genotypes ranged between 1.21 (highly tolerant) to 1.76 (highly sensitive) with a mean of 1.53. Consequently, for TI_{MDA} indicated that not only WT (wild type) but also G8 and G5 were tolerant genotypes both under control and cold stress (Table 1).

Levitt (1980) defined the electrolyte leakage (EL) method as one of the most reliable protocols for evaluating the chilling and freezing tolerance in plants. To measure membrane injury, electrolyte leakage (EL) is an important indicator of membrane damage. Therefore, we evaluated EL analysis in twenty genotypes with one wild type (WT- LA 1777) and three commercial cultivars (CV) under cold stress. G5 and G8 were more cold tolerance (CT) than the other genotypes except for WT and CV under cold stress. Among the genotypes, differences and CTI can be suitable indicator to select the tolerant and sensitive genotype. Similar finding reported Cao et al. (2015) after the cold treatment, exhibited lowest level of EL among the genotypes, these lines found higher cold tolerance than others. In a similar study, Ma et al. (2018), stated that EL cell membrane permeability increased with cold stress in tomato. Xia et al. (2018), they exposed tomato genotypes to cold stress at 4°C for 3 days. They found that in wild types mutant types and also in transgenic tomato genotypes EC increased with cold in all genotypes. Zhao et al. (2009), has found that chilling susceptibility tomato cultivars had higher differences the coefficient between chilling injury and electrolyte leakage under the cold stress. The

Table 1. Means of electrolyte leakage (EL), dry matter yield (DM) and malondialdehyde (MDA) value under the control condition (CC) and cold stress(T), differences (%) and cold tolerance index (TI) for all tomato genotypes.

Genotypes	Electrolyte leakage (EL)				Dry matter yield (DM)				Malondialdehyde (MDA)			
	CC	T	Diff.(%)	TI	CC	T	Diff.(%)	TI	CC	T	Diff.(%)	TI
G1	37±2.3	46±1.5	24.3	1.24	3.0±0.5	2.6±0.4	-13.3	0.87	56±3	91±4	62.5	1.63
G2	47±2.1	60±2.1	27.7	1.28	2.0±0.6	1.5±0.5	-25.0	0.75	52±3	79±5	51.9	1.52
G3	41±1.0	57±2.0	39.0	1.39	2.0±0.4	1.6±0.4	-20.0	0.8	49±4	80±3	63.3	1.63
G4	53±2.1	69±2.1	30.2	1.30	3.0±0.7	2.3±0.6	-23.3	0.77	47±4	69±5	46.8	1.47
G5	52±2.5	62±2.5	19.2	1.19	3.0±0.6	2.5±0.5	-16.7	0.83	42±3	55±5	31.0	1.31
G6	46±1.5	64±1.7	39.1	1.39	3.0±0.6	2.3±0.2	-23.3	0.77	47±2	72±4	53.2	1.53
G7	50±2.0	67±2.5	34.0	1.26	4.0±0.6	3.1±0.4	-22.5	0.78	48±5	64±4	33.3	1.33
G8	43±2.5	52±2.1	20.9	1.21	4.0±0.8	3.5±0.5	-12.5	0.88	43±4	55±3	27.9	1.28
G9	47±1.5	59±2.3	25.5	1.26	2.0±0.6	1.4±0.7	-30.0	0.7	53±3	86±4	62.3	1.62
G10	48±2.1	61±2.1	27.1	1.27	3.0±0.6	2.2±0.4	-26.7	0.73	54±2	86±4	59.3	1.59
G11	48±2.1	59±1.7	22.9	1.23	2.3±0.8	1.8±0.4	-21.7	0.78	63±2	96±3	52.4	1.52
G12	42±2.3	58±2.1	38.1	1.38	3.1±0.7	2.3±0.4	-25.8	0.74	55±3	88±5	60.0	1.6
G13	46±2.1	68±2.5	47.8	1.48	3.5±0.6	2.3±0.6	-34.3	0.66	46±3	81±4	76.1	1.76
G14	50±1.7	69±2.6	38.0	1.38	2.6±0.7	1.9±0.6	-26.9	0.73	55±4	96±3	74.5	1.75
G15	41±2.1	52±1.5	26.8	1.27	2.9±0.6	2.4±0.4	-17.2	0.83	54±2	79±3	46.3	1.59
G16	38±2.5	52±2.1	36.8	1.37	3.3±0.8	2.5±0.6	-24.2	0.76	51±2	77±4	51.0	1.55
G17	37±2.0	51±2.6	37.8	1.38	4.2±0.5	3.1±0.3	-26.2	0.74	58±3	89±4	53.4	1.53
G18	48±2.5	62±2.5	29.2	1.29	2.9±0.8	2.1±0.5	-27.6	0.72	44±2	71±3	61.4	1.61
G19	49±2.3	61±1.5	24.5	1.24	3.6±0.5	2.7±0.3	-25.0	0.75	51±3	80±3	56.9	1.57
G20	49±1.5	66±2.3	34.7	1.35	2.7±0.8	2.0±0.5	-25.9	0.74	59±2	91±4	54.2	1.54
WT	45±1.5	59±2.0	31.1	1.31	3.0±0.9	2.6±0.6	-13.3	0.87	34±2	41±2	20.6	1.21
Com-1	43±2.1	53±2.5	23.3	1.26	2.0±0.5	1.8±0.3	-10.0	0.9	45±2	69±4	53.3	1.53
Com-2	42±2.5	51±2.6	21.4	1.28	4.0±0.6	3.5±0.4	-12.5	0.88	48±3	70±3	45.8	1.46
Com-3	40±2.6	53±2.1	32.5	1.33	3.4±0.7	2.8±0.6	-17.6	0.82	42±2	67±3	59.5	1.6
Means	45.1	58.8	30.5	1.31	3.0	2.4	-21.7	0.78	49.8	76.3	52.4	1.5
Significance	***[a]	***	***	***	***	***	***	***	***	***	***	***

[a] Levels of significance are represented by at *** $P<0.001$.

results of this study correspond to Caffagni et al. (2014), examined EL analysis of fourteen tomato genotypes at different temperatures (5, 3, and 1°C) and at five time points (2, 4, 8, 24, and 72 h). They reported that differences in cold tolerance between the accessions were the most apparent when the plants were exposed to 1°C for 24 h; the EL values ranged between 26.4 and 71.0 %.

Dry matter yields are one of the most important parameters in response to cold stress. Percentage of growth (TI_{DW}) both under nonstress (TIc) and stress condition (TI_S) is reliable indicator of stress tolerance (Foolad and Lin, 2001). When the cold tolerance index of the dry matter yields (TI_{DM}) of the genotypes were examined, it was determined that the G1 and G8 could be tolerant to cold stress. Dry matter yields of genotypes have decreased as a result of cold stress. Foolad and Lin (2000), reached similar conclusion when they evaluated the tomato accessions for DM and TI under control conditions and cold stress. Furthermore, they also determined that there was a positive correlation (r=0.68, $P<0.01$) between under cold stress and vegetative growth tolerance index. Similarly, Foolad and Lin (2001) evaluated the genetic control of cold tolerance (CT) in tomato *L. esculentum* breeding lines. They determined that there was a significant correlation between DM under cold stress (DMs) and TI among the lines. Similarly, Foolad and Lin (2001) measured plant vigour via germination tolerance index (TI_G) and vegetative growth index (TI_{VG}) under cold stress. When they compared both of them, they found that TI_G and TI_{VG} were good indicators of relative CT, but they may not be used alone good selection for cold tolerance breeding. Gökmen (2006) points out that different low temperature and duration applications can show significant differences in dry matter production in some genotypes depending on the time spent at low temperature and the degree of low temperature in tomato genotypes. Liu et al. (2018) reported that in their study on photoscent rates at different irrigation levels under low temperature conditions, dry matter yields decreased with cold application. The findings obtained in this study were consistent with these results. Considering the changing environmental

factors in dry matter yields, it shows that the genotypes with the highest dry matter yield can be low temperature tolerant.

Lipid peroxidation (MDA) is evaluated in studies of plant mechanisms and aceppted as an indicator in various stresses like as cold stress. Thus we evaluated with MDA content of the genotypes at vegetative growth stage. Compared with MDA content of tomato genotypes, it was found that the differences of MDA content increased in all genotypes because of cold treatment. But WT and G8 had lower difference rates of MDA content and smaller rising cold TI than the other genotypes. Duan et al. (2012), studied the contribution of thylakoid ascorbate peroxidase (tAPX) to protect the plant under cold stress in wild type (WT) and transgenic plants. They found that the lower level of MDA was measured in transgenic plants compared with WT plants after 12h cold treatment. And they also suggested that MDA and EL are good markers of the oxidative stress suffered by plants. Malekzadeh et al. (2014) used different concentration of Gamma-aminobutyric acid (GABA) in tomato seedling under cold stress. They found that under cold stress there was an increase in MDA content in tomato seedling. They suggested that applying GABA can protect tomato seedlings against cold stress. Similarly, Xia et al. (2017) in their study, They found that MDA content was lower transgenic (DWF:OX2) genotypes than in wild-type as well as mutant type. Similarly, Li et al. (2015) compared grafted and ungrafted plants under cold stress. They detected that MDA content was increased in both ungrafted and grafted plants in the first 24 h after treatment. Liu et al. (2018) reported that cold applications increased lipid peroxidation rates in their study on photosensitive rates at different irrigation levels under low temperature conditions.

Findings obtained were consistent with these results. It should be taken into account that genotypes with the least change in MDA ratio at low temperatures may be tolerant, but fluctuate according to genotypes.

3.2. Reproductive stage

3.2.1. Pollen viability

Due to cold stress, all genotypes pollen viability rates decreased. Statistically significant differences ($p < 0.01$) were found among group of within the tested genotypes. Pollen viability of the G8 and WT were higher than the others (Table 2).

Table 2. Means of pollen viability (PV) and pollen germination (PG) value under the control condition (CC) and cold stress (T), differences (%) and tolerance index (TI) for all tomato genotypes.

Genotypes	Pollen viability (PV)				Pollen germination (PG)			
	CC	T	Diff. (%)	TI	CC	T	Diff. (%)	TI
G1	91±5	79±7	-13.2	87.0	86±3	71±4	-17.4	82.7
G2	86±3	67±7	-22.1	78.0	90±3	70±8	-22.2	78.3
G3	93±4	77±6	-17.2	83.0	83±4	62±5	-25.3	75.3
G4	89±4	70±4	-21.3	79.0	91±3	73±6	-19.8	80.3
G5	91±6	69±8	-24.2	76.0	91±4	66±6	-27.5	76.3
G6	86±5	76±6	-11.6	88.0	89±2	68±6	-23.6	76.3
G7	91±4	70±7	-23.1	77.0	89±3	67±8	-24.7	75.3
G8	89±6	80±5	-10.1	90.0	93±4	75±6	-19.4	81.3
G9	94±4	75±6	-20.2	80.0	89±4	69±5	-22.5	78.3
G10	92±4	73±7	-20.7	79.0	92±2	76±5	-17.4	83.0
G11	90±4	75±5	-16.7	83.0	95±3	78±7	-17.9	82.3
G12	93±2	74±6	-20.4	80.0	89±4	69±6	-22.5	78.3
G13	92±4	70±5	-23.9	76.0	91±5	69±5	-24.2	76.3
G14	95±6	77±7	-18.9	81.0	88±4	69±6	-21.6	78.3
G15	85±5	61±7	-28.2	72.0	90±3	65±6	-27.8	72.0
G16	89±3	69±8	-22.5	78.0	93±3	66±5	-29.0	71.0
G17	90±3	65±5	-27.8	72.0	90±4	71±5	-21.1	79.3
G18	91±3	75±4	-17.6	82.0	93±4	70±6	-24.7	75.3
G19	89±4	63±5	-29.2	71.0	92±3	65±6	-29.3	71.3
G20	88±5	65±7	-26.1	74.0	88±4	67±7	-23.9	76.0
WT	94±2	86±4	-8.5	91.5	95±2	84±4	-11.6	88.3
Com-1	94±4	83±3	-11.7	88.3	95±3	82±4	-13.7	85.7
Com-2	92±6	82±3	-10.9	89.1	96±2	81±4	-15.6	83.7
Com-3	93±3	76±5	-18.3	82	92±3	79±5	-14.1	86.3
Means	90.7	73.2	-19.4	80.7	90.8	71.3	-21.5	78.8
Significance	**[a]	**	**	**	***	***	***	***

[a] Level of significance are represented by at ***P*<0.01 and ****P*<0.001.

3.2.2. Pollen germination

In all genotypes, pollen germination rates decreased during the cold stress. There were significant differences (P<0.001) among group of the genotypes, and so cold tolerance exists within the tested genotypes. Pollen germination of the WT and CV was higher than the others and they evaluated in the same group. On the other hand, G1, G11, G10 and G8 found much higher than the others (Table 2).

The pollen viability is the most important criteria at reproductive stage under the cold stress. When the plants were exposed to low temperature, pollen damage happened. Therefore it caused poor pollen viability. Our results indicated that there was decrease in polen viability under cold stress. Among the genoypes, two genotypes (G8 and G1) were classified high pollen viability under cold stress condition. Likewise, higher pollen viability rate were also detected in WT and commercial varieties (Com-1 and Com-2). Similar results were obtained by Picken (1984) who found that poor pollen viability was recorded at low temperatures. Domínguez et al. (2005) who investigated five populations pollen performance at low temperature. They found that there were no differences in pollen viability among the populations except for NNNC that showed a higher mean percentage of viability. Similar results were obtained by Maisonneuve et al. (1986) and Zamir and Gadish (1987).

Pollen germination is severely reduced at temperatures below 10°C. To assess pollen germination rate, whole genotypes screened under cold stress at reproductive stage. As expected, the genotypes showed lower percentage of pollen viability and percentage of pollen germination under cold stress. In our study, wild type (LA1227) and all the commercial varieties exhibited higher means of pollen germination than the other genotypes. Nevertheless, genotypes No. G11, G10, G8 and G1 showed better response among the genotypes except for WT and commercial varieties. Moreover, these genotypes had higher cold tolerance index than others. It was observed that pollen germination of all genotypes was affected by low temperature applications. Keleş (2006) also reported that the germination percentage may be effective in distinguishing genotypes from each other in pepper. The data we obtained in tomato were consistent with these results. It is thought that the germination percentage may be effective in distinguishing genotypes from each other. Some authors reported that low temperatures affected pollen viability and germination of sensitive genotypes compared to tolerant genotypes. Zamir and Gadish (1987), Mulcahy et al. (1996) and Domínguez et al. (2005) conducted their experiment in segregating populations by using pollen selection. Researchers also stated that pollen selection may determine both vegetative and reproductive stage to tolerance for cold stress.

As a result of all these evaluations, it indicates that genotypes determined as tolerants can not only create more dry matter under cold stress, but also show high fertilization and fruit set with a high rate of live pollen and germination.

4. Conclusion

As a result, cold stress tolerance of twenty genotypes was evaluated with different physiological parameters during vegetative growth and reproduction. These parameters (EL, MDA, DM, PV and PG) have been succesfully used to screen cold stress in tomatoes. Furthermore, results of all these parameters indicated that when breeding for improved tolerant genotypes, both stages are necessary for selecting cold tolerant genotypes in tomato. Besides, cold tolerance index could be effectively used for evaluating cold tolerance in tomatoes. G1, G5 and G8 genotypes could be tolerant to cold stress as shown by physiological parameters indicators EL, MDA, DM at vegetative groth stage and also PV and PG at reproductive stage among the genotypes. These genotypes will be valuable for breeding programs as sources of cold stress tolerance.

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References

Atayee, A.R., & Noori, M.S. (2020). Alleviation of cold stress in vegetable crops. *Journal of Scientific Agriculture*, 4:38-44.

Barrero-Gil, J., Huertas, R., Rambla, J.L., Granell, A., & Salinas, J. (2016). Tomato plants increase their tolerance to low temperature in a chilling acclimation process entailing comprehensive transcriptional and metabolic adjustments. *Plant, Cell & Environment*, 39:2303-2318.

Boyacı, H.F., Oğuz A., Ünlü, M., Eren, A., Topçu, V., Erkal, S. (2009). Relationship between some parameters of vegetative and generative developments of parthenocarp and non-parthenocarp eggplants (*Solanum melongena* L.). *Derim,* 26:28-39.

Cao, X., Jiang, F., Wang, X., Zang, Y., Wu, Z. (2015). Comprehensive evaluation and screening for chilling-tolerance in tomato lines at the seedling stage. *Euphytica*, 205:569-584.

Caffagni, A., Pecchioni, N., Francia, E., Pagani, D., Milc, J. (2014). Candidate gene expression profiling in two contrasting tomato cultivars under chilling stress. *Biologia Plantarum*, 58:283-295.

Domínguez, E., Cuartero, J., & Fernández-Muñoz, R. (2005). Breeding tomato for pollen tolerance to low temperatures by gametophytic selection. *Euphytica*, 142:253-263.

Duan, M., Feng, H.L., Wang, L.Y., Li, D., & Meng, Q.W. (2012). Overexpression of thylakoidal ascorbate

peroxidase shows enhanced resistance to cold stressin tomato. *Journal of Plant Physiology*, 169:867-877.

Elizondo, R., & Oyanedel, E. (2010). Field testing of tomato chilling tolerance under varying light and temperature conditions. *Chilean Journal of Agricultural Research*, 70:552-558.

FAO, (2021). Food and Agriculture Organization (FAO). FAO Statistical Databases (FAOSTAT): Crops and livestock products. Available at: http://www.fao.org/faostat/en/#data/QC (Accessed: 02 July 2021).

Foolad, M.R., & Lin, G.Y. (2000). Relationship between cold tolerance during seed germination and vegetative growth in tomato: germplasm evaluation. *Journal of the American Society for Horticultural Science*, 125:679-683.

Foolad, M.R., Lin, G.Y. (2001). Genetic analysis of cold tolerance during vegetative growth in tomato, Lycopersicon esculentum Mill. *Euphytica*, 122:105-111.

Funatsuki, H., Kawaguchi, K., Matsuba, S., Sato, Y., & Ishimoto, M. (2005). Mapping of QTL associated with chilling tolerance during reproductive growth in soybean. *Theoretical and Applied Genetics*, 111:851-861.

Gökmen, Ö.Ö. (2006). Investigation of Low Temparature Stress in tomato in Respect to Antioxidative Mechanisms. MSc Thesis, Çukurova University, Adana (in Turkish).

Keleş, D., (2007). Characterization of Different Pepper Genotypes and Low Temperature Tolerance. PhD Thesis, Çukurova University, Adana (in Turkish).

Levitt, J. (1980). Responses of Plants to Environmental Stress, Volume 1: Chilling, Freezing, and High Temperature Stresses. Academic Press.

Li, Y., Tian, X., Wei, M., Shi, Q., Yang, F., & Wang, X. (2015). Mechanisms of tolerance differences in cucumber seedlings grafted on rootstocks with different tolerance to low temperature and weak light stresses. *Turkish Journal of Botany*, 39:606-614.

Liu, G., Du, Q., Jiao, X., & Li, J. (2018). Irrigation at the level of evapotranspiration aids growth recovery and photosynthesis rate in tomato grown under chilling stress. *Acta Physiologiae Plantarum*, 40:1-11.

Lutts, S., Kinnet, J.M., & Bouharmont, J., (1996). NaCl induced senescence in leaves of rice cultivars differing in salinity resistance. *Annual Botany,* 78:389-398.

Ma, X., Chen, C., Yang, M., Dong, X., Lv, W., & Meng, Q. (2018). Cold-regulated protein (SlCOR413IM1) confers cold stresstolerance in tomato plants. *Plant Physiology and Biochemistry*, 124:29-39.

Maisonneuve, B., Hogenboom, N.G., & Den Nijs, A.P.M. (1986). Pollen selection in breeding tomato (*Lycopersicon esculentum* Mill.) for adaptation to low temperature. *Euphytica*, 35:983-992.

Malekzadeh, P., Khara, J., & Heydari, R. (2014). Alleviating effects of exogenous Gamma-aminobutiric acid on tomato seedling under chilling stress. *Physiology and Molecular Biology of Plants*, 20:133-137.

Mulcahy, D.L., Sari-Gorla, M., & Mulcahy, G.B. (1996). Pollen selection-past, present and future. *Sexual Plant Reproduction*, 9:353-356.

Ражаметов, Ш.Н., Янг, Ы., Чо, М.Ч., Чэ, С.Ё., & Жеонг, Х.Б. (2020). Screening of pepper (*Capsicum L.*) seedlings tolerance to low temperature. *Аграрный научный журнал*, 11:78-82.

Peel, M.C., Finlayson, B.L., & McMahon, T.A. (2007). Updated world map of the Köppen-Geiger climate classification. *Hydrology and Earth System Sciences*, 11:1633-1644.

Picken, A.J.F. (1984). A review of pollination and fruit set in the tomato (*Lycopersicon esculentum* Mill.). *Journal of Horticultural Science*, 59: -13.

Ronga, D., Rizza, F., Badeck, F.W., Milc, J., Laviano, L., Montevecchi, G., Pecchioni, N., & Francia, E. (2018). Physiological responses to chilling in cultivars of processing tomato released and cultivated over the past decades in Southern Europe. *Scientia Horticulturae*, 231:118-125.

Sayyari, M., (2012). Improving chilling resistance of cucumber seedlings by salicylic acid. *American-Eurasian Journal of Agricultural & Environmental Sciences,* 12: 04-209.

Xia, X.J., Fang, P.P., Guo, X., Qian, X.J., Zhou, J., Shi, K., Yu, J.Q. (2018). Brassinosteroid-mediated apoplastic H_2O_2-glutaredoxin 12/14 cascade regulates antioxidant capacity in response to chilling in tomato. *Plant, Cell & Environment*, 41:1052-1064.

Zamir, D., & Gadish, I. (1987). Pollen selection for low temperature adaptation in tomato. *Theoretical and Applied Genetics*, 74:545-548.

Zhao, D.Y., Shen, L., Fan, B., Liu, K.L., Yu, M.M., Zheng, Y., Ding, Y., Sheng, J.P. (2009). Physiological and genetic properties of tomato fruits from 2 cultivars differing in chilling tolerance at cold storage. *Journal of Food Science*, 74:C348-C352.

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RESEARCH PAPER

Estimating the Breeding Potential of Purple Sweet Corn Lines with Topcross Mating Design

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Abstract

The estimation of breeding potential of early generation lines is a prerequisite in corn breeding. Breeders could use a methodology called topcross for this purpose. The present study aimed (i) to investigate the breeding potential of purple sweet corn lines with topcross design, and (ii) to determine correlation between fresh ear yield and yield traits in topcross hybrids (TH). Hundred and eighteen S2 generation purple sweet corn lines were crossed with the tester, the Ant-224-E-1 yellow sweet corn inbred lines in isolated cross block (ICB), in Antalya in 2020. Hundred and twenty-one hybrids (118 topcross hybrids and 3 commercial hybrid controls) were obtained and analyzed with an 11 × 11 triple lattice design in 2021. It was determined that the number of days to anthesis (DtoA), plant height (PH), ear height (EA), general plant appearance (PA) and fresh ear yield (FEY) were statistically significant at $p < 0.001$ across the hybrids. The DtoA, PH, EH, PA and FEY varied between 90.7-103.0 days, 105.6-217.9 cm, 38.2-77.9 cm, 1.7-3.7 scores, and 4970.2-13472.9 kg ha^{-1}, respectively. Sixty-three topcross hybrids exhibited higher FEY when compared to the trial average. While 16 PSC lines exhibited statistically significant positive general combining ability (GCA) for FEY, 20 PSC lines exhibited negative GCA for FEY. Eighteen PSC lines were selected based on 15% selection intensity and yield trait criteria. It was determined that these lines had a significant PSC hybrid potential as parents in future PSC breeding programs.

1. Introduction

Corn is significant crop with various specialty varieties such as sweet corn (*Zea mays* saccrata Sturt.) (Hallauer, 2001). Although most cultivated sweet corn kernels are yellow, sweet corn breeding programs to achieve corn in different colors, especially purple, were initiated globally due to their rich phytochemical content. The breeding potential of the lines should be analyzed in corn breeding programs. The topcross mating design serves this purpose (Çeçen et al., 1998; Özkaynak and Samancı, 2003; Paterniani et al., 2006; Aydın et al., 2007; Aguiar et al., 2008; Nelson and Goodman, 2008; Erdal et al., 2010; Marcondes et al., 2015). Prediction of the combining ability of new inbred lines is important and topcross-mating design is one of the commonly preferred designs (Rahimi and Sadeghi, 2017). It was reported that the General Combination Ability (GCA) of the parental line could be determined based on the performance of the topcross hybrids with the same parental line (Zystro et al., 2021). Hallauer and Miranda (1981) reported that the topcross combining ability could lead to differences in F_1 hybrid performance and trial mean. It was suggested that the general combining abilities of the lines could be determined with an accepted tester (Davis, 1927; Jenkins and Brunson,

1932). It was also reported that homozygous lines with different genetic properties could be employed as testers to determine the line GCA (Russell and Eberhart, 1975; Hoegemeyer and Hallauer, 1976).

The present study aimed (i) to investigate the breeding potential of the S_2 generation PSC lines with topcross, and (ii) to determine their agronomical performances for the analyzed traits.

2. Material and Methods

2.1. Plant material

The purple sweet corn (PSC) breeding program was initiated in Turkey in 2017, similar to the global trends. One hundred eighteen inbred S_2 generation PSC lines were developed between 2017 and 2020. To obtain topcross hybrids, 118 PSC lines were used as female parents, and also the Ant-224-E-1 yellow sweet corn inbred line was used as both male parents and the tester. Three commercial hybrids were used as controls in topcross hybrid (TCH) analysis. The PSC lines were the crosses of the combination of purple waxy corn genotype procured from Thailand and standard BATEM yellow sweet corn lines. The sweet corn lines used in the current study were "su" type sweet corn.

2.2. Experimental design

2.2.1. Obtaining the hybrids

The study was conducted in 2020 and 2021 at Batı Akdeniz Agricultural Research Institute (BATEM) fields in Antalya, Türkiye (36°52'N, 30°45'E). The climate in Antalya is classic Mediterranean climate, and the climate data for the study period are presented in Figure 1. In the first year of the research, 118 PSC lines were crossed with the tester to obtain 118 isolated topcross hybrids. The topcross hybrids (TCH) were planted as 4 rows of female and 2 rows of male plants at 5 m distance. The female parents were detasseled by hand to produce topcross hybrids. The male parent (tester) was planted in 5-day interval to obtain maximum grain count with best pollination. The ears of the 118 TCH hybrids included both purple and yellow seeds, and these seeds were individually selected by hand. The yellow seeds were removed and purple seeds were selected for field analysis in the subsequent year.

2.2.2. Hybrid analysis

In 2021, the field experiments were conducted with 121 genotypes (118 PSC TCH and 3 commercial controls) to analyze the TCH with a 11×11 triple lattice design in three replicates in Antalya. The hybrids were planted on March 09, 2021. The plots included two 5 m long rows with a space of 0.7 m and 0.5 m between the rows and the plants, respectively. 80 kg ha^{-1} nitrogen, phosphorus and potassium were applied before sowing based on the soil tests, and nitrogen (170 kg ha^{-1}) was applied 4 times. Weed control was conducted with herbicides with an active ingredient of tembotrione and isoxadifen-ethyl. The topcross hybrids were harvested by hand in the 75 milk-line stage, about 24 to 26 days after the silks emerged (Olsen et al., 1990; Öktem, 2008) between July 5 and 15 in 2021. The traits were analyzed to estimate breeding potential of the PSC lines, including the number of days to anthesis (DtoA), plant height (PH, cm), ear height (EH, cm), plant appearance (PA, 1-5 points), ear length (EL, cm),

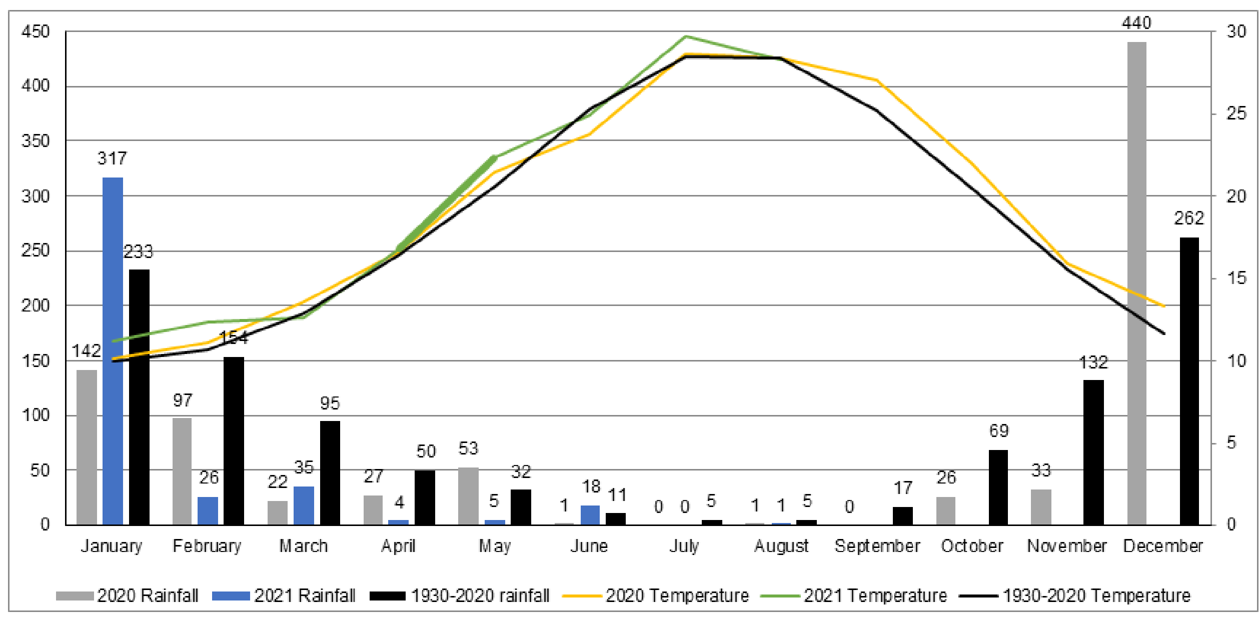

Figure 1. Climatic data for 2020 and 2021 in Aksu, Antalya (Rainfall: mm, Temperature: °C).

ear diameter (ED, cm), and fresh ear yield (FEY, kg ha^{-1}). The DtoA was calculated when 50% of plant anthers appeared in each plot. The PH, EH, PA, EL, ED were measured in ten random plants in each plot. PH and EH were determined by calculating the distance between ground and the top of the tassel and the first ear, respectively. PA was scored between 1 (best) and 5 (worst) during the milk stage. The ear length and ear dimater, which are important yield related traits, were measured from 10 plants presented each plot and mean of these data was recorded as ear length and ear diameter for each plot. EL was measured as the distance between the base and tip of the ear. ED was measured on the central section of the ear. FEY was determined by scaling all ear except the husk in each plot, and then the finding was converted to fresh ear yield per hectare (kg ha^{-1}).

2.3. Data analyses

The statistical data analysis was conducted with the SAS 9.0 Statistics Software. Analysis of Variance (ANOVA) was conducted on triple lattice experimental design criteria with the Fisher's least significant difference post hoc test. General Combining Ability was also estimated with the formula given below (Ferreira et al., 2009).

$$gi = ci - c$$

where; gi: the impact of the general combining ability of the lines, ci: mean of each hybrid, and c: overall mean of topcross hybrids.

3. Results and Discussion

3.1. Topcrosses

One hundred and twenty one genotypes (118 TH and 3 control varieties) were scrutinized to determine GCA of the PSC lines in Antalya in 2021. The trait data for the THs and controls are presented in Table1. DtoA, PH, EH, PA and FEY were significant in the studied genotypes at P<0.01, while EL and ED were insignificant.

The DtoA of TH varied between 90.7 and 103.1 days (average 98.3 days). All TH were in the same DtoA category. Thus, it could be suggested that it could be beneficial to conduct future breeding programs with these PSC lines as parents. It could be also noted that the earliest Jubilee and Adapare commercial control DtoAs were 90.7 and 92.1 days, respectively. TCH-29 had the longest flowering time (103.1 days; Table 1). Although the DtoA data were similar to those reported by Pecina-Martínez et al. (2013) and Mendoza et al. (2019), it was longer than those reported in certain studies (Turgut and Balcı 2002; Kara and Akman, 2002; Ji et al., 2010; Özata 2013; Tuan et al., 2016; Aboyousef et al., 2018; Ibrahim and Ghada, 2019; Ismail et al., 2020; Dermail et al., 2020; Reis et al., 2020; Tuan et al., 2021). The longer DtoA observed in the present study could be early plantation of the genotypes (on March 9) to avoid drought stress during the flowering period. The climate data demonstrated that the air temperature in March and April in 2021, when the experiments were conducted, were well lower than optimum temperature requirements of corn. Furthermore, sweet corn is more succeptible to abiotic stress factors such as low temperatures when compared to dent corn. It took a long time for the PSC lines to reach total temperature demand; and thus, DtoAs were extended. However, the DtoA of the purple sweet corn lines varied between 60 and 65 days between 2017 and 2021 (data not presented and unpublished).

There were statistically significant differences between the PH figures in the current study (P<0.01). The mean PH was measured at 188.5 cm. While the TCH-54 exhibited the shortest plant height (150.6 cm), TCH-08 had the highest plant height (217.9 cm) (Table 1). Furthermore, twenty-seven topcross hybrids (TCH-6, TCH-10, TCH-12, TCH-21, TCH-22, TCH-28, TCH-35, TCH-36, TCH-41, TCH-46, TCH-47, TCH-48, TCH-49, TCH-50, TCH-51, TCH-65, TCH-70, TCH-71, TCH-74, TCH-75, TCH-90, TCH-93, TCH-102, TCH-106, TCH-110, TCH-112 and TCH-117) were classified as the most significant group (above 199.19 cm). The plant height data were higher when compared to certain studies (Turgut and Balci, 2002; Bozolkalfa et al., 2004; İdikut et al., 2005; Mahato et al., 2018). However, TH plant height was similar to those reported in the literature (Ji et al., 2010; Özata, 2013; Ibrahim and Ghada, 2019; Mendoza et al., 2019; Islam et al., 2020; Arsyad and Basunanda, 2020; Gavriç and Omerbegoviç, 2021; Tuan et al., 2021).

There were statistically significant differences between thegenotypes based on first ear height (P<0.01) (Table 1). The ear height varied between 38.2 and 77.9 cm (average 60.0 cm). While TCH-94 had the lowest ear height (38.2 cm), TCH-6 ear height was the highest (77.9 cm). Certain studies reported similar first ear height figures (Turgut and Balcı, 2002; Bozolkalfa et al., 2004; Idikut et al., 2005; Ji et al., 2010; Özata, 2013; Tuan et al., 2016; Mahato et al., 2018; Ibrahim and Ghada, 2019; Mendoza et al., 2019; Dermail et al., 2020; Arsyad and Basunanda, 2020; Tuan et al., 2021).

There were statistically significant differences between the topcross hybrids employed in the current study based on the plant appearance (PA) variable (P<0.01) (Table 1). The mean genotype PA score was 2.9. While TCH-71 exhibited the best PA (1.7), TCH-53 scored the worst in PA (3.7). The scores of the TCH-121, 120, 94, 108, 119 and 54 were below the mean trial FEY score. The TCH-36, TCH-70 and TCH-75, which exhibited the best PA scores, were in the highest FEY group statistically. Nevertheless, ten out of 14 TCHs (TCH-8, TCH-10, TCH-32, TCH-36, TCH-46, TCH-50, TCH-70, TCH-

Table 1. Average data of topcross hybrids and checks for yield and yield related traits.

Hybrids number	Number of days to anthesis (day)	Plant height (cm)	Ear heigth (cm)	Plant appearance (1-5)	Ear length (cm)	Ear diameter (cm)	Fresh ear yield (kg ha^{-1})
TCH1	97.8	187.5	70.2	2.6	16.46	3.64	8905.0
TCH2	102.0	167.0	52.8	3.5	16.58	4.14	9319.5
TCH3	97.7	170.4	70.8	3.4	19.24	4.04	9150.2
TCH4	99.3	183.5	61.4	3.4	18.08	4.22	9049.6
TCH5	101.3	185.3	65.6	3.2	17.70	3.89	8229.9
TCH6	99.5	199.7	77.9	3.0	17.51	3.99	7758.4
TCH7	97.8	193.7	55.7	2.9	17.52	4.22	12054.7
TCH8	99.7	217.9	71.8	2.3	19.00	4.10	10735.6
TCH9	101.1	196.7	75.1	3.1	20.29	3.94	8925.5
TCH10	98.4	214.8	61.3	2.4	17.78	4.12	9790.8
TCH11	98.3	190.8	67.2	3.2	17.50	3.65	8155.7
TCH12	97.0	201.6	58.9	2.7	19.38	4.27	9689.3
TCH13	98.4	195.1	59.0	2.6	18.20	4.17	8855.7
TCH14	98.7	182.4	52.5	3.1	16.58	4.36	9384.5
TCH15	97.2	192.4	57.3	3.0	18.27	3.89	9530.6
TCH16	97.2	188.4	55.4	2.8	18.73	3.78	10561.7
TCH17	94.8	161.3	53.2	3.0	16.70	3.89	7132.8
TCH18	100.9	163.1	49.9	3.0	16.83	3.61	9194.2
TCH19	98.5	182.6	60.1	3.1	18.14	4.40	10742.2
TCH20	101.0	182.2	45.5	2.3	16.27	3.91	5530.4
TCH21	96.4	210.8	68.3	2.8	19.14	4.35	7340.2
TCH22	96.9	200.4	63.1	2.5	18.20	4.27	9613.1
TCH23	97.0	181.0	65.1	2.9	15.34	4.08	8484.6
TCH24	100.8	191.3	73.3	3.0	17.40	4.12	9919.8
TCH25	99.9	198.1	64.0	3.1	17.16	3.97	8493.1
TCH26	98.1	187.1	65.9	2.9	17.44	4.29	13057.3
TCH27	98.5	196.1	60.2	2.7	17.10	4.11	11348.0
TCH28	97.2	201.4	66.9	3.0	17.41	4.04	10074.5
TCH29	103.1	191.7	65.7	3.0	17.04	3.59	7108.8
TCH30	102.8	194.2	53.2	2.8	17.11	3.88	9476.9
TCH31	98.6	185.7	57.7	2.7	18.90	4.23	12486.8
TCH32	97.2	198.4	64.2	2.1	16.84	4.01	9880.5
TCH33	98.5	193.5	64.1	3.0	17.17	3.69	9883.9
TCH34	97.6	197.4	60.9	2.9	16.92	3.78	10358.1
TCH35	100.1	202.3	57.7	2.7	15.54	3.82	10606.4
TCH36	98.0	199.2	66.4	2.0	16.54	4.21	12728.7
TCH37	95.6	183.8	52.5	2.9	17.05	4.03	10415.5
TCH38	94.4	155.6	46.9	3.5	14.78	4.20	10406.9
TCH39	95.9	190.7	64.8	3.1	16.37	3.66	9741.4
TCH40	99.1	190.1	65.9	2.8	16.49	4.30	9059.6
TCH41	101.6	199.3	63.3	2.2	15.32	3.48	8886.1
TCH42	98.2	172.1	50.6	3.3	17.58	3.59	10375.0
TCH43	95.2	196.8	62.9	3.4	18.11	3.88	10882.9
TCH44	96.5	196.0	64.8	2.8	16.42	4.33	9157.7
TCH45	96.1	178.9	63.4	3.2	17.17	3.81	8617.8
TCH46	102.5	201.0	73.6	1.9	18.05	3.67	10192.4
TCH47	94.1	199.2	65.9	2.6	18.21	4.19	10600.5
TCH48	97.8	202.2	65.1	3.0	17.80	3.95	7415.5
TCH49	98.4	213.0	67.0	2.7	18.83	4.18	11045.3
TCH50	97.0	212.6	71.7	2.5	20.61	4.39	9717.2
TCH51	96.6	203.4	70.6	2.5	20.72	4.06	8712.8
TCH52	98.5	180.1	60.8	3.3	19.92	4.01	7323.7
TCH53	99.2	181.4	56.4	3.7	17.86	4.22	9453.4
TCH54	98.6	150.6	51.0	3.5	15.39	3.93	8827.7
TCH55	98.7	178.5	57.0	3.3	18.69	3.89	9305.5
TCH56	97.8	185.3	53.9	3.0	17.62	4.43	9777.1
TCH57	100.9	181.4	49.5	3.2	17.08	3.90	6294.5
TCH58	97.4	196.9	66.8	2.7	16.84	4.11	9890.1
TCH59	99.5	184.5	61.3	2.9	16.52	3.60	5968.1
TCH60	97.8	183.8	62.9	3.1	19.36	3.77	8114.5
TCH61	102.5	190.6	58.5	3.2	17.47	3.65	6022.1
TCH62	99.6	192.6	64.1	2.5	17.41	3.74	8216.9
TCH63	98.5	187.3	57.2	3.3	18.12	3.97	9626.8
TCH64	98.3	189.4	61.7	3.0	16.77	4.01	7808.4

Table 1. Average data of topcross hybrids and checks for yield and yield related traits (conti.).

Hybrids number	Number of days to anthesis (day)	Plant height (cm)	Ear height (cm)	Plant appearance (1-5)	Ear length (cm)	Ear diameter (cm)	Fresh ear yield (kg ha^{-1})
TCH65	98.8	202.1	55.1	2.9	17.39	3.56	8125.1
TCH66	99.0	184.5	56.7	3.0	18.52	4.17	8234.7
TCH67	100.5	182.0	69.6	2.7	17.06	3.93	7386.4
TCH68	101.0	188.5	57.7	3.0	19.12	3.84	8771.0
TCH69	97.1	179.7	52.1	3.1	16.88	3.95	6819.8
TCH70	99.1	202.9	68.3	2.5	18.10	3.73	11509.5
TCH71	101.5	201.7	71.8	1.7	16.56	3.70	10330.3
TCH72	100.6	192.0	58.8	3.0	16.79	3.68	7893.9
TCH73	99.1	189.6	53.6	3.3	18.45	4.37	7156.5
TCH74	100.1	200.6	61.8	3.0	15.80	3.86	9670.4
TCH75	94.7	207.3	65.8	2.4	18.02	3.88	13472.9
TCH76	97.2	187.5	56.4	3.2	13.67	4.23	10027.5
TCH77	98.2	196.7	66.9	3.2	14.90	4.08	10040.2
TCH78	99.2	171.9	63.2	3.1	19.11	3.96	9426.4
TCH79	95.5	187.9	55.1	3.0	16.09	4.23	10379.6
TCH80	97.8	174.4	56.9	2.8	16.29	4.28	10831.4
TCH81	98.1	178.3	58.5	3.0	16.71	4.57	9823.4
TCH82	97.0	164.4	55.5	3.1	14.08	3.99	8769.6
TCH83	96.9	186.0	51.0	3.2	16.75	3.94	7072.6
TCH84	95.5	170.4	52.3	2.9	15.67	4.03	7122.7
TCH85	97.9	178.6	46.3	2.7	16.71	4.35	8285.6
TCH86	98.5	186.5	53.7	2.9	15.17	4.16	7399.5
TCH87	97.2	193.0	62.2	2.5	17.53	3.76	9378.0
TCH88	95.5	194.7	63.2	3.0	17.39	4.07	8108.1
TCH89	99.1	188.8	54.7	3.0	17.40	3.59	9293.6
TCH90	96.5	211.6	73.8	2.7	17.74	4.40	10960.4
TCH91	95.2	181.0	49.6	3.1	19.97	4.04	8168.6
TCH92	98.6	180.2	53.6	3.0	21.02	3.97	5930.3
TCH93	101.9	207.0	60.9	3.0	18.15	4.16	7670.0
TCH94	102.2	164.2	38.2	3.6	17.87	3.63	5565.0
TCH95	98.6	186.5	46.4	2.8	15.30	4.25	9375.3
TCH96	98.4	178.2	46.8	3.0	15.02	4.02	10045.1
TCH97	97.8	175.0	55.1	3.0	18.65	3.96	7817.5
TCH98	96.9	194.4	67.0	3.0	17.51	3.81	8358.0
TCH99	98.0	189.1	54.2	2.9	15.81	3.91	9165.7
TCH100	97.9	182.3	48.5	3.0	18.56	3.60	6506.9
TCH101	98.3	187.7	49.8	3.0	17.30	4.16	10242.6
TCH102	98.1	203.8	72.8	2.6	18.02	4.31	11858.6
TCH103	100.0	184.3	59.0	3.2	17.23	3.82	8768.9
TCH104	98.7	188.1	62.1	2.9	17.13	3.53	10295.1
TCH105	97.1	169.3	49.8	3.3	16.13	3.87	6326.7
TCH106	97.3	217.2	74.7	1.7	17.12	4.15	7908.7
TCH107	99.9	185.1	62.0	3.3	17.99	3.57	10334.1
TCH108	98.4	177.1	50.1	3.4	17.76	3.62	6909.9
TCH109	96.5	190.5	64.5	3.2	17.66	3.80	9599.5
TCH110	99.4	203.0	66.8	3.0	16.93	3.46	8530.4
TCH111	97.0	188.0	72.6	2.9	18.43	4.09	8806.5
TCH112	96.8	201.7	63.0	2.6	16.53	4.16	11958.3
TCH113	99.0	187.0	58.2	3.1	15.52	3.97	9813.1
TCH114	98.3	191.0	60.0	3.1	17.15	4.15	9962.2
TCH115	99.2	179.3	52.0	3.1	17.85	4.35	9504.0
TCH116	99.6	187.5	62.1	2.9	16.20	4.17	6846.9
TCH117	97.8	199.5	69.0	2.8	16.81	4.18	7600.8
TCH118	99.0	176.3	61.4	3.1	19.35	4.00	7634.6
Batem Tatlı	97.2	174.5	51.4	3.5	18.64	3.81	12348.0
Adapare	92.1	161.3	47.3	3.5	17.11	3.50	8185.1
Jübile	90.7	159.5	43.7	3.6	14.44	3.18	4970.2
Mean	98.2	188.5	60.0	2.9	17.37	3.98	9080.23
LSD	2.97**	19.46**	15.41**	0.80**	ns	ns	213.9**
CV%	1.7	6.12	15.27	16.30	11.55	9.78	14.01

** and ns, significant at $p \leq 0.01$ and not significant, respectively.

71, TCH-75 and TCH-87) in the best PA group, exhibited higher FEY when compared to the trail mean score.

There were no statistically significant differences between the topcrosses based on ear length and diameter (Table 1). The mean TCH ear length was 17.4 cm and TCH varied between 13.7 cm (TCH-76) and 21.0 cm (TCH-92). The current study EL data were similar to those reported by previous studies (Turgut and Balcı, 2002; Bozokalfa et al., 2004; Ji et al., 2010; Özata, 2013; Tuan et al., 2016; Mahato et al., 2018; Ibrahim and Ghada, 2019; Mendoza et al., 2019; Dermail et al., 2020; Mollah et al., 2020; Islam et al., 2020; Arsyad and Basunanda, 2020; Gavric and Omerbegovic, 2021; Tuan et al., 2021).

The mean TCH ear diameter was 3.98 cm and varied between 3.18 cm (Jubile F1) and 4.57 cm (TCH-81). The current study ED findings were consistent with previous study findings (Turgut and Balcı, 2002; Bozokalfa et al., 2004; Ji et al., 2010; Özata, 2013; Tuan et al., 2016; Mahato et al., 2018; Ibrahim and Ghada, 2019; Mendoza et al., 2019; Dermail et al., 2020; Mollah et al., 2020; Tuan et al., 2021).

There were statistically significant differences between TCH fresh ear yield (FEY) ($P<0.01$). It was observed that the mean of FEY was 9080.2 kg ha^{-1} (Table 1). There were also significant differences between the genotype FEY figures in the present study. While Jubilee exhibited the lowest FEY (4970.2 kg ha^{-1}), the highest FEY was measured in TCH-75 (13472.9 kg ha^{-1}). Ten TCHs (TCH-75, TCH-26, TCH-36, TCH-31, TCH-119, TCH-7, TCH-112, TCH-102, TCH-70 and TCH-27) were in the same group with the best FEY statistically. The comparison of these findings with the previous studies conducted with sweet or purple corn revealed that FEY figures were higher in the current study when compared to certain studies (Tuan et al., 2016; Mendoza et al., 2019; Mollah et al., 2020). The plant materials in these studies were either population or open pollinated genotypes, which led to lower mean data when compared to the current study findings determined with hybrids.

3.2. Determination of the combining ability of topcross hybrids

The tester with a broad genetic base was crossed with the PSC lines to estimate the impact of the general combining ability. The GCA of the PSC lines for FEY varied between -3564 kg ha^{-1} (TCH-20) and 4377 kg ha^{-1} (TCH-75). The GCA estimates for PSC lines are presented in Table 2 and Figure 2. The analysis of the GCA findings revealed that 63 out of 118 THs exhibited higher FEY when compared to the trial mean (Table 2). The top 19 TH with the highest FEY were selected for GCA in the trial. Similarly, Sezer and Sürmeli (2003) also selected 19 lines in their study where 124 inbred lines were analyzed for topcross combining abilities. Finally, it was determined that 16 TCHs (TCH-75, TCH-26, TCH-36, TCH-31, TCH-7, TCH-112, TCH-102, TCH-70, TCH-27, TCH-49, TCH-90, TCH-43, TCH-80, TCH-19, TCH-47 and TCH-37) among the selected 19 TCHs had statistically positive and significant effects (Table 2). It was estimated that the hybrids of the lines with high GCA for FEY would have high FEY potential, similar to previous study findings (Rawlings and Thompson, 1962; Hallauer and Miranda, 1988; Aydın et al., 2007; Erdal et al., 2010).

The lines with a negative GCA should not be excluded from breeding programs (Rahimi ve and Sadeghi, 2017). Clovis et al. (2015) suggested that the selection of the lines with a negative GCA during flowering as male and female parents provided a significant earliness advantage. Lonnquist and Lindsey (1964) reported that hybrids obtained with high x low yield lines provided higher yields when compared to hybrids of high × high and low × low lines. Thus, lines with a statistically negative significant GCA for FEY (TCH-20, TCH-94, TCH-92, TCH-59, TCH-61, TCH-57, TCH-105, TCH-100, TCH-116, TCH-83, TCH-29, TCH-17, TCH-73, TCH-52, TCH-67, TCH-118, TCH-6, TCH-106 and TCH-62) were considered to have breeding potential in the present study.

To estimate of breeding potential of PSC lines, not only the GCA data but also all selection criteria presented Table 1 were considered. Thus, eighteen TCHs were selected based on all findings and 15% selection criteria in the trial (Table 3).

4. Conclusion

In the current study, the breeding potential of 118 S_2 purple sweet corn topcross lines was estimated in Antalya conditions. The PSC lines exhibited good general combining ability for FEY. Lines with high GCA were selected for testing in future breeding studies and included in diallel crosses for the determination of special combining abilities. The PSC lines also exhibited excellent variance for FEY and yield traits. Eighteen lines were selected noy only based on positive or negative statistical significance in fresh ear yield but also other analyzed observation criteria such as plant height, ear height, plant appearance, ear length, and ear diameter. The seeds of the promising PSC lines should be reproduced, and these lines should be employed as purple sweet corn hybrid parents in future PSC breeding programs.

References

Aboyousef, H.A., Gamea, H.A.A., & El Mohamed, A.M. (2018). Estimates of combining ability for grain yield

Table 2. Estimates of general combining ability of purple sweet corn inbred lines for fresh ear yield (kg ha^{-1}).

Topcross hybrids number	GCA value	Topcross hybrids number	GCA value	Topcross hybrids number	GCA value
TCH1	-190.0 ns	TCH41	-208.9 ns	TCH80	1736.4 *
TCH2	224.5 ns	TCH42	1280.0 ns	TCH81	728.4 ns
TCH3	55.2 ns	TCH43	1787.9 **	TCH82	-325.4 ns
TCH4	-45.4 ns	TCH44	62.7 ns	TCH83	-2022.4 *
TCH5	-865.1 ns	TCH45	-477.2 ns	TCH84	-1972.3 ns
TCH6	-1336.6 *	TCH46	1097.4 ns	TCH85	-809.4 ns
TCH7	2959.7**	TCH47	1505.5 *	TCH86	-1695.5 ns
TCH8	1640.6 ns	TCH48	-1679.5 ns	TCH87	283.0 ns
TCH9	-169.5 ns	TCH49	1950.3 **	TCH88	-986.9 ns
TCH10	695.8 ns	TCH50	622.2 ns	TCH89	198.6 ns
TCH11	-939.3 ns	TCH51	-382.2 ns	TCH90	1865.4 *
TCH12	594.3 ns	TCH52	-1771.3 **	TCH91	-926.4 ns
TCH13	-239.3 ns	TCH53	358.4 ns	TCH92	-3164.7 **
TCH14	289.5 ns	TCH54	-267.3 ns	TCH93	-1425.0 ns
TCH15	435.6 ns	TCH55	210.5 ns	TCH94	-3530.0 **
TCH16	1466.7 ns	TCH56	682.1 ns	TCH95	280.3 ns
TCH17	-1962.2 *	TCH57	-2800.5 **	TCH96	950.1 ns
TCH18	99.2 ns	TCH58	795.1 ns	TCH97	-1277.5 ns
TCH19	1647.2 *	TCH59	-3126.9 **	TCH98	-737.0 ns
TCH20	-3564.6 **	TCH60	-980.5 ns	TCH99	70.7 ns
TCH21	-1754.8 ns	TCH61	-3072.9 **	TCH100	-2588.1 *
TCH22	518.1 ns	TCH62	-878.1*	TCH101	1147.6 ns
TCH23	-610.4 ns	TCH63	531.8 ns	TCH102	2763.6 **
TCH24	824.8 ns	TCH64	-1286.6 ns	TCH103	-326.1 ns
TCH25	-601.9 ns	TCH65	-969.9 ns	TCH104	1200.1 ns
TCH26	3962.3 **	TCH66	-860.3 ns	TCH105	-2768.3 **
TCH27	2253.0 **	TCH67	-1708.6 *	TCH106	-1186.3 *
TCH28	979.5 ns	TCH68	-324.0 ns	TCH107	1239.1 ns
TCH29	-1986.2 **	TCH69	-2275.2 ns	TCH108	-2185.1 ns
TCH30	381.9 ns	TCH70	2414.5 **	TCH109	504.5 ns
TCH31	3391.8 **	TCH71	1235.3 ns	TCH110	-564.6 ns
TCH32	785.5 ns	TCH72	-1201.1 ns	TCH111	-288.5 ns
TCH33	788.9 ns	TCH73	-1938.5 *	TCH112	2863.3 **
TCH34	1263.1 ns	TCH74	575.4 ns	TCH113	718.1 ns
TCH35	1511.4 ns	TCH75	4377.9 **	TCH114	867.2 ns
TCH36	3633.7 **	TCH76	932.5 ns	TCH115	409 ns
TCH37	1320.5 *	TCH77	945.2 ns	TCH116	-2248.1 **
TCH38	1311.9 ns	TCH78	331.4 ns	TCH117	-1494.2 ns
TCH39	646.4 ns	TCH79	1284.6 ns	TCH118	-1460.4 *
TCH40	-35.4 ns				

**, * and ns, significant at p≤0.01, p≤0.05, and not significant, respectively.

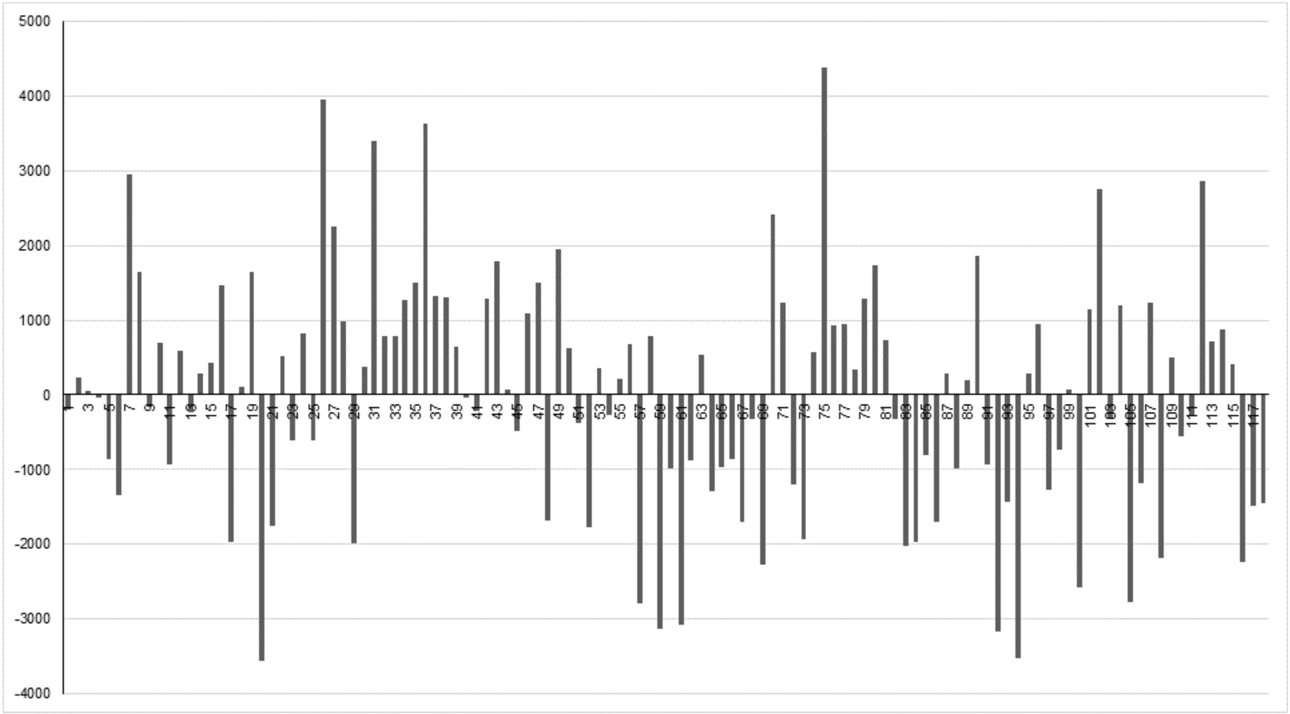

Figure 2. Showing of general combining ability value of purple sweet corn inbred lines for fresh ear yield.

Table 3. The number and traits of selected hybrids based on general combining ability and yield related traits as selection criteria.

No	Hybrids number	Number of days to anthesis (day)	Plant height (cm)	Ear heigth (cm)	Plant appearance (1-5)	Ear length (cm)	Ear diameter (cm)	Fresh ear yield (kg ha^{-1})
1	TCH19	98.5	182.6	60.1	3.1	18.14	4.40	10742*
2	TCH26	98.1	187.1	65.9	2.9	17.44	4.29	13057*
3	TCH31	98.6	185.7	57.7	2.7	18.90	4.23	12486*
4	TCH36	98.0	199.2	66.4	2.0	16.54	4.21	12728*
5	TCH43	95.2	196.8	62.9	3.4	18.11	3.88	10882*
6	TCH47	94.1	199.2	65.9	2.6	18.21	4.19	10600*
7	TCH49	98.4	213.0	67.0	2.7	18.83	4.18	11045*
8	TCH52	98.5	180.1	60.8	3.3	19.92	4.01	7323**
9	TCH62	99.6	192.6	64.1	2.5	17.41	3.74	8216**
10	TCH67	100.5	182.0	69.6	2.7	17.06	3.93	7386**
11	TCH70	99.1	202.9	68.3	2.5	18.10	3.73	11509*
12	TCH75	94.7	207.3	65.8	2.4	18.02	3.88	13473*
13	TCH90	96.5	211.6	73.8	2.7	17.74	4.40	10960*
14	TCH97	97.8	175.0	55.1	3.0	18.65	3.96	7817**
15	TCH102	98.1	203.8	72.8	2.6	18.02	4.31	11858*
16	TCH106	97.3	217.2	74.7	1.7	17.12	4.15	7908**
17	TCH112	96.8	201.7	63.0	2.6	16.53	4.16	11958*
18	TCH118	99.0	176.3	61.4	3.1	19.35	4.00	7634**

*, ** represent positive and negative general combining ability, respectively.

and other agronmic traits in yellow maize hybrids. Archives of Agriculture Sciences Journal, 1:113-121.

Aguiar, C.G., Schuster, I., Amaral Júnior, A.T., Scapim, C.A., & Vieira, E.S.N. (2008). Heterotic groups in tropical maize germplasm by test crosses and simple sequence repeat markers. G*enetics and Molecular Research*, 7:1233-1244.

Arsyad, F., & Basunanda, P. (2020). Identification of agronomic characters effecting cob weight of the families BTP1-× purple sweet corn (*Zea mays* L. *Saccharata Sturt*) using path analysis. *Earth and Environmental Science*, 484:012011.

Aydın, N., Gökmen, S., & Yıldırım, A. (2007). Determination of combining ability of inbred corn lines in terms of grain yield and some other characteristics by topcrossing method. *Journal of Agricultural Science,* 13:120-127.

Bozokalfa, M.K., Eşiyok, D., & Uğur, A. (2004). Determination of yield quality and plant characteristic of some sweet corn (*Zea mays* L. *var. saccharata*) varieties as main and second crop in ege region. *Ege University Journal of Agriculture Faculty*, 41:11-19.

Clovis, L.R., Scapim, C.A., Pinto, R.J.B., Bolson, E., & Senhorinho, H.J.C. (2015). Evaluation of S3 maize inbred lines for off-season cropping with testers adapted. *Revista Caatinga*, 28:109-120.

Çeçen, S., Çakmakçı, S., & Turgut, İ. (1998). A comparison of some maize inbred lines and their testcrosses under the second crop conditions. *Turkish Journal of Agriculture and Forestry*, 22:209-213.

Davis, R.L. (1927). Report of the Plant Breeder. Report of the Plant Breeder: 14-15.

Dermail, A., Suriharn, B., Chankaew, S., Sanitchon, J., & Lertrat, K. (2020). Hybrid prediction based on SSR-genetic distance, heterosis and combining ability on agronomic traits and yields in sweet and waxy corn. S*cientia Horticulturae,* 259:108817.

Erdal, Ş., Pamukçu, M., Savur, O., Soysal, M., Toros, A., & Tezel, M. (2010). Determination of combining ability of standard sweet corn (*Zea mays* L. var. s*accharata sturt*) inbred lines in terms of fresh ear yield by topcrossing method. *Derim*, 27:10-21.

Ferreira, E.A., Paterniani, M.E.A.G.Z., Duarte, A.P., Gallo, P.B., Sawazaki, E., Azevedo Filho, J.A.D., & Guimarães, P.D.S. 2009. Performance of maize top cross hybrids from S3 lines in three environments in São Paulo State. *Bragantia,* 68:319-327.

Gavric, T., & Omerbegovic, O. (2021). Effect of transplanting and direct sowing on productive properties and earliness of sweet corn. *Chilean Journal of Agricultural Research*, 81:39-45.

Hallauer, A.R. (2001). Specialty Corns. 2nd Edition, CRC Press LLC, Boca Raton, Florida 33431.

Hallauer, A., & Miranda, J. (1981). Quantitative Genetics in Maize Breeding. Iowa State Univ. Press, Ames, Iowa, Quantitative Genetics in Maize Breeding. Iowa State Univ. Press, Ames, Iowa.

Hallauer, A.R., Russel, W.A., & Lamkey, K.R., (1988). Corn Breding, Iowa State University Digital Repository, Agronomy Publication.

Hoegemeyer, T., & Hallauer, A.R. (1976). Selection among and within Full-sib families to develop single-crosses of maize 1. *Crop Science*, 16: 76-81.

Ibrahim, A.I.A., & Ghada, A.A., (2019). Evaluation of some Sweet corn hybrids for agronomic traits and technological parameters under different planting dates. *Journal of Food Sciences; Suez Canal University,* 6:49-63.

Islam, S., Ferdausi, A., Sweety, A.Y., Das, A., Ferdoush, A., & Haque, M.A. (2020). Morphological characterization and genetic diversity analyses of plant traits contributed to grain yield in maize (*Zea mays* L.). *Journal of Bioscience and Agriculture Research*, 25:2047-2059.

Ismail, M.R., El-Hosary, A.A., & El-Badawy, M.E.M. (2020). Genetic analysis of some topcrosses of yellow maize. *Electronic Journal of Plant Breeding*, 11:1099-1105.

İdikut, L., Cesur, C., & Tosun S. (2005). Effect of planting dates and growing techiques on green yield and some charecters of sweet corn. *KSU Journal of Science and Engineering,* 8:95-100.

Jenkins, M., & Brunson, A. (1932). Methods of testing inbred lines of maize in crossbred combinations, *Agronomy Journal*, 24:523-530.

Ji, H.C., Lee, H.B., & Yamakawa, T. (2010). Major Agricultural characteristics and antioxidants analysis of the new developed colored waxy corn hybrids. *Journal of the Faculty of Agriculture, Kyushu University,* 55:55–59.

Kara, B., & Akman, Z. (2002). Effect of tiller, leaf and tassel removal on yield and ear characters in sweet corn (*Zea mays saccharata Sturt.*). *Akdeniz University Journal of Agriculture Faculty*, 15:9-18.

Lonnquist, J.H., & Lindsey, M.F. (1964). Topcross versus S1 line performance in corn (*Zea mays L.*). *Crop Science,* 4:580-584.

Mahato, A., Shahi, J.P., Singh, P.K., & Kumar, M. (2018). Genetic diversity of sweet corn inbreds using agro-morphological traits and microsatellite markers. *3 Biotech*, 8:1-9.

Marcondes, M.M., Faria, M.V., Neumann, M., Marcondes, M.M., Silva, C.A., Vascoski, V.L., & Rizzardi, D.A. (2015). Desempenho agronômico e forrageiro de linhagens S4 de milho em topcrosses com híbrido simples. *Semina: Ciências Agrárias*, 36:2395-2406.

Mendoza-Mendoza, C.G., Mendoza-Castillo, M.C., Castillo-González, F., Sánchez-Ramírez, F.J., Delgado-Alvarado, A., & Pecina-Martínez, J.A., (2019). Agronomic performance and grain yield of mexican purple corn populations from Ixtenco, Tlaxcala. *Maydica*, 2019: 64-73.

Mollah, A., Bahrun, A.H., Sarahdibha, M.P., Dariati, T., Riadi, M., & Yanti, C.W.B. (2020). Growth and production of purple waxy corn (Z*ea mays ceratina Kulesh*) on the application of NPK fertilizers and humic acid. *Earth and Environmental Science*, 575:012118.

Nelson, P.T., & Goodman, M.M. (2008). Evaluation of elite exotic maize inbreds for use in temperate breeding. *Crop Science*, 48:85-92.

Olsen, J.K., Giles, J.E., & Jordan, R.A. (1990). Post-harvest carbohydrate changes and sensory quality of three sweet corn cultivars. *Scientia Horticulturae*, 44:179-189.

Öktem, A., & Öktem. A.G. (2006). determination of yield characteristics of some sweet corn (*Zea mays saccharata Sturt*) genotypes under Harran Plain conditions. *Uludağ University Journal of Agriculture Faculty,* 20:33-46

Öktem, A. (2008). Effect of water shortage on yield, and protein and mineral compositions of drip-irrigated sweet corn in sustainable agricultural systems. *Agricultural Water Management,* 95:1003-1010.

Özata, E. (2013). Effect of Different Plant Densities and Nitrogen Doses on Fresh Ear Yield and Yield Components of Sweet Corn (*Zea mays* maccharata Sturt.). PhD Thesis, Ankara University, Ankara (in Turkish).

Özerkişi, E. (2016). Effect of Different Plant Space on Fresh Ear Yield and Quality of Sweet Corn (*Zea mays* L. saccharata Sturt.) under Tekirdag Ecological Conditions. MSc Thesis, Namık Kemal University, Tekirdağ (in Turkish).

Özkaynak, E., & Samancı, B. (2003). Comparison of popcorn (*Zea mays everta* Sturt.) lines and their Testcrosses for yield and yield-related traits. *Mediterranean Agricultural Sciences*, 16:35-42.

Paterniani, M.E.A.G.Z., Luders, R.R., Duarte, A.P., Gallo, P.B., & Sawazaki, E. (2006). Performance of maize triple cross-way hybrids in three environments in São Paulo State, Brazil. *Bragantia*, 65:597605.

Pecina-Martínez, J.A., Del Carmen Mendoza-Castillo, M., Castillo-Gonzalez, F., López-Santillán, J.A., Mendoza-Rodriguez, M., & Reyes-Mendez, C.A. (2013). Genetic potential of S1 lines derived from native maize populations of Tamaulipas, México. *Maydica*, 58:127-134.

Rahimi, M., & Sadeghi, F. (2017). Top cross analysis of S7 maize lines to evaluation combining ability of some agronomic traits. *Bulletin of the Institute of Tropical Agriculture, Kyushu University*, 40:51-61.

Rawlings, J.O. & Thompson, D.L. (1962). Performance level as criterion fort he choice of maize testers. *Crop Science,* 2:217-220.

Reis, E.F.D., Candido, W.D.S., Costa, M.L., Ragagnin, A.L.S.L., & Somera, A. (2020). Partially inbred maize lines in topcrosses and hybrid performance. *Revista Ceres*, 67:216-222.

Russell, W., & Eberhart, S. (1975). Hybrid Performance of selected maize lines from reciprocal recurrent and testcross selection programs. *Crop Science*, 15:1-4.

Sezer, İ., & Sürmeli, A. (2003). Search of the combining abilitiy of some plant traits of maize inbreed lines suitable for obtaining hybride corn (*Zea mays* L.) in main cropping in the blacksea region. *Anadolu Tarım Bilimleri Dergisi,* 18:24-31 (in Turkish).

Tuan, P.Q., Hung, N.T., Long, N.V., Anh, N.T.N., & Liet, V.V. (2016). Evaluation of purple waxy corn lines for hybrid variety development. *Vietnam Journal of Agricultural Science,* 14:328-337.

Tuan, P.Q., Trung Duc, N., Anh, N.T.N., Binh, V.T.X., & Liet, V.V. (2021). Development and selection of purple sweet corn inbred lines. *Nguyen Thi Nguyet and Binh, Heliyon,* 1-12.

Turgut, İ. & Balcı, A. (2002). Effect of different sowing dates on fresh ear yield and yield components of sweet corn (*Zea mays saccharata* Sturt.) cultivars under Bursa conditions. *Uludağ University Journal of Agriculture Faculty,* 16:79-91 (in Turkish).

Zystro, J., Peters, T., Miller, K., & Tracy, W. F. (2021). Classical and genomic prediction of hybrid sweet corn performance in organic environments. *Crop Science*, 61:1698-1708.

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REWIEW PAPER

Current Progress on the Responses of Eggplant to Ultra-Low Temperatures during Production

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Abstract

Cold stress has an adverse effect on eggplant growth and is a yield-limiting factor. Low temperatures are prevalent during early spring cultivation in temperate climates, and they have a negative impact on eggplant growth and development. Temperatures below the eggplant's optimum growth temperature (22-30ºC) are considered low and detrimental to growth and development. In this review, we described how eggplants respond to moderately low and cold temperatures at different scales. We compiled literature on the current understanding of physiological, cellular responses to cold stress in eggplant as well as the transcriptional regulation during cold stress. Subsequently, we also highlight the genetic and molecular evidence, particularly the function of cold-responsive genes in strengthening cold tolerance in eggplant. Lastly, we covered the role of mineral nutrients and useful microorganisms in alleviating the consequences of cold stress in eggplant roots. Agronomic management practices such as the use of AMF species may mitigate the detrimental effects of low temperature and the enhancement of crop varieties with high yield throughout cold stress.

1. Introduction

Every year, there is a significant loss of crop yield and quality due to a range of abiotic stresses such as drought, salinity, high, and low temperature. Plant growth, development and yield are significantly affected by temperature (Prabhavathi and Rajam, 2007; Zhou et al., 2018). In the eggplant (*Solanum melongena*), a thermophilic vegetable and one the most important in the Mediterranean region and Asia, low temperatures affect productivity (eggplant yield and quality) particularly during early spring in those regions. Thus, the top eggplant producing countries (China, India, Egypt and Turkey) are located in Asia and in Mediterranean regions (Alam and Salimullah, 2021). Eggplant is ranked in the top ten vegetables with high anthocyanin content particularly in fruit peels. This particularity is mostly noticed in dark/purple skin eggplant cultivars. Furthermore, anthocyanins as important secondary metabolites play an important role in human health (Jiang et al., 2016). In contrast to other *Solanaceous* crops, eggplant is substantially more sensitive to low temperature (Wan et al., 2014). The growth and development of eggplant is optimum under this following range of temperatures; from 22 to 30ºC. Under low temperatures, the chilling damage devastates mostly freezing-sensitive eggplants. It is often followed by abnormal growth with disordered metabolism. During the winter season, low temperatures limit root growth, reduce plant vigor, and produce deformed fruits (Concellón et al., 2005; Zhou et al., 2018; Alam and Salimullah, 2021). It is also reported that low temperature stress in eggplant throughout the cool season (temperature below 15ºC) engenders gradual loss of pollen fertility and leads to the development of seedless

fruit (Nothmann and Koller, 1975; Abak and Guler, 1994; Makrogianni et al., 2017). The combination of insufficient light, low temperature, and/or high humidity under greenhouse cultivation decrease fruit yield and quality in eggplant (Acciarri et al., 2002). For instance, in North-China, it is reported that eggplant lower yield and poor quality subsist due to the cultivation in unheated greenhouses during winter season characterized by low temperatures and reduced light intensity (Gao et al., 2008).

In recent years, more attention has been given low temperature stress due to its adverse effects and its frequent occurrence in early spring (Yang et al., 2020). Thus, growing cold-tolerant varieties is affordable way to avoid huge losses arising from cold stress. Eggplant (*Solanum melongena* L.) originated in the subtropical climatic region. Moreover, it is famously grown in temperate climatic zones, both in the open field and in under covers. In temperate latitudes, eggplant seedlings growing usually starts in greenhouses (Pohl et al., 2019). Eggplant needs a temperature of approximately 15ºC (night) and 23-25ºC (day) for satisfactory development and yield; unless plant growth rate slows and fruit set is hampered. Heating in the Mediterranean Basin's low-cost and low-input greenhouses has not expanded due to the high cost of fuel. During the winter, for instance, temperatures are typically kept to a minimum, with nighttime temperatures sometimes falling below 10-15ºC (Makrogianni et al., 2017). It has been reported that treating flower buds with phytohormones alleviates the negative effects of suboptimal weather conditions on fruit production in the Mediterranean region, particularly during the winter season in unheated greenhouses. Unfortunately, because of the cost of both chemicals and labor, this treatment method is more expensive (Acciarri et al., 2002).

In this review, we compiled various published research on eggplant's growth and development-related responses to moderate low and cold temperature. It is well known that plants have built effective responses at the biochemical, physiological and morphological levels enabling them to mitigate and/or adapt to abiotic/biotic and disadvantageous environmental conditions. Thus, such responses to stress are complex and involve numerous factors such as signaling, hormones, transcription factors, and secondary metabolites.

2. Physiological changes during eggplant cultivation under low temperatures

It is acknowledged that unfavorable environmental conditions inhibit fruit set and growth in a variety of vegetable crops. High or low temperature and humidity, low light intensity, strong wind and heavy rain all have a negative impact on several stages of the reproductive cycle, including pollen formation, dissemination, and germination, fertilization, and seed maturation. As a result, they reduce fruit production (Donzella et al., 2000). It is reported that temperature lower than 17ºC causes a slowdown in eggplant growth and when it gets lower than 10ºC metabolic and physiological disturbance occurs. Moreover, low temperature is detrimental to the cell membrane system leading to the increase of relative conductivity with a change in Malondialdehyde (MDA) content and electrolyte leakage (Chen et al., 2011; Lv et al., 2017). Cold stress reduces the hydraulic conductance and osmotic potential in the cell, resulting in stomatal control setback (Latef et al., 2016). These physiological changes have a negative impact on pigment composition, chloroplast development and chlorophyll fluorescence which reduces photosynthetic efficiency (Pasbani et al., 2020).

Chilling temperatures generally refers to low temperatures in the range of 0-15ºC and that are somehow non-freezing. These kinds of temperatures are common during early spring in temperate regions. Thus, they are often detrimental to the productivity of chilling-sensitive plants (Pohl et al., 2019). According to reports, chilling stress induces a reduction of cell membranes' activities as well as the root respiratory intensity. Hence, undesirable active oxygen is produced in the eggplant. Accordingly, numerous enzymes of plant defense system, such as catalase (CAT), superoxide dismutase (SOD) and peroxidase (POD) as well as the antioxidants (ascorbic acid and reduced glutathione) are promptly employed to scavenge reactive oxygen species (ROS) (Gao et al., 2008). At the cellular level, accelerated ROS production alters homeostasis due to stress factors (Gao et al., 2008; Pohl et al., 2019). Additionally, adverse effect on the photosynthetic apparatus in tropical and subtropical plants are known to be caused by chilling temperatures as well as the reduction of plant photon energy utilization efficiency and acceleration of the oxygen generation resulting in low photosynthetic rate (Gao et al., 2008). By assessing the acclimation mechanisms of several eggplant (*Solanum melongena* L.) cultivars to chilling stress, Pohl et al. (2019) demonstrated that less chilling-sensitive eggplant cultivars were able to modify antioxidant system during exposure to low temperature stress (6ºC) compared to control plants kept at 18°C. Thus, eggplant's defense systems were quite enough to scavenge the surplus of ROS. Briefly, the antioxidant defense system played a vital role in the tolerance potential of eggplant cultivars and peroxidases (POX) and soluble sugars (SS) contributed to the anti-oxidative mechanisms involved in the stress response. Eggplant cultivars engage in metabolic efforts to raise SS content in leaf tissues. SS are reported in several publications as a major player in stress response and changes in ROS balance. They serve as signaling molecules and osmoprotectants to cellular membranes, not forgetting their role as energy providers and main building units. Sugars are also referred to as protectors of chloroplasts and photosynthesis

stabilizers in stress conditions (Peshev et al., 2013; Gangola and Ramadoss, 2018; Pohl et al., 2019).

It was determined by Xia et al. (2013) that under salt stress physiological changes of sensitive and tolerant eggplant seedlings are slightly different. Particularly in the leaves of sensitive eggplant materials, they observed a high increase of malondialdehyde (MDA) and O_2 contents, enzyme activities such as SOD and POD also rise as well as an increase in soluble protein and proline contents. All above mentioned increases changes are less significant in tolerant eggplant materials. The authors concluded that under cold stress, tolerant materials can mitigate cold damage by regulating enzyme activities to maintain plant metabolism and growth at normal rate. Likewise, Xia et al. (2018) advanced that under cold stress, the chlorophyll content in eggplant leaves diminish. However, an increase of proline, MDA, soluble sugar and soluble protein contents in the seedlings is noticed. Authors also reported the existence of high correlations between the electric conductivity (EC) and the MDA/soluble sugar content. Similarly, Lv et al. (2017) have confirmed that under cold stress, cold tolerant eggplant materials display high activity increase of POD, SOD, CAT as well as and a moderate increase of electrolyte leakage (EL) and MDA content compared to cold sensitive eggplant materials (Figure 1). Furthermore, soluble sugar, electric conductivity and MDA have been reported as suitable for assessing eggplant seedling cold tolerance (Xia et al., 2018).

It was also reported by Wang et al. (2009) that cold stress alters photosystem II, the second type of photosynthesis known for capturing the energy from sunlight and employing it to extract electrons from water molecules. Moreover, it is recognized that during rapid changes in temperatures (low/high) an immediate cellular acclimation reaction is initiated in chloroplasts. It consists of changes in thylakoid-located mechanisms as well as the stroma and transport activities alteration throughout the chloroplast envelope. Thus, these reactions reinstate homeostatic level and assist in plant stress tolerance (Schwenkert et al., 2022). Eggplants are good for the human diet due to their high antioxidant and low carbohydrate content (Makrogianni et al., 2017). However, some studies have demonstrated that low-temperature stress has an effect on antioxidant content. Pohl et al., (2019) demonstrated, for instance, that during chilling acclimation at extremely low temperatures (6°C), eggplant seedlings, particularly chilling-sensitive cultivars, exhibit an increase in antioxidant activity, followed by an increase in malondialdehyde (MDA) and H_2O_2 content.

3. Molecular mechanisms of low temperature response in eggplant

A comprehensive understanding of the molecular mechanisms governing low temperature tolerance is necessary to ensure high yield and quality productivity of eggplant and can facilitate the creation of crop varieties that can mitigate the severity of cold stress on productivity (Yang et al., 2020; Zhou et al., 2020). The sensing and signalling of low temperature stress is a complex process remodeling biochemical, morphological and physiological processes in plants. Numerous transcriptional factors, core genes and pathways associated with the regulation of plant growth, development and response to low temperature have been reported in literature (Mehrotra et al., 2020; Yang et al., 2020). Table 1 shows a number of cold-responsive genes in eggplant. However, more studies are needed before developing stress-tolerant eggplant varieties.

Previously, quantitative genetics was also tried in stress-related studies in eggplants. For instance Yang et al. (2017) utilized high throughput sequencing to investigate the role of MicroRNAs (miRNAs) in plant development and stress responses in wild eggplant (*Solanum*

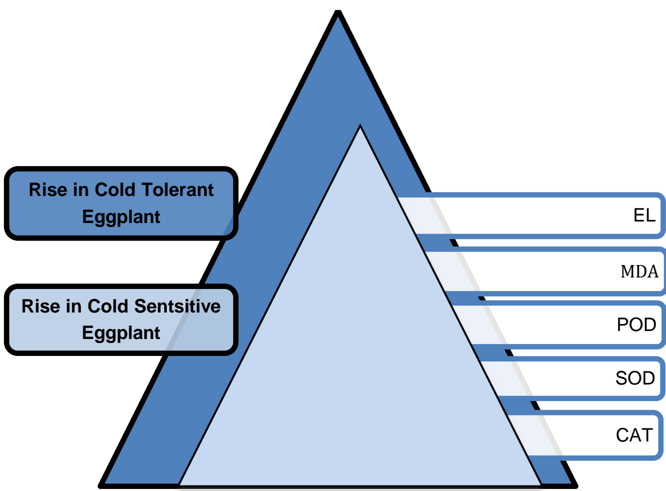

Figure 1. Major physiological changes of cold sensitive and tolerant eggplants under cold stress.

Table 1. Gene related to low temperature stress in eggplant.

No	Gene	Family/Subfamily and/or Superfamily	Function	Mechanism of action	Reference
1	*SmAP2/ERF* genes	*AP2/ERF*[b] superfamily	Transcription factor	Response to plant hormones and stress conditions such as response to low temperature.	(Li et al., 2021)
2	*SmNAC* genes (*SmNAC19, SmNAC75* and *SmNAC31*)	*NAC* family/ATAF subfamily	Transcription factor	The control of a broad range of responses to different abiotic stresses.	(Wan et al., 2021)
3	A total of 20 Hsf (*SmeHsf*) genes were identified	*SmeHsf* family	Transcription factors	Under stress conditions, Hsfs are implicated in a variety of protein homeostasis. They improve tolerance to a variety of abiotic stresses, including low temperature stress.	(Wang et al., 2020)
4	*CTR1* and *EBF1/2*	N/A	Transcription factor	Response to low temperature and ethylene signal transduction during cold stress.	(Yang et al., 2020)
5	*SmWRKY* genes (*SmWRKY26* and *SmWRKY32*)	*WRKY* gene family	Transcription factors	They favorably regulate the response to cold stress.	(Yang et al., 2020)
6	*SmICE1a*	*CBF*[a] family	Transcription factor	It trigger expression of SmCBF2, SmCBF1 and SmCBF3 and it plays a crucial role in cold response.	(Zhou et al., 2020)
7	*SmMYB113*	*CBF*[a] family/	Transcription factors	It is involved in cold-controlled anthocyanin biosynthesis.	(Zhou et al., 2019)
8	*SmCBFs* (*SmCBF1, SmCBF2* and *SmCBF3*)	*CBF*[a] family/ CBF-*DREB* subfamily	Transcription factors	They are engaged in abiotic stress response in eggplant including in response to low temperature.	(Zhou et al., 2018)
9	P_{SAG12}-*IPT*	*IPT* gene	N/A	The overexpression of the PSAG12-IPT gene defers leaf senescence and also triggers abiotic stress tolerance.	(Xiao et al., 2017)
10	*SmCHS*[d], *SmCHI*[c], *SmF3H*[e], and *SmDFR*[f]	N/A	Anthocyanin biosynthesis genes	Up-regulation under cold stress.	(Jiang et al., 2016)

[a]*CBFs*: The C-repeat binding factors, [b]*AP2/ERF*: APETALA2/Ethylene Response Factor, [c]*SmCHI*: chalcone isomerase, [d]*SmCHS*: chalcone synthase, [e]*SmF3H*: flavanone 3-hydroxylase, [f]*SmDFR*: dihydroflavonol 4-reductase.

aculeatissimum) subjected to low temperature stress. They discovered nine significant miRNAs that are expressed in response to low temperature stress. Furthermore, many target genes of miRNAs involved in the chilling response were identified. Thus, target genes were engaged in a variety of roles, such as the expression of anti-stress proteins and antioxidant enzymes. Such studies contribute to the broad knowledge on cold-tolerance regulation mechanisms as well as a theoretical background for future research on low temperature stress in eggplant.

4. Reaction of transgenic eggplant to low temperature stress

Several studies have found that eggplant transgenic lines have some level of tolerance to a set of abiotic stresses, including low temperature stress. Wan et al. (2014) discovered that the expression of Arabidopsis cold-regulated 15A (*AtCOR15A*) and C-repeat binding factor 3 (*AtCBF3*) genes in transgenic eggplants (*Solanum melongena*) increases significantly under extreme low temperature (2 ± 1ºC) stress. Consequently, it improved eggplant chilling tolerance. Likewise, Prabhavathi and Rajam (2007) tested transgenic eggplant seeds with increased polyamines (PA) particularly due to exogenous adc (arginine decarboxylase) gene (introduced through primary transformants self-pollination) in low temperature tolerance assays performed *in vivo* and *in vitro* growth conditions. They discovered that after being exposed to extra low temperatures (6–8ºC), transgenic seeds germinated 4 days and one week earlier *in vivo* and *in vitro*, respectively, when compared to control seeds. Thus, transgenic lines showed increased tolerance to low temperatures and other abiotic stresses, as well as rapid growth. Furthermore, Acciarri et al. (2002) studied the growth and fruit development of *DefH9-iaaM* transgenic hybrid eggplants (parthenocarpic hybrids) grown during early spring production in two regions of Italy with average temperatures ranging

from 7 to 17ºC. They discovered a significant yield increase (up to six-fold higher) when compared to controls (commercial hybrids and open-pollinated parthenocarpic cultivars).

Donzella et al. (2000) conducted a similar study in which they assessed the productivity of transgenic eggplant hybrids (containing the parthenocarpic gene *DefH9-iaaM*) under cold stress (unheated greenhouse). Transgenic eggplant was compared to controls (untransformed eggplant hybrids) and commercial parthenocarpic cultivars treated or untreated with phytohormones, which trigger fruit set and growth (the transformation of an ovary into a fruit) in greenhouse-grown eggplant. In both cases, they discovered that the productivity of transgenic eggplant was still higher. It was proved that transgenic parthenocarpic eggplants rise winter production and can be beneficial to all Mediterranean region where unheated greenhouses still prevail (Donzella et al., 2000; Acciarri et al., 2002). Furthermore, it was reported by Ying et al. (2009) that at low temperatures (~12.8ºC), the eggplant parthenocarpic gene is expressed normally, resulting in the formation and development of parthenocarpic fruits at 100%. Thus, under cold stress, eggplant resources with parthenocarpic ability demonstrated a greater ability to bear multitude fruits without pollination than non-parthenocarpic resources that require pollination. Parthenocarpy can overcome flower and fruit loss as well as increase eggplant yield, and lower the production costs (Lv et al., 2017).

6. Low temperature stress mitigation approaches in eggplant

Grafting is one the approaches used to mitigate the impact of abiotic stresses such as low temperature during preharvest. Handful studies conducted in eggplant have identified rootstock-scion combinations able to improve the low temperature tolerance. For instance, Darré et al. (2021) in their experiment, a cold-tolerant rootstock (Java) were combined with (cv. Monarca) scion and they found that the grafting enhanced plant vigor and fruit growth rate by reducing time to harvest by 10 to 15%. Thus, grafted eggplant showed reduced contents of dry matter content and phenolic compounds (~15–20% each), as well as lower respiration (~60%) than controls. Obviously, it results in higher tolerance to chilling injury of grafted eggplants. Remarkably, eggplant growth performance under cold stress was improved significatively. Gao et al. (2008) have reported a successful improvement of cold tolerance in eggplant seedling scions and rootstocks. The authors found that after a chilling treatment at 10/4ºC (day/night), chilling-tolerant rootstocks (Hiranasu) in which they grafted a cold tolerant scion “Jinong 2000” resulted in stronger cold tolerant eggplant with a more improved scion tolerance. In the same manner, it was reported by Gao et al. (2006) that a rootstock with stronger cold tolerance improves eggplant root activity as well as the cold resistance. This conclusion was obtained after evaluating physiological and biochemical property changes of grafted eggplant seedling roots under low temperature stress. Yan et al. (2009) also contributed to evaluate grafted eggplant seedlings tolerance to winter cold stress. They employed a rootstock/scion interchangeability between two eggplant cultivars; one tolerant (Hiranasu) and other susceptible (Daidaro) to cold stress. After measuring several physiology criterions (such as MDA content, electrolyte leakage, and essential osmotic adjustment substances) before and after low temperature treatment in grafted seedling leaves, it was concluded that only the tolerant cultivar was able to enhance cold tolerance through both its root and shoot.

The arbuscular mycorrhizal fungi (AMF) have been employed as a mitigation approach to cold stress in eggplant, particularly in *Solanum melongena*. Pasbani et al. (2020) inoculated and evaluated several AMF species on three ranges of temperature including low temperatures (15ºC and 5ºC). They found that three from four inoculated AMF species (*Claroideoglomus etunicatum*, *Funneliformis mosseae*, and *Rhizophagus irregularis*) alleviated cold stress in *S. melongena* despite their low level of root colonization. Thus, they activated antioxidant defense systems, improved photochemical reactions as well as the accumulation of protecting molecules (free phenolics and prolines), and also reduced membrane damages. Cytokines are known to play a role in the regulation of plant abiotic stress tolerance and adaptation. Chen et al. (2015) tested the effect of an exogenous and synthetic cytokinin, 6-benzylaminopurine (6-BA), on eggplant (*Solanum melongena*) seedling growth, osmoregulation responses, and antioxidant defense system under low temperature stress. Moreover, exogenous 6-BA pretreatment (through spray application) has also been shown to significantly reduce the low-temperature-induced decrease in eggplant growth. As shown in Figure 2, it also improves the activities of several enzymes and helps to reduce the activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT). Finally, the overall results show that 6-BA pretreatment improves eggplant seedlings' low temperature tolerance.

7. Research perspective and conclusion

High-throughput mRNA sequencing techniques and genome-wide sequencing in broad sense provide clues on the roles and regulatory mechanisms of genes involved in stress responses and can be used for rapid and efficient transcriptome characterization. In the future, the use of these techniques will contribute to a greater understanding of stress regulation in eggplants. A

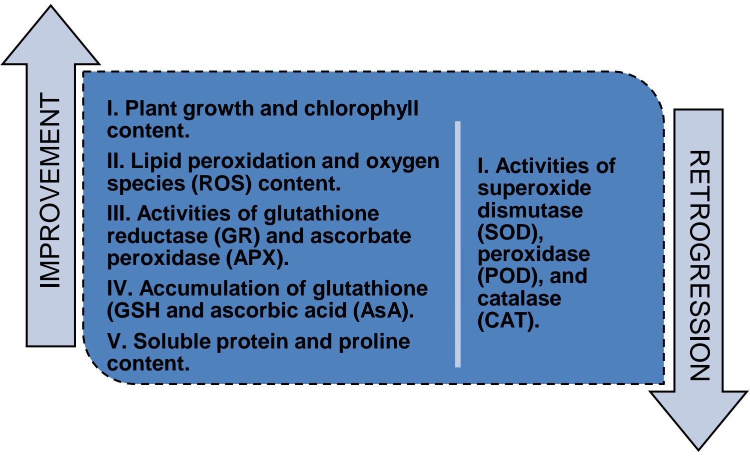

Figure 2. Exogenous application of 6-BA and related improvement under low temperature stress.

mentioned above, the discovery of stress-regulated miRNAs is critical for understanding how eggplant responds to various stresses. So far, only a few studies using these techniques in eggplant have been published and future research in eggplant will be oriented toward the use of high throughput sequencing to understand cold stress regulation mechanisms. Low temperature is among the stresses that endanger eggplant throughout its entire growth phase, resulting in the suppression of growth and development and a drop in productivity (Yang et al., 2020). This review summarizes current knowledge in key areas concerning eggplant low temperature stress tolerance. In addition, a description and repercussions of genetic, biochemical and physiological changes with regard to low temperature are presented. The development of transgenic parthenocarpic eggplants, which ameliorate low-temperature induced deficiencies in fruit set, as well as the use of grafting and arbuscular mycorrhizal fungi, were addressed as potential techniques to boost eggplant productivity in suboptimal conditions often seen during winter particularly in the Mediterranean region and some part of Asia. Furthermore, ectopic expression of genes such as AtCOR15A and AtCBF3 can help eggplants resist low temperature stress.

References

Abak, K., & Guler, H.Y. (1994). Pollen fertility and the vegetative growth of various eggplant genotypes under low temperature greenhouse conditions. *Acta Horticulturae*, 366:85–92.

Acciarri, N., Restaino, F., Vitelli, G., Perrone, D., Zottini, M., Pandolfini, T., Spena, A., & Rotino, G.L. (2002). Genetically modified parthenocarpic eggplants: improved fruit productivity under both greenhouse and open field cultivation. *BMC Biotechnology*, 2:4.

Alam, I., & Salimullah, M. (2021). Genetic engineering of eggplant (*Solanum melongena* L.): Progress, controversy and potential. *Horticulturae*, 7:1-29.

Chen, J., Wu, X., Yao, X., Zhu, Z., Xu, S., & Zha, D. (2015). Exogenous 6-benzylaminopurine confers tolerance to low temperature by amelioration of oxidative damage in eggplant (*Solanum melongena* L.) seedlings. *Brazilian Journal of Botany*, 39:409–416.

Chen, S., Zimei, L., Cui, J., Jiangang, D., Xia, X., Liu, D., & Yu, J. (2011) Alleviation of chilling-induced oxidative damage by salicylic acid pretreatment and related gene expression in eggplant seedlings. *Plant Growth Regulation*, 65:101–108.

Concellón, A., Añón, M.C., & Chaves, A.R. (2005) Effect of chilling on ethylene production in eggplant fruit. *Food Chemistry* 92:63-69.

Darré, M., Valerga, L., Zaro, M.J., Lemoine, M.L., Concellón, A., & Vicente, A.R. (2021). Eggplant grafting on a cold-tolerant rootstock reduces fruit chilling susceptibility and improves antioxidant stability during storage. *Journal of the Science of Food and Agriculture*, doi:10.1002/jsfa.11682.

Donzella, G., Spena, A., & Rotino, G.L. (000). Transgenic parthenocarpic eggplants: superior germplasm for increased win2ter production. *Molecular Breeding* ,6:79–86.

Gangola, M.P., & Ramadoss, B.R. (2018). Sugars play a critical role in abiotic stress tolerance in plants. pp. 17–38. In: Wani, S.H. (ed.) Biochemical, physiological and molecular avenues for combating abiotic stress tolerance in plants (1st Edition). Academic Press, Cambridge, MA.

Gao, Q., Wu, Y., Xu, K., & Gao, H. (2006). Responses of grafted eggplant seedling roots to low temperature stress. *Ying Yong Sheng Tai Xue Bao*, 17:390–394.

Gao, Q.H., Xu, K., Wang, X.F., & Wu, Y. (2008). Effect of grafting on cold tolerance in eggplant seedlings. *Acta Horticulturae*, 771:167–174.

Jiang, M., Liu, Y., Ren, L., Lian, H., & Chen, H. (2016). Molecular cloning and characterization of anthocyanin biosynthesis genes in eggplant (*Solanum melongena* L.). *Acta Physiologiae Plantarum* 38:163.

Latef, A.A.H.A., Hashem, A., Rasool, S., Abd_Allah, E.F., Alqarawi, A.A., Egamberdieva, D., Jan, S., Anjum, N.A., & Ahmad, P (2016) Arbuscular mycorrhizal symbiosis and abiotic stress in plants: A review. *Journal of Plant Biology,* 59:407–426.

Li, D., He, Y., Li, S., Shi, S., Li, L., Liu, Y., & Chen, H. (2021). Genome-wide characterization and expression analysis of AP2/ERF genes in eggplant

(*Solanum melongena* L.). *Plant Physiology and Biochemistry,* 167:492–503.

Lv, L.L., Li, W., Xiao, X.O., & Gao, X.M. (2017). Grey Correlative Degree Analysis on the Cold-Resistant Traits of Parthenocarpic Eggplant. *Journal of Agricultural Science,* 9:95–104.

Makrogianni, D.I., Tsistraki, A., Karapanos, I.C., & Passam, H.C. (2017). Nutritional value and antioxidant content of seed-containing and seedless eggplant fruits of two cultivars grown under protected cultivation during autumn-winter and spring-summer. *Journal of the Science of Food and Agriculture,* 97:3752–3760.

Mehrotra, S., Verma, S., Kumar, S., Kumari, S., & Mishra, B.N. (2020). Transcriptional regulation and signalling of cold stress response in plants: an overview of current understanding. *Environmental and Experimental Botany*, 180:104243.

Nothmann, J., & Koller, D. (1975). Effects of Low-Temperature Stress on Fertility and Fruiting of Eggplant (*Solanum melongena*) in a Subtropical Climate. *Experimental Agriculture*, 11:33–38.

Pasbani, B., Salimi, A., Aliasgharzad, N., & Hajiboland, R. (2020). Colonization with arbuscular mycorrhizal fungi mitigates cold stress through improvement of antioxidant defense and accumulation of protecting molecules in eggplants. *Scientia Horticulturae*, 272:109575.

Peshev, D., Vergauwen, R., Moglia, A., Hideg, E., & Van den Ende, W. (2013). Towards understanding vacuolar antioxidant mechanisms: a role for fructans? *Journal of Experimental Botany,* 64:1025–1038.

Pohl, A., Komorowska, M., Kalisz, A., & Sękara, A. (2019). Eggplant seedlings modify antioxidant system during acclimation to low temperature. *Agrochimica,* 63:151–167.

Prabhavathi, V.R., & Rajam, M.V. (2007). Polyamine accumulation in transgenic eggplant enhances tolerance to multiple abiotic stresses and fungal resistance. *Plant Biotechnology,* 24:273–282.

Schwenkert, S., Fernie, A.R., Geigenberger, P., Leister, D., Möhlmann, T., Naranjo, B., & Ekkehard Neuhaus, H. (2022). Chloroplasts are key players to cope with light and temperature stress. *Trends in Plant Science* https://doi.org/10.1016/j.tplants.2021.12.004.

Wan, F.-X., Gao, J., Wang, G.-L., Niu, Y., Wang, L.-Z., Zhang, X.-G., Wang, Y.-Q& Pan, Y. (2021). Genome-wide identification of NAC transcription factor family and expression analysis of ATAF subfamily members under abiotic stress in eggplant. *Scientia Horticulturae,* 289:110424.

Wan, F., Pan, Y., Li, J., Chen, X., Pan, Y., Wang, Y., Tian, S., & Zhang, X. (2014). Heterologous expression of Arabidopsis C-repeat binding factor 3 (AtCBF3) and cold-regulated 15A (AtCOR15A) enhanced chilling tolerance in transgenic eggplant (*Solanum melongena* L.). *Plant Cell Reports,* 33:1951–1961.

Wang, J., Hu, H., Wang, W., Wei, Q., Hu, T., & Bao, C. (2020). Genome-wide identification and functional characterization of the heat shock factor family in eggplant (*Solanum melongena* L.) under abiotic stress conditions. *Plants*, 9:915.

Wang, M., Gao Z., Huang, R., Lü G., Zhang, W., & Du, S. (2009). Studies on cold shock stress effect of photosystem II and its thermodynamics analyse in eggplant. *Acta Horticulturae Sinica*, 36:261–266.

Xia, L.C., Ying, L.B., Ying, S.B., Xu, L., Nan, L.Y., Yue, L.Z. (2018). Effects of low temperature on physiological properties of eggplant seedlings and selection of cold-tolerance indicators. *Fujian Journal of Agricultural Sciences,* 33:930–936.

Xia, X., Chun, Y.X., Shi, Z.D., Wen, Z.Z., & Shuang, X. (2013). Studies on physiological mechanism of eggplant under low temperature stress. *Acta Agriculturae Shanghai,* 29:45–49.

Xiao, X.O., Zeng, Y.M., Cao, B.H., Lei, J.J., Chen, Q.H., Meng, C.M., & Cheng, Y.J. (2017). P_{SAG12}-IPT overexpression in eggplant delays leaf senescence and induces abiotic stress tolerance. *The Journal of Horticultural Science and Biotechnology,* 92:349–357.

Yan, Z.X., & Kun, X. (2009). Effect of interaction between rootstock and scion on chilling tolerance of grafted eggplant seedlings under low temperature and light conditions. *Scientia Agricultura Sinica,* 42:3734–3740.

Yang, Y., Liu, J., Zhou, X., Liu, S., & Zhuang, Y. (2020). Identification of WRKY gene family and characterization of cold stress-responsive WRKY genes in eggplant. *Peer Journal*, https://doi.org/10.7717/peerj.8777

Yang, X., Liu, F., Zhang, Y., Wang, L., & Cheng, Y. (2017). Cold-responsive miRNAs and their target genes in the wild eggplant species *Solanum aculeatissimum*. *BMC Genomics*, 18:1000.

Yang, Y., Liu, J., Zhou, X., Liu, S., & Zhuang, Y. (2020). Transcriptomics analysis unravels the response to low temperature in sensitive and tolerant eggplants. Scientia Horticulturae 271:109468.

Ying, Z., Zhong, L.F., Hui, C.Y., & Yong, L. (2009). Characteristics of eggplant parthenocarpy at low temperature. *China Vegetables*, 2:16–20.

Zhou, L., He, Y., Li, J., Li, L., Liu, Y., & Chen, H.Y. (2020). An eggplant SmICE1a gene encoding MYC-type ICE1-like transcription factor enhances freezing tolerance in transgenic *Arabidopsis thaliana*. *Plant Biology*, 22:450–458.

Zhou, L., He, Y., Li, J., Liu, Y., & Chen, H. (2019). CBFs function in anthocyanin biosynthesis by interacting with MYB113 in eggplant (*Solanum melongena* L). *Plant and Cell Physiology*, 61:416–426.

Zhou, L., Li, J., He, Y., Liu, Y., & Chen, H. (2018). Functional characterization of SmCBF genes involved in abiotic stress response in eggplant (*Solanum melongena*). *Scientia Horticulturae*, 233:14–21.