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## Determination of Heat Tolerance of Interspecific (*Cucurbita maxima* x *Cucurbita moschata*) Inbred Line of Squash ‘*Maxchata*’ and Its Parents through Photosynthetic Response

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### ABSTRACT

Development of heat tolerant cultivars is needed to combat the challenges of global warming and food supply to increasing population. This study was conducted to determine the extent of heat tolerance of newly developed interspecific inbred line of squash named as ‘*Maxchata*’ through its photosynthetic attributes compared to its parents *C. maxima* and *C. moschata*. Plants of these three genotypes were subjected to three different temperatures i.e 30 °C day/25 °C night as control, 37 °C day/32 °C night as moderate heat stress and 42 °C day/37 °C night as severe heat stress, for seven days. Results showed that various gas exchange attributes such as net photosynthesis rate ( $P_N$ ), stomatal conductance ( $g_s$ ) and transpiration rate ( $E$ ) as well as maximum photochemical efficiency of PSII ( $F_v/F_m$ ) dropped significantly with increasing temperature, while intercellular CO<sub>2</sub> concentration ( $C_i$ ) increased showing the nonstomatal limitations. Further, chlorophyll pigments also degraded with heat shocks resulting in higher Chl *a* to *b* ratio and decreased chlorophyll to carotenoids ratio. However, these trends were more abrupt in *C. maxima*, chased by ‘*Maxchata*’ and then *C. moschata*. *C. moschata* had the best photosynthetic machinery to sustain the heat regimes, followed by ‘*Maxchata*’, while *C. maxima* was the most susceptible. Hybrid ‘*Maxchata*’ with some degree of heat tolerance might have ability to cope with the climate change.

Keywords: Chlorophyll florescence; Cucurbits; Gas exchange; Heat stress; Pumpkin; Thermotolerance

## Kendilenmiş Türler Arası Melez Kabak Hattı ‘*Maxchata*’ (*Cucurbita maxima* x *Cucurbita moschata*)’nın ve Ebeveynlerinin Fotosentetik Tepki Yoluyla Sıcaklık Toleransının Belirlenmesi

### ESER BİLGİSİ

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**ÖZET**

Artan nüfus için gıda temini ve küresel ısınmanın oluşturduğu zorluklarla mücadele için sıcaklık stresine toleranslı çeşit geliştirmek gerekmektedir. Bu çalışma, *Maxchata* olarak bilinen ve yeni geliştirilmiş kendilenmiş türler arası melez kabak hattının fotosentetik karakteristikleri yoluyla sıcaklık stresine karşı tolerans seviyesinin belirlenmesi ve ebeveynlerinin (*C. maxima* ve *C. moschata*) performansı ile karşılaştırılması amacıyla yapılmıştır. Bu üç genotipe ait bitkiler 7 gün süre ile 30/25 °C gündüz/gece (kontrol), 37/32 °C gündüz/gece (orta sıcaklık stresi) ve 42/37 °C gündüz/gece (şiddetli sıcaklık stresi) sıcaklık derecesine maruz bırakılmıştır. Sonuçlar, sıcaklık artışına bağlı olarak net fotosentez oranı ( $P_N$ ), stoma iletkenliği ( $g_s$ ), terleme oranı ( $E$ ) ve PSII ( $F_v/F_m$ )'nin maksimum fitokimyasal etkinliği gibi bazı gaz değişim karakteristiklerinin önemli derecede düştüğünü, bununla birlikte hücrelerarası karbondioksit ( $C_i$ ) konsantrasyonunun arttığını (stomadan kaynaklanmayan sınırlamaları) göstermiştir. Bunun ötesinde, yüksek sıcaklık şokuna bağlı olarak birlikte klorofil pigmentleri parçalanmış ve sonuçta Chl  $a/b$  oranı yükselmiş ve klorofil/karotenoid oranı azalmıştır. Ancak, bu değişim *C. maxima*'da daha belirgin olmuş, bunu *Maxchata*' takip etmiş ve ardından *C. moschata* gelmiştir. *C. moschata* sıcaklık rejimlerine karşı en iyi fotosentetik mekanizmaya sahip bulunmuş, bunu *Maxchata*' izlemiş, ancak en hassas *C. maxima* olmuştur. Belli derecede sıcaklık toleransına sahip olan melez 'Maxchata'nın iklim değişikliği etkisine karşı yararlı olabileceği düşünülmektedir.

Anahtar Kelimeler: Klorofil floresansı; Cucurbits; Gaz değişimi; Sıcaklık stresi; Kabak; Termotolerans

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**1. Introduction**

Heat stress is considered as an important yield limiting factor of the plants worldwide. Temperature is one of the main ecological variables that determine the natural geographical and seasonal distribution of plants (Berry & Bjorkman 1980). According to recent climate models prediction, the higher rates of population growth and emission of greenhouse gases may cause the global mean temperature to rise 0.3°C per decade reaching to approximately 1°C and 3°C above the present value by 2025 and 2100, respectively (Jones 1999; IPCC 2007; Wahid et al 2007). With global warming, expansion of the arid and semiarid regions is also expected. The industrialization and urbanization further aggravate the situation. This scenario forces all the scientists, especially plant breeders to strive for the development of high yielding heat tolerant cultivars to combat this challenging situation.

In an attempt to address this problem, we tried to bring innovation in squash that belongs to family "*Cucurbitaceae*". The cucurbit species are among the 10 leading vegetable crops all around the world. China ranks first, followed by Iran and Turkey among the cucurbits producing countries (FAO 2011). Chinese squash (*Cucurbita moschata*) is best

adapted to hot climate and is successfully cultivated in the tropical and subtropical regions (Balkaya et al 2010). On the other hand, Indian squash (*Cucurbita maxima*) is widely grown in comparatively warm temperate areas of the world (Paris 1994; Malik 1996; Bisognin 2002). Generally, *C. maxima* is more famous for its brighter yellowish orange flesh color, taste, texture and other quality attributes than *C. moschata*. But it is more susceptible to diseases and heat stress. Hence, we developed an interspecific inbred line by crossing these two species, followed by selfing to combine the good attributes of both of them. This novel interspecific inbred line has been named as '*Maxchata*'.

The response of photosynthesis to temperature is a central facet of plant response to climate exhibiting variation among species and studies and has been considered the best indicator of thermotolerance of plants (Berry & Bjorkman 1980; Pastene & Horton 1996; Wahid et al 2007; Djanaguiraman et al 2011; Lin et al 2012; Zeng et al 2012). Possible related biochemical and physiological processes that are affected by heat stress include the activity of photosynthetic enzymes such as Rubisco, membrane integrity and permeability especially that of thylakoids, photorespiration, photophosphorylation,

electron flow in chloroplast and stomatal conductance and assimilate translocation (Dinar & Rudich 1985; Jensen 2000; Camejo & Torres 2001). Generally, high temperature greatly affects the photochemical reaction in the thylakoids lamellae and carbon metabolism in the stroma of chloroplast (Wise et al 2004). The function of PSII in the thylakoids, as determined by the chlorophyll fluorescence, is highly sensitive to temperature (Camejo et al 2005). Heat stress may disturb the oxygen evolving complex resulting in imbalance electron flow and generation of reactive oxygen species that result in cellular injury (De Ronde et al 2004). Likewise, pigment content of light harvesting chlorophyll-carotenoids antenna complexes of PSI and PSII also undergo changes under heat stress and degradation of chlorophyll *a* and *b* is more pronounced (Camejo & Torres 2001). Further, the leaf gas exchange capacity and maximum photochemical efficiency of PSII of the plant is also highly correlated with the thermotolerance of plant (Hall 1992; Maxwell & Johnson 2000; Hassan 2006). Net photosynthesis rate, stomatal conductance and transpiration rates are generally reduced with high temperatures, while intercellular carbon dioxide concentrations ( $C_i$ ) either increase or decrease depending upon stomatal and non stomatal limitations.

The present study was conducted to determine various photosynthetic characteristics of the newly developed interspecific inbred line of squash 'Maxchata' as well as its parents *i.e.* *C. maxima* and *C. moschata* and further to unveil the secret of thermotolerance of 'Maxchata' by its photosynthetic response at different temperature regimes to know about its adaptability and efficiency to cope with the challenging global climate change.

## 2. Materials and Methods

### 2.1. Plant material and experimental conditions

The plants of *C. maxima*, *C. moschata* and 'Maxchata' hybrid were raised in pots containing mixture of peat moss, vermiculite and perlite (3:1:1, v/v/v) at Zhejiang University, P.R. China during spring of 2012. The seedlings were kept in growth

chamber (MLR352H Sanyo, Panasonic biomedical sale group, Europe BV) initially with growth conditions of 30 °C day/25 °C night temperatures, 10 hour dark and 14 hour light (100  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and 70% humidity. When seedlings were reached to 3-4 leaf stage, the raised plants were divided into three equal groups. One group was subjected to the same growing temperature as control stock. The second group was subjected to moderate heat shock at 37 °C day/32 °C night temperatures for 7 days. While the last group was raised in severe heat stress conditions with 42 °C day/37 °C night temperatures for 7 days. These temperature ranges were selected on the basis of plants response in some pre-experiments. The experiment was designed in completely randomized design with four replications. There were total nine different treatments *i.e.* three different genotypes with three different temperatures. Each replication had four plants of each genotype. After 7 days of exposure, the data on the below mentioned various photosynthetic parameters were collected.

### 2.2. Chlorophyll extraction and quantification

Chlorophylls and carotenoids were extracted with mortar and pestle in chilled 100% methanol. After centrifugation, the Chlorophyll *a*, *b* and carotenoids were determined using spectrophotometer (UV-2550 UV-VIS, Shimadzu corporation, Kyoto, Japan) at wavelengths of 665.2 nm (Chl *a*), 652 nm (Chl *b*) and 470 nm (Carotenes). The concentrations were computed according to Porra et al (1989).

### 2.3. $\text{CO}_2$ assimilation

Net photosynthesis rate ( $P_N$ ) was measured with a portable photosynthesis system of infrared gas analyzer (LI-6400XTR, LI-COR Inc., NE, USA) using photon flux of 1200  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . This machine also gave the simultaneous readings for intercellular  $\text{CO}_2$  concentrations ( $C_i$ ), transpiration rate ( $E$ ) and stomatal conductance ( $g_s$ ).

### 2.4. Chlorophyll fluorescence

Chlorophyll fluorescence was measured with a non portable Chlorophyll fluorometer (D-91090 EffeLtrich Heinz Walz, Germany). Plants were first

stabilized in dark for 15 minutes and then minimal fluorescence ( $F_0$ ) was recorded. Next, a saturation pulse of  $6000 \mu\text{mol m}^{-2} \text{s}^{-1}$  for 0.8 second was applied for recording maximal fluorescence ( $F_m$ ). The maximum photochemical efficiency of photosystem II ( $F_v/F_m$ ) was determined by using  $F_v/F_m$  option button of ImagingWin v2.39 software.

### 2.5. Statistical analysis

All the measurements were repeated four times and the data was subjected to statistical analysis by using SAS software (SAS 9.1, SAS Institute, NC, USA). One way ANOVA was conducted to reveal the significant differences among the genotypes at each temperature range. The mean  $\pm$  Standard deviation (SD) were compared by Tukey's test. Comparisons with P values  $<0.01$  were rated as significantly different.

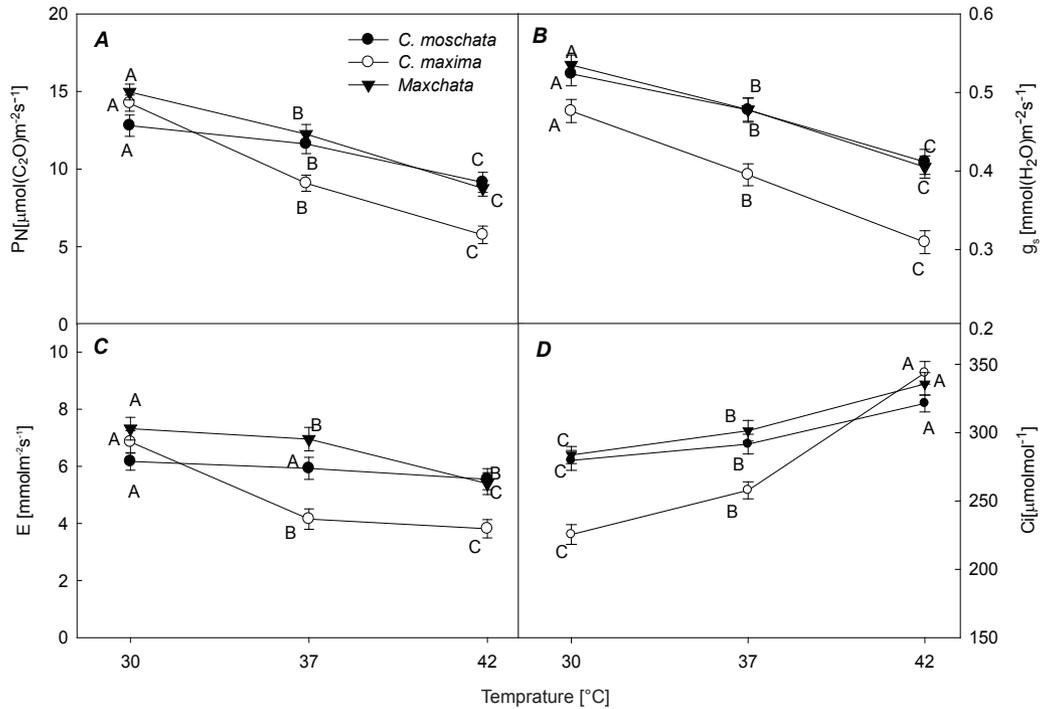
## 3. Results and Discussion

### 3.1. Effect of heat stress on $P_N$ , $g_s$ , $E$ and $C_i$

Net photosynthesis rate ( $P_N$ ) exhibited significant differences among selected genotypes under heat stress (Figure. 1A).  $P_N$  decreased from  $12.7 \mu\text{mol m}^{-2} \text{s}^{-1}$  to  $11.6 \mu\text{mol m}^{-2} \text{s}^{-1}$  at  $37^\circ\text{C}$  and to  $9.1 \mu\text{mol m}^{-2} \text{s}^{-1}$  at  $42^\circ\text{C}$  in *C. moschata*. On the other hand, *C. maxima* had  $P_N$   $14.24 \mu\text{mol m}^{-2} \text{s}^{-1}$  at  $30^\circ\text{C}$ ,  $9.09 \mu\text{mol m}^{-2} \text{s}^{-1}$  at  $37^\circ\text{C}$  and  $5.77 \mu\text{mol m}^{-2} \text{s}^{-1}$  at  $42^\circ\text{C}$ . However, 'Maxchata' showed reduction from  $14.96 \mu\text{mol m}^{-2} \text{s}^{-1}$  to  $12.25 \mu\text{mol m}^{-2} \text{s}^{-1}$  and  $8.76 \mu\text{mol m}^{-2} \text{s}^{-1}$  under moderate and severe heat stress respectively. Stomatal conductance ( $g_s$ ) reduced from  $5.24 \text{mmol (H}_2\text{O) m}^{-2} \text{s}^{-1}$  to  $4.77 \text{mmol (H}_2\text{O) m}^{-2} \text{s}^{-1}$  at  $37^\circ\text{C}$  and to  $0.411 \text{mmol (H}_2\text{O) m}^{-2} \text{s}^{-1}$  at  $42^\circ\text{C}$  in *C. moschata*, from  $0.476 \text{mmol (H}_2\text{O) m}^{-2} \text{s}^{-1}$  to  $0.395 \text{mmol (H}_2\text{O) m}^{-2} \text{s}^{-1}$  at  $37^\circ\text{C}$  and to  $0.309 \text{mmol (H}_2\text{O) m}^{-2} \text{s}^{-1}$  at  $42^\circ\text{C}$  in *C. maxima*, and from  $0.53 \text{mmol (H}_2\text{O) m}^{-2} \text{s}^{-1}$  to  $0.478 \text{mmol (H}_2\text{O) m}^{-2} \text{s}^{-1}$  at  $37^\circ\text{C}$  and to  $0.405 \text{mmol (H}_2\text{O) m}^{-2} \text{s}^{-1}$  at  $42^\circ\text{C}$  in 'Maxchata' (Figure 1B, 1C). Likewise, transpiration rate ( $E$ ) was decreased from  $6.17 \text{mmol m}^{-2} \text{s}^{-1}$  to  $5.92 \text{mmol m}^{-2} \text{s}^{-1}$  and to  $5.54 \text{mmol (H}_2\text{O) m}^{-2} \text{s}^{-1}$  at  $37^\circ\text{C}$  and  $42^\circ\text{C}$ , respectively in *C. moschata*, from  $6.85 \text{mmol m}^{-2} \text{s}^{-1}$  to  $4.15 \text{mmol m}^{-2} \text{s}^{-1}$  at  $37^\circ\text{C}$  and to  $3.8 \text{mmol m}^{-2} \text{s}^{-1}$

at  $42^\circ\text{C}$  in *C. maxima*, and from  $7.31 \text{mmol m}^{-2} \text{s}^{-1}$  to  $6.95 \text{mmol m}^{-2} \text{s}^{-1}$  at  $37^\circ\text{C}$  and to  $5.39 \text{mmol m}^{-2} \text{s}^{-1}$  at  $42^\circ\text{C}$  in 'Maxchata'. The decrease was more pronounced in *C. maxima* followed by 'Maxchata' as compared to *C. moschata*. Intercellular  $\text{CO}_2$  concentration ( $C_i$ ) increased by 4.2%, 14.3% and 6.25% at  $37^\circ\text{C}$ , and 10.2%, 33.3%, and 11.3% at  $42^\circ\text{C}$  in *C. moschata*, *C. maxima* and 'Maxchata', respectively (Figure 1D).

The ability to sustain leaf gas exchange under heat stress is the best indicator of the heat tolerance of the plant species (Wahid et al 2007). Our results showed that  $P_N$ ,  $g_s$  and  $E$  were reduced in all selected genotypes under heat stress, while  $C_i$  increased (Figure 1A-D). However, there was significant difference among the genotypes in the rates of change with increasing temperature. *C. maxima* exhibited more drastic response, followed by Maxchata and then *C. moschata* indicating the extent of heat tolerance of each genotype. These findings are in line with many investigations in which heat effect on photosynthesis was studied (Hammes & Jager 1990; Camejo et al 2005; Guo et al 2009). The reduction of  $P_N$  indicates the stress condition in plants resulting from photoinhibition which may suggest the decreased photochemical efficiency of PSII. Changes in stomatal conductance ( $g_s$ ) are related to both the control of water loss and  $\text{CO}_2$  assimilation for maintenance of photosynthesis rate (Zhang et al 2003). This phenomenon contributes to decline in transpiration rate ( $E$ ) due to stomatal closure. Reduced transpiration rate ( $E$ ) may result in reduction of cooling effect of water transpiration leading to more heat injury. However, according to Whiteman & Koller (1967) the rise in temperature triggers the transpiration rate, which can lead to reduction in turgor of guard cells, resulting stomatal closure hence  $g_s$  and  $E$  are interdependent parameters. Further, the reduction of  $g_s$  may limit the  $\text{CO}_2$  fixation rate, consequent with decrease of its concentration in the substomatal cavities and in the intercellular spaces, often called stomatal limitation. Our results did not point to stomatal limitations as  $C_i$  was increased under heat stress. Thus, if there is increase in  $C_i$  despite reduction of  $g_s$ , it may suggest



**Figure 1- Effect of heat stress on net photosynthesis rate ( $P_N$ ) (A), stomatal conductance ( $g_s$ ) (B), transpiration rate (E) (C), intercellular  $\text{CO}_2$  concentration ( $C_i$ ) (D). Values are means  $\pm$  SD (n = 4). Different letters indicate significant difference (P < 0.01)**

Şekil 1- Sıcaklık stresinin net fotosentez oranı ( $P_N$ ) üzerine etkisi. (A), stoma iletkenliği ( $g_s$ ) (B), terleme oranı (E) (C), hücreler arası  $\text{CO}_2$  konsantrasyonu ( $C_i$ ) (D). Rakamlar ortalama  $\pm$  SD (n = 4)'dir. Farklı harfler ile gösterilen ortalamalar arasındaki farklılık önemlidir (P < 0.01)

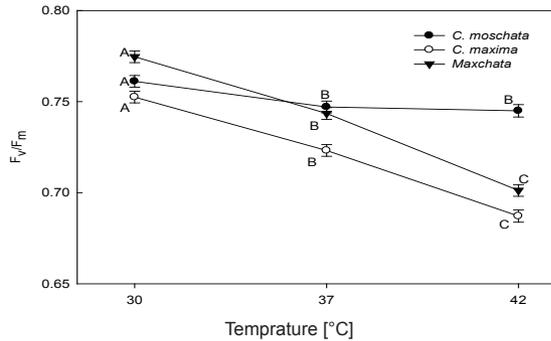
nonstomatal limitations. High temperature enhances the rate of respiration and hence supplementing  $C_i$  (Whiteman & Koller 1967). However, Rubisco limitation could be the major limitation to  $\text{CO}_2$  fixation during photosynthesis. Jensen (2000) reported that at high temperature, Rubisco activase is unable to keep pace with a faster deactivation of Rubisco resulting in lesser availability of its activated state to fix  $\text{CO}_2$ . Similarly, the inhibition of  $P_N$  and  $g_s$  had also been correlated with this deactivation of Rubisco in many species. In addition, heat stress may decrease the affinity of this key photosynthetic enzyme for  $\text{CO}_2$  and may increase oxygenase activity instead of carboxylation leading to reduced photosynthesis and increased

photorespiration (Crafts-Brander & Salvucci 2000; 2002; Morales et al 2003; Wahid et al 2007).

### 3.2. Change in maximum photochemical efficiency of PSII

Maximum photochemical efficiency of PSII ( $F_v/F_m$ ) was determined to evaluate the heat tolerance of the selected genotypes. There were no large differences in  $F_v/F_m$  values under increasing heat shock in *C. moschata* which had 0.761, 0.747 and 0.745 at 30 °C, 37 °C and 42 °C, respectively (Figure 2). *C. maxima* had 0.752, 0.723 and 0.687  $F_v/F_m$  values at 30 °C, 37 °C and 42 °C, respectively. 'Maxchata' had  $F_v/F_m$  values of 0.775, 0.744 and 0.701 at 30 °C, 37 °C and 42 °C, respectively. Although both

*C. maxima* and 'Maxchata' exhibited reduction in  $F_v/F_m$ , 'Maxchata' had comparatively higher values.



**Figure 2-** Effect of heat stress on maximum photochemical efficiency of PSII ( $F_v/F_m$ ). Values are means  $\pm$  SD ( $n = 4$ ). Different letters indicate significant difference ( $P < 0.01$ )

Şekil 2- Sıcaklık stresinin PSII( $F_v/F_m$ )'nin maksimum fotokimyasal etkinlik üzerine etkisi. Rakamlar ortalama  $\pm$  SD ( $n = 4$ )'dir. Farklı harfler ile gösterilen ortalamalar arasındaki farklılık önemlidir ( $P < 0.01$ )

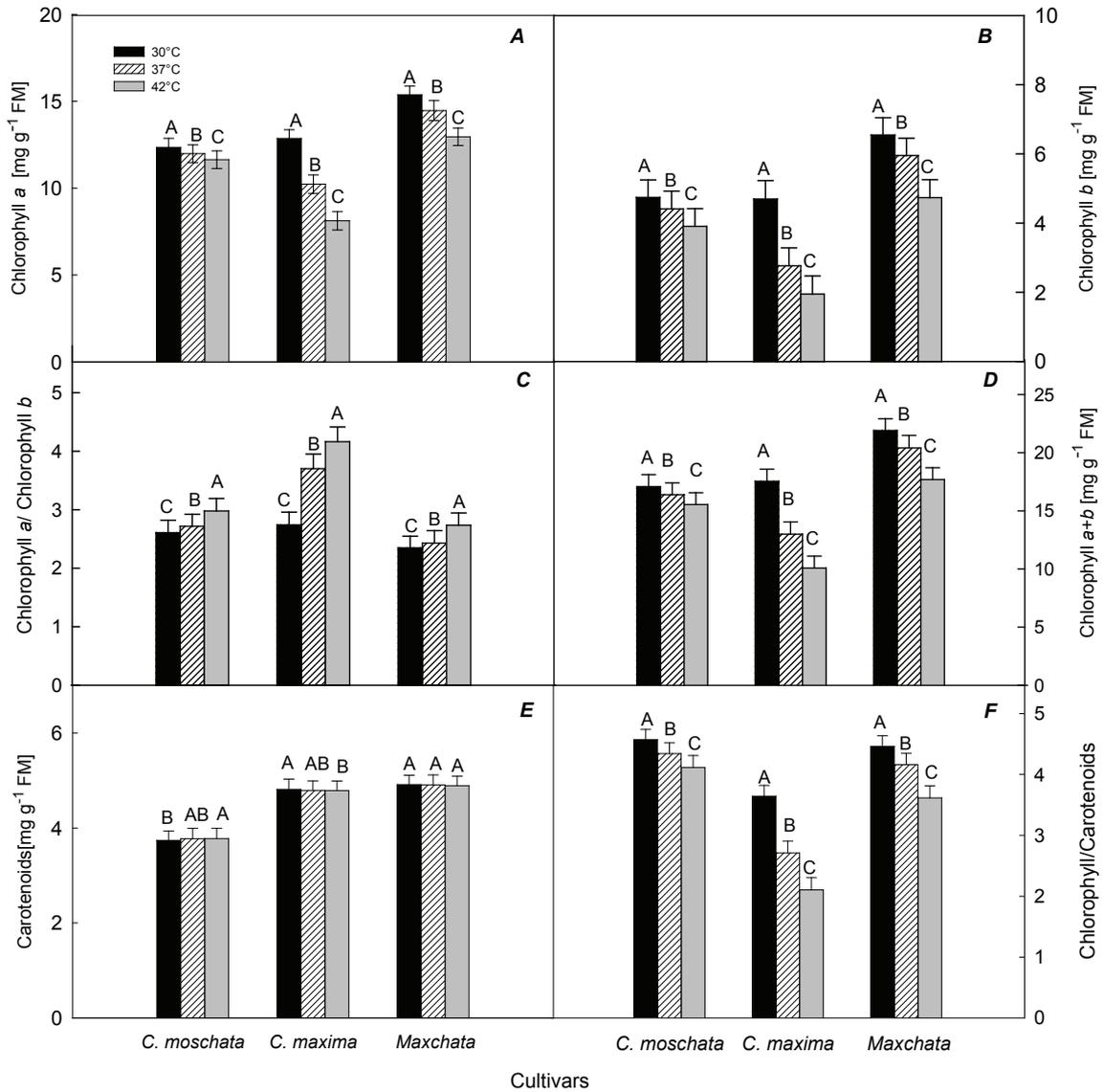
Chlorophyll fluorescence is also one of the heat responsive plant attribute. It is used to estimate the potential efficiency of PSII, which is more heat sensitive than PSI. It also reflects the interruption of electron donation to PSII reaction center due to heat inactivation of Oxygen Evolving System (OES) (Maxwell & Johnson 2000; De Ronde et al 2004). This may result in more reactive oxygen species (ROS) generations, which in turn injure or inactivate PSII causing photoinhibition (Rohacek 2002; Guo et al 2006; Ogwenon et al 2009; Pires et al 2011). Hence, the decrease in  $F_v/F_m$  points to the photoinhibitory damage in response to high temperature and may lead to reduced  $P_N$  (Sinsawat et al 2004; Guo et al 2009). Many researchers have used  $F_v/F_m$  to distinguish differences in heat tolerance between species (Wolf et al 1990; Camejo et al 2005; Guo et al 2006; Reed et al 2012; Zeng et al 2012). Keeping in view the trend observed in previous studies, the lowest  $F_v/F_m$  of *C. maxima* indicates that this species is heat sensitive in line with other photosynthetic characteristics. The relatively constant values of *C. moschata* under

increasing heat stress confirm its heat tolerance. 'Maxchata' plants exhibited intermediary response.

### 3.3. Changes in chlorophyll and carotenoid contents

Both Chl *a* and *b* showed significant reduction in all selected genotypes with increasing heat stress (Figure 3A, 3B). Chl *a* decreased from 12.35 mg g<sup>-1</sup> FM to 11.98 mg g<sup>-1</sup> FM at 37 °C and to 11.63 mg g<sup>-1</sup> FM at 42 °C in *C. moschata*, from 12.85 mg g<sup>-1</sup> FM to 10.23 mg g<sup>-1</sup> FM at 37 °C and to 8.12 mg g<sup>-1</sup> FM at 42 °C in *C. maxima* and from 15.36 mg g<sup>-1</sup> FM to 14.46 mg g<sup>-1</sup> FM at 37 °C and to 12.95 mg g<sup>-1</sup> FM at 42 °C in 'Maxchata'. Chl *b* degraded more drastically under high temperature than Chl *a*, especially in *C. maxima*, followed by 'Maxchata' and then *C. moschata*. Chl *b* in *C. moschata* was 4.74 mg g<sup>-1</sup> FM, 4.41 mg g<sup>-1</sup> FM and 3.90 mg g<sup>-1</sup> FM at 30 °C, 37 °C and 42 °C, respectively (Figure 3B). Chl *b* in *C. maxima* was 4.69 mg g<sup>-1</sup> FM, 2.76 mg g<sup>-1</sup> FM and 1.95 mg g<sup>-1</sup> FM at 30 °C, 37 °C and 42 °C, respectively. 'Maxchata' had Chl *b* content of 6.54 mg g<sup>-1</sup> FM, 5.95 mg g<sup>-1</sup> FM and 4.74 mg g<sup>-1</sup> FM at 30 °C, 37 °C and 42 °C, respectively. The increasing Chl *a*/Chl *b* ratio also reflected the same trend (Figure 3C). The total Chl content was higher in 'Maxchata' than its parents (Figure 3D). *C. maxima* was more prone to total chlorophyll degradation under heat shock that chlorophyll loss was 25.9% at 37 °C and 42.6% at 42 °C, respectively. On the other hand 'Maxchata' had lower chlorophyll degradation (6.8% and 19.2%), while *C. moschata* had the lowest degradation (4.1% and 9.02%) at 37 °C and 42 °C, respectively. Carotenoids were comparatively stable at all heat regimes. Although, close observation reveals that there was slight increase in carotenoids in *C. moschata* and other two genotypes faced trivial fall with rising temperature (Figure 3E). Thus taken the contents of chlorophyll and carotenoids together, the chlorophyll to carotenoids ratio dropped more drastically in *C. maxima*, followed by 'Maxchata' and then *C. moschata* with high temperatures (Figure 3F).

Destruction of photosynthetic pigments under heat stress also provides a clue to determine the



**Figure 3-** Effect of heat stress on chlorophyll a (Chl a) (A), chlorophyll b (Chl b) (B), chlorophyll a/b (Chl a/b) (C), Total chlorophyll (Chl) (D), carotenoids (Car) (E), chlorophyll/carotenoids (Chl/Car) (F). Values are means  $\pm$  SD ( $n = 4$ ). Different letters indicate significant difference ( $P < 0.01$ )

Şekil 3- Sıcaklık stresinin klorofil a (Chl a) (A), klorofil b (Chl b) (B), klorofil a/b (Chl a/b) (C), toplam klorofil (Chl) (D), karotenoidler (Car) (E), klorofil/karotenoidler (Chl/Car) (F). Rakamlar ortalama  $\pm$  SD ( $n = 4$ )'dir. Farklı harfler ile gösterilen ortalamalar arasındaki farklılık önemlidir ( $P < 0.01$ )

heat tolerance of plants. Our findings showed that all of the genotypes experienced chlorophyll degradation with increasing heat stress, but it was more pronounced in *C. maxima* confirming its heat sensitivity, followed by 'Maxchata'. Chl *b* dropped steadily in *C. moschata* but drastically in *C. maxima*, resulting in comparatively higher Chl *a* to *b* ratio. The ratio of Chl *a* to *b* is an indicator of functional pigment equipment and light acclimation of photosynthetic apparatus. Light harvesting complex around PSII contains more Chl *b*. So, it may have some contribution in photochemical efficiency of PSII and may correlate with reduction in  $F_v/F_m$  (Reed et al 2012). Comparatively more decline in chlorophyll to carotenoids ratio under heat stress in *C. maxima* further confirms its sensitivity. 'Maxchata' gave better result than *C. maxima* in this respect. Camejo & Torres (2001) also reported more reduction in Chlorophyll to carotenoids content in heat sensitive cultivars of tomato than tolerant ones.

#### 4. Conclusions

The heat stress affected almost all the photosynthetic attributes of the selected squash genotypes. The reduction in  $P_N$ ,  $g_s$  and  $E$  were more drastic in *C. maxima*, followed by 'Maxchata' and then *C. moschata*. The increase in  $C_i$  indicated high temperature induced non-stomatal limitations. In addition, reduction in chlorophyll degradation and chlorophyll fluorescence also confirmed the heat sensitivity of *C. maxima* and tolerance of *C. moschata*. 'Maxchata' showed intermediate response among its parents showing some potential of heat tolerance to confront climate change with global warming. The results suggest that some heat tolerance related characters might have been transferred from *C. moschata* to this inbred line. However, there is a need to further explore the genetic and biochemical bases of this difference for understanding the mechanism of thermotolerance.

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Abbreviations and Symbols	
Car	Carotenoids
$C_i$	Intercellular CO <sub>2</sub> concentration
Chl	Chlorophyll
$E$	Transpiration rate
$F_0$	Minimal fluorescence
$F_m$	Maximal fluorescence
$F_v$	Variable fluorescence
$F_v/F_m$	Maximum photochemical efficiency of PSII
$g_s$	Stomatal conductance
$P_N$	Net photosynthesis rate
PS	Photosystem
Rubisco	Ribulose 1,5-bisphosphate carboxylase/oxygenase

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