



## Effect of temperature dynamics on phenological and horticultural performance of different citrus mandarin genotypes growing under a temperature gradient tunnel

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**सार** – खट्टे फल एक महत्वपूर्ण फल फसल है और मुख्य रूप से दुनिया के उष्णकटिबंधीय और उपोष्णकटिबंधीय क्षेत्रों में उगाए जाते हैं। खट्टे फल की गुणवत्ता तापमान में वृद्धि के प्रति अत्यधिक सुभेद्य है, और इसलिए, उत्पादकों की लाभप्रदता पर प्रभाव डालती है। इस प्रमुख उद्देश्य के साथ, तीन मुख्य मंदारिन जीनोटाइप अर्थात् जी1- किन्नो, जी2- डेज़ी और जी3- डब्ल्यू मर्कॉट पर एक अध्ययन किया गया, जिन्हें तापमान ग्रेडिएंट टनल (टीजीटी) के भीतर परिवेश की स्थितियों से +1.51, +2.03 और +2.29 के उच्च तापमान व्यवस्था में उजागर किया गया था। जीनोटाइप का फेनोलॉजिकल व्यवहार, यानी बड बस्ट (बीबीसीएच1) से फल परिपक्वता (बीबीसीएच97) की शुरुआत परिवेशीय स्थितियों की तुलना में जी1 में 1-15 दिन, जी2 में 1-29 दिन और जी3 में 1-17 दिन आगे बढ़ गई। उच्च तापमान का फलन घनत्व (5-54 संख्या / 3), पेड़ की उपज (5.81-60.20 किग्रा / पेड़), उपज दक्षता (9.26-58.52 किग्रा / 3), फल वजन, आकार और ऑर्गनोलेप्टिक लक्षणों, यानी टीएसएस पर निराशाजनक प्रभाव पड़ा। बागवानी लक्षणों और पौधों की वृद्धि विशेषताओं, यानी पौधे की ऊंचाई, कैनोपी वॉल्यूम, फल स्कैरिंग और पत्ती जलने पर हल्के प्रभावों से पता चला है कि किनो ने उच्च तापमान के लिए उच्च सहनशीलता का प्रदर्शन किया। इसके अलावा, बहुभिन्नरूपी विश्लेषण विभिन्न तापमान व्यवस्थाओं के तहत मंदारिन के अनुरूप अंतर्संबंध और बागवानी लक्षणों के बीच विभेदक संघों को दर्शाता है। इस प्रकार, हम निष्कर्ष निकालते हैं कि भविष्य के जलवायु परिवर्तन परिदृश्य में बढ़े हुए तापमान का साइट्रस किस्मों के फेनोलॉजिकल और बागवानी प्रदर्शन पर नकारात्मक जेनोटाइपिक-विशिष्ट प्रभाव पड़ेगा। यह विभेदक किस्म की प्रतिक्रिया लाभदायक उत्पादन के लिए बागों के बेहतर प्रबंधन में साइट्रस उत्पादकों और भविष्य की गर्म जलवायु से उत्पन्न चुनौतियों के अनुकूल होने में वैज्ञानिकों की मदद कर सकती है।

**ABSTRACT.** Citrus is an important fruit crop and is primarily cultivated in tropical and subtropical regions of the world. The quality of the citrus fruit is highly vulnerable to a rise in temperature, and hence, impacts the profitability of the growers. With this key objective, a study was conducted on three main mandarin genotypes, i.e. G1- Kinnow, G2- Daisy and G3- W Murcott, which were exposed to an elevated temperature regime of +1.51, +2.03 and +2.29 °C from ambient conditions within a Temperature Gradient Tunnel (TGT). The phenological behaviour of the genotypes, i.e. the beginning of bud burst (BBCH1) to fruit maturity (BBCH97) was advanced by 1-15 days in G1, 1-29 days in G2 and 1-17 days in G3 as compared to ambient conditions. The elevated temperature had a depressing effect on fruiting density (5-54 no/m<sup>3</sup>), tree yield (5.81-60.20 kg/tree), yield efficiency (9.26-58.52 kg/m<sup>3</sup>), fruit weight, size and organoleptic traits, i.e. TSS. Kinnow exhibited high tolerance for elevated temperatures, as revealed by mild effects on horticultural traits, and plant growth characteristics, i.e. plant height, canopy volume, fruit scaring and leaf burning. Moreover, multivariate analysis depicts differential associations between horticultural traits and the analogous interconnection of mandarins under different temperature regimes. Thus, we conclude that an increased temperature in the future climate change scenario will have negative genotypic-specific effects on the phenological and horticultural performance of citrus cultivars. This differential varietal response can help citrus growers in better management of orchards for profitable production and scientists to adapt to the challenges posed by a futuristic warming climate.

**Key words** – BBCH, Citrus, Climate change, Elevated temperature, Phenophases, TGT.

## 1. Introduction

Plant species have historically demonstrated their ability to adapt to constantly changing environmental conditions i.e. elevated temperatures, drought, flooding, and soil salinity (Rasera *et al.*, 2023). However, the current climate shift is unprecedented and poses a potential threat to the adaptation of many plant species (Mitra, 2021). Citrus is one of the most popular fruit crops globally, cultivated in more than 140 countries and climate change poses a serious threat to the citriculture industry (Vincent *et al.*, 2020). The optimum temperature range for successful citrus cultivation is estimated to fall within 22-34 °C. The recent report by NOAA (National Oceanic and Atmospheric Administration) has shown that the average rate of increase in the temperature of the Earth since 1880 is ~0.08 °C per decade (Anonymous, 2023). The Intergovernmental Panel on Climate Change (IPCC, 2007) has predicted an increase in global temperature by 1.8-4.0 °C due to an increase in anthropogenic emissions of greenhouse gases. So, this continuing increase in atmospheric temperature hurts the cultivation of subtropical fruits (Vincent *et al.*, 2020).

Khalid *et al.* (2022) have reported a consensus on the occurrence of earlier budburst and flowering of citrus fruit crops as a consequence of global warming in the last decades. At the field level, the phenological shifts of perennial citrus trees resulting from climate change are considered highly probable due to the inherent limitations in artificially manipulating cropping seasons on an annual basis and the fixed physiological processes that coincide with specific environmental conditions (Luedeling *et al.*, 2009). Generally, the increase in temperature and shifting of plant phenophases can be seen as a “cause-and-effect” relationship (Zheng *et al.*, 2006). However, these variations in terms of the occurrence of phenological stages due to an increase in temperature have already been reported amongst different citrus species (Kwon *et al.*, 2021). A rise in temperature beyond the critical range affects the temperature-related biological response in citrus (Zandalinas *et al.*, 2017), which can be an increase in the rate of transpiration and photosynthesis, disruption of cell membrane stability, and exacerbation of oxidative stress (Balfagón *et al.*, 2020). The rise in temperature coupled with water stress at critical phenological stages of citrus leads to a decrease in the fruit set in the trees, reduces fruit growth and size, increases in fruit acidity, reduces the fruit peel thickness and increases the premature fruit drop (Shafqat *et al.*, 2020). The nutraceutical value of citrus fruits is a critical determinant of their overall fruit quality and consumer acceptability. An increase in temperature above the optimal range leads to significant deterioration of fruit quality traits such as fruit abscission, reduced fruit size and organoleptic quality parameters (Zandalinas *et al.*,

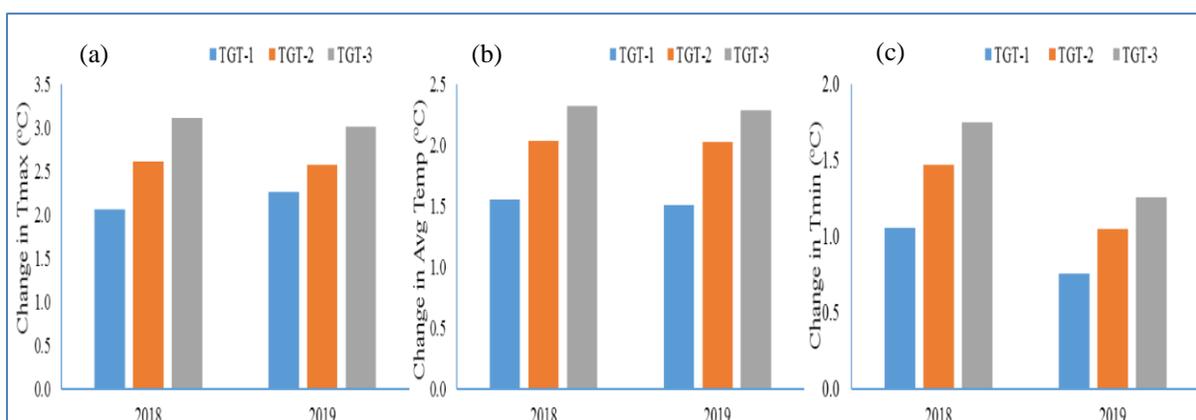
2017). In addition to their pleasant taste and aroma, citrus fruits are a good source of vitamins and a full pack of powerful antioxidants. The deleterious effects of increased temperature on yield efficiency and organoleptic fruit quality have been reported amongst the various citrus genotypes cultivated in the different production regions. Moreover, these factors ultimately diminish the commercial value of the fruit yield (Balfagón *et al.*, 2022).

Under natural conditions, plants respond to environmental changes by shifting the timing of life cycle events. An understanding of this shifting of phenological stages response is helpful to better predict how citrus will respond to future climate changes. For profitable management of orchards, most farmers use a combination of calendar dates and phenological events. Thus, this information can help in the better management of orchards in response to changing temperature conditions. A series of experiments have been conducted in both field and controlled environments, i.e. OTCs, TGTs, FACE facilities, to assess the impacts of climate change on fruit crops around the globe. However, under Indian conditions, relatively limited work has been done to assess the impact of elevated temperatures on citrus cultivation. From this perspective, it is imperative to assess the impact of elevated temperatures on the phenological and horticultural performance of different commercial citrus cultivars to check their genotypic and phenotypic plasticity. Thus, the current study was designed to analyze the effect of elevated temperature on two main citrus cultivation perspectives viz., phenophases and horticultural behavior of mandarin genotypes.

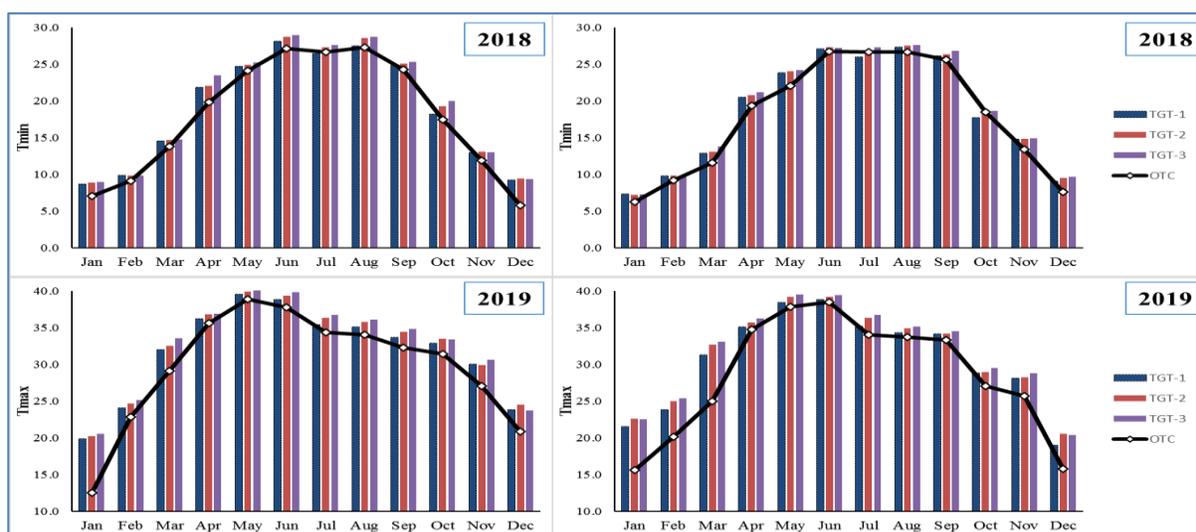
## 2. Data and methodology

### 2.1. Study area and plant material

The experiment was conducted at Research Farm, Department of Climate Change and Agricultural Meteorology, Punjab Agricultural University, Ludhiana (located at 30° 54' N latitude, 75° 48' E longitude and 247 m above mean sea level). This crop production area is characterised by a subtropical region with a dry and hot summer. The annual maximum and minimum temperatures are 29-30 °C and 16-17 °C, respectively, with an annual rainfall of 760 mm (Prabhjyot-Kaur *et al.*, 2016). The soil of the experimental site was found to be sandy loam textured with a neutral pH value of 8.2, electrical conductivity ranging from 0.12-0.18 ds/m, and organic carbon ranging from 0.02-0.18%. The daily data of minimum and maximum temperatures for two years were obtained from the observatory of the Department of Climate Change and Agricultural Meteorology at Punjab Agricultural University, Ludhiana. During 2018, the



**Figs. 1(a-c).** Change in A) maximum temperature (Tmax), B) Average temperature and C) minimum temperature gradient (Tmin) at ambient and elevated temperature conditions during an experimental period of two years



**Fig. 2.** Monthly progress of average minimum temperature (Tmin) and maximum temperature (Tmax) (°C) within the TGT (Temperature Gradient Tunnel) and in open field conditions (OTC) during the citrus growing phase. Jan= January, Feb= February, Mar= March, Apr=April, Jun= June, Jul= July, Aug= August, Sep= September, Oct= October, Nov= November, Dec= December

average maximum temperature under ambient conditions was recorded as 29.7 °C, with extreme values reaching as high as 38.9 °C. The minimum temperature averaged at 17.9 °C, with extreme values falling below 5.8 °C (Fig. 1). While in 2019, the average maximum temperature was 28.4 °C, with an extreme value of 38.5 °C the average minimum temperature was 17.8 °C, with an extreme value of 6.2 °C.

The experimental genotypes consisted of three mandarin citrus cultivars, viz Kinnow, Daisy and W. Murcott. About one-and-a-half-year nursery plants of each cultivar planted in 26-inch cemented pots were kept inside the compartments of a temperature gradient tunnel (TGT) having varied temperature regimes in July 2016 (Fig. 2). Three replicates of each genotype were placed in each

compartment of TGT (3 TGT compartments x 3 genotypes x 3 replicates of each genotype). Similarly, nine plants (3 genotypes x 3 replicates of each genotype) having similar genotypic constitutions were also placed outside TGT to serve as controls for comparative analysis. The plants were allowed to grow for about one and a half years under such conditions, and the phenological and horticultural traits were recorded during 2018-19.

## 2.2. Temperature Gradient Tunnel (TGT)

The temperature gradient tunnel (TGT), having dimensions of 30 m in length, 5 m in breadth and 3 m in height, was used for the present investigation. The TGT is made up of galvanised iron pipes and covered with

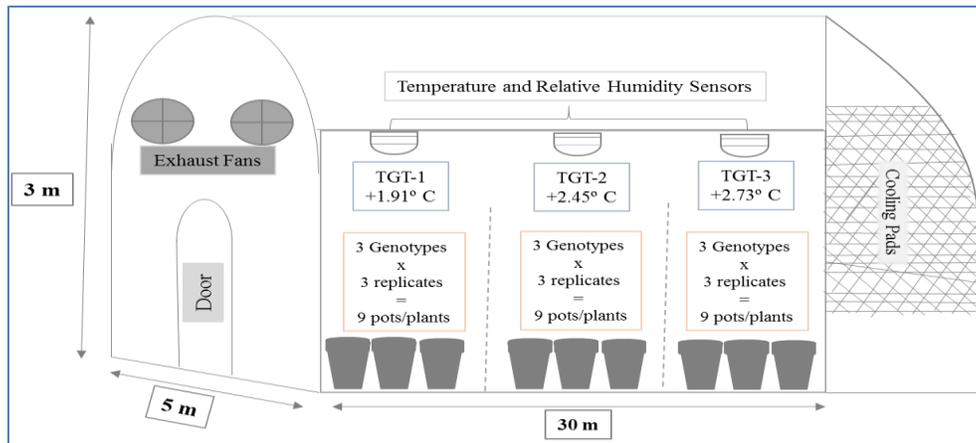


Fig. 3. Diagrammatic representation of the layout of the experiment within the temperature gradient tunnel (TGT)

polythene sheets having >85% transparency. The air inside the TGT was naturally warm due to solar radiation. At one end of the tunnel, a forced air hydro-cooling system with cooling pads was fitted to produce a cool draft and at the second end of the TGT, two exhaust fans were installed to create a thermal gradient by sucking air from the inlet and releasing it from the outlet. The TGT was divided into three compartments at the distances of 5 m, 15 m and 25 m from the cooling system side and designated as TGT-1, TGT-2 and TGT-3, respectively (Fig. 3). Data was monitored with the help of temperature sensors fitted in each TGT compartment and recorded at five intervals throughout the growing season with the help of a data logger (Delta-T make).

### 2.3. Phenological survey

A phenological event is a point in the annual life cycle of a plant, generally marking the start or endpoint of a phenophase, and can be recorded as a calendar date. The phenological event monitoring approach is a traditional method and is often described using the European-based Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie (BBCH) coding (Meier, 2001). In this work, codification and description of different growth stages was done according to a standard formatted citrus-specific BBCH scale (Agusti *et al.*, 2000) and monitoring of these stages were performed on a daily basis. This BBCH scale includes the following principal growth stages, *i.e.* from starting with the beginning of bud swelling (BBCH1) to ending when the winter dormancy periods start (BBCH97). The full description of BBCH coding (from BBCH1 to BBCH97) is given in Table 1. Eventually, the mean date of the onset of each phenophase was calculated as an average of the three plants and the data was expressed in terms of its advancement from the plants kept as control outside the TGT.

### 2.4. Horticultural traits

The growth and yield characteristics of the plants are affected by the ambient meteorological conditions. Hence, to analyze the effect of temperature changes on the citrus genotypes, some of the important characteristics were computed as:

#### 2.4.1. Tree canopy volume (CV)

It is expressed in cubic meters and was estimated using the methodology proposed by Roose *et al.* (1986) as:

$$CV = \frac{4}{6\pi} hr^2 \quad (1)$$

where, h= height of tree (m), r = sum of east-west and north-south direction (m)/4.

#### 2.4.2. Yield efficiency (YE)

It is expressed in kg/cm<sup>2</sup> TCSA and was determined according to the formula described below by Westwood (1993):

$$YE = \frac{\text{Fruit yield } (\frac{\text{kg}}{\text{tree}})}{\text{TCSA } (\text{cm}^2)} \quad (2)$$

where, TCSA= trunk cross-sectional area of the tree.

However, the fruits were collected from different directions of each replicate (plant) for the determination of their horticultural performance. Fruits were cut into two halves, and fresh juice was extracted using a juice extractor and sieved through a muslin cloth to remove pulp/ peel debris. The total soluble content (TSS) of each fruit sample was determined using a digital hand

TABLE 1

Dates of onset of different phenological phases and their duration in terms of days for of three mandarin genotypes under open field and elevated temperature conditions.

BBCH code	Description	G <sub>1</sub> : Kinnow				G <sub>2</sub> : Daisy				G <sub>3</sub> : W Murcott			
		OTC	TGT-1	TGT-2	TGT-3	OTC	TGT-1	TGT-2	TGT-3	OTC	TGT-1	TGT-2	TGT-3
1	Beginning of bud swelling	8 Feb (0)*	6 Feb (2)	5 Feb (3)	4 Feb (4)	17 Feb (0)	15 Feb (3)	10 Feb (8)	3 Feb (15)	15 Feb (0)	13 Feb (3)	10 Feb (6)	9 Feb (7)
7	Beginning of bud burst	15 Feb (0)	12 Feb (3)	11 Feb (4)	9 Feb (7)	24 Feb (0)	22 Feb (3)	18 Feb (7)	13 Feb (12)	16 Feb (0)	15 Feb (2)	14 Feb (3)	12 Feb (5)
39	Shoots about 90% of final length	16 Mar (0)	15 Mar (2)	13 Mar (3)	8 Mar (9)	28 Mar (0)	26 Mar (3)	18 Mar (11)	13 Mar (16)	20 Mar (0)	19 Mar (2)	15 Mar (6)	10 Mar (11)
60	First flowers open	15 Mar (0)	13 Mar (2)	8 Mar (8)	4 Mar (11)	20 Mar (0)	18 Mar (3)	14 Mar (7)	8 Mar (13)	19 Mar (0)	18 Mar (2)	10 Mar (10)	6 Mar (14)
65	Full flowering: 50% of flowers open	22 Mar (0)	19 Mar (3)	15 Mar (6)	10 Mar (11)	30 Mar (0)	28 Mar (3)	22 Mar (9)	14 Mar (17)	30 Mar (0)	26 Mar (5)	21 Mar (9)	14 Mar (17)
69	End of flowering: all petals fallen	1 Apr (0)	28 Mar (4)	25 Mar (7)	16 Mar (13)	8 Apr (0)	7 Apr (2)	30 Mar (10)	23 Mar (15)	5 Apr (0)	2 Apr (4)	27 Mar (10)	22 Mar (14)
71	Fruit set; beginning of ovary growth and fruitlets	2 Apr (0)	29 Mar (5)	26 Mar (8)	20 Mar (14)	11 Apr (0)	10 Apr (2)	4 Apr (8)	27 Mar (15)	7 Apr (0)	6 Apr (2)	29 Mar (10)	24 Mar (14)
72	Green fruit surrounded by sepal crown	10 Apr (0)	8 Apr (2)	2 Apr (9)	27 Mar (14)	13 Apr (0)	12 Apr (2)	7 Apr (7)	1 Apr (13)	14 Apr (0)	10 Apr (5)	4 Apr (11)	29 Mar (17)
73	Beginning of physiological fruit	13 Apr (0)	11 Apr (2)	8 Apr (6)	2 Apr (12)	16 Apr (0)	14 Apr (2)	13 Apr (3)	9 Apr (7)	17 Apr (0)	14 Apr (3)	10 Apr (7)	8 Apr (9)
74	Fruits are about 40% of the final size. Dark green fruit	11 Aug (0)	8 Aug (3)	8 Aug (3)	2 Aug (9)	31 Jul (0)	29 Jul (2)	24 Jul (7)	20 Jul (11)	11 Aug (0)	7 Aug (4)	3 Aug (8)	1 Aug (10)
79	Fruits about 90% of the final size	5 Oct (0)	1 Oct (4)	1 Oct (4)	26 Sep (9)	12 Sep (0)	9 Sep (3)	6 Sep (6)	3 Sep (9)	7 Oct (0)	5 Oct (2)	5 Oct (2)	1 Oct (6)
83	Fruit ripe for picking	28 Dec (0)	24 Dec (4)	18 Dec (11)	14 Dec (14)	17 Nov (0)	14 Nov (3)	12 Nov (5)	9 Nov (8)	30 Dec (0)	28 Dec (2)	23 Dec (7)	20 Dec (10)
85	Advanced ripening; increase in intensity of variety-specific coloration	6 Jan (0)	4 Jan (2)	30 Dec (7)	24 Dec (13)	22 Nov (0)	21 Nov (1)	19 Nov (3)	17 Nov (5)	12 Jan (0)	10 Jan (2)	5 Jan (7)	2 Jan (10)
89	Fruit ripe for consumption; fruit has a typical taste	13 Jan (0)	12 Jan (1)	8 Jan (5)	1 Jan (12)	22 Nov (0)	21 Nov (2)	18 Nov (4)	15 Nov (7)	20 Jan (0)	19 Jan (1)	11 Jan (9)	6 Jan (14)
97	Winter dormancy period	11 Feb (0)	9 Feb (2)	5 Feb (6)	2 Feb (9)	15 Feb (0)	13 Feb (2)	9 Feb (6)	5 Feb (10)	11 Feb (0)	10 Feb (1)	9 Feb (2)	5 Feb (6)

refractometer and was expressed in terms of degree Brix (°B) with a correction factor. The Titratable Acidity (TA) expressed in per cent was estimated by titrating the 2 ml juice with a standardised N/10 sodium hydroxide (NaOH) solution, using phenolphthalein dye (two drops) as an indicator (AOAC, 2000).

### 2.5. Statistical analysis

One-way ANOVA analysis was carried out using “lme4” and “lmerTest” packages of R Studio (R Core

Team, 2021) and p p-value ( $\leq 0.05$ ) is considered for post-hoc comparisons. The Pearson's correlation coefficient (r) was calculated according to the following formula:

$$r = \frac{n(\sum xy) - (\sum x)(\sum y)}{\sqrt{[\sum nx^2 - (\sum x)^2][\sum ny^2 - (\sum y)^2]}} \quad (3)$$

where, n = number,  $\sum x$  = total of the first variable,  $\sum y$  = total of the second variable,  $\sum xy$  = sum of the first and the second variable,  $\sum x^2$  = sum of squares of the first variable,  $\sum y^2$  = the sum of the squares of the second variable.

The correlation matrix was visualized using a correlogram and was generated using a “corplot” package. The red colour represents negative correlation, and blue represents positive correlation. The intensity of color represents the degree and magnitude of correlation.

The principal component analysis (PCA) was calculated using the R packages “Performance Analytics”, “FactoMineR”, and “factoextra”. The vectors of PCA represent positive and negative associations among traits. Heatmap analyses were carried out using a “pheatmap” package of R. The color intensity of heatmap cells represents the degree of change, and colour bands reveal the differential association of traits. The rows represent genotypes, and columns represent the studied traits, which are expressed as the difference between the individual genotypes of each treatment (within a column).

### 3. Results and discussion

The data of maximum, minimum and average temperature recorded from each compartment of TGT and experimental site for two consecutive years shows interannual and seasonal variations (Fig. 3). The elevated temperature gradient was created inside the three compartments (within TGT) of the tunnel. During the two study years, the average annual temperature gradient of the elevated temperature were respectively 1.56 °C (TGT-1), 2.04 °C (TGT-2) and 2.32 °C (TGT-3) more than ambient of 29.71 °C during 2018 whereas, during 2019 it was 1.51 °C (TGT-1), 2.03 °C (TGT-2), and 2.29 °C (TGT-3) higher than ambient of 28.44 °C recorded under ambient field conditions.

#### 3.1. Effect of elevated temperature on different phenophases

Phenological observations were recorded on principal growth stages using a BBCH (Biologische Bundesanstalt, Bundessortenamt and Chemical industry) scale adapted for citrus trees (Agustí *et al.*, 1997). The phenological calendar of citrus mandarin genotypes Kinnow, Daisy and W Murcott inside TGT and ambient conditions are presented in Table 1. Though the three genotypes behaved differently in their respective phenological stages, there was an advancement in the timing of the phenophases under the three elevated temperature regimes as compared to ambient conditions. The maximum advancement in the phenological dates of the buds and flowers was observed under TGT-3, wherein maximum elevation in temperature as compared to open was achieved. Amongst the genotypes, the periods of bud swelling were almost synchronous and a week earlier under TGT-3. During the BBCH1 stage (1<sup>st</sup> fortnight of February), the beginning of bud appearance of genotypes Kinnow, W Murcott and

Daisy was advanced by 2-4, 3-15 and 3-7 days, respectively, within the TGT as compared to open ambient conditions. The 50% full blooming (BBCH65), which was observed on 22 March (in Kinnow) and 30 March (in Daisy and W Murcott) in open conditions, was advanced by 11 days (in Kinnow) and 17 days (in Daisy and W Murcott). The fruit set (BBCH71) in the three genotypes under ambient conditions advanced by 14-15 days under TGT-3, by 8-10 days under TGT-2 and by 2-5 days under TGT-1. The variety-specific colour (BBCH85) in the three genotypes under ambient conditions was advanced by 11-14 days under TGT-3, by 6-8 days under TGT-2 and by 2-3 days under TGT-1, while the stage BBCH89 when the fruit is ripe for consumption, i.e. has typical taste and firmness, was advanced by 8-14 days under TGT-3, by 6-10 days under TGT-2 and by 1-2 days under TGT-1.

Amongst the three genotypes, the advancement in the phenological stages was more pronounced in Daisy, while less in the Kinnow genotype. Under the TGT-3 temperature elevation in genotype Daisy, W Murcott and Kinnow, the bud stages (BBCH1 and BBCH7) were advanced by 12-15, 5-7 and 4-7 days, respectively; the flowering stages (BBCH60, BBCH65 and BBCH69) were advanced by 13-17, 14-17 and 11-13 days, respectively; fruit set (BBCH71 and BBCH72) stages were advanced by 13-15, 14-17 and 14 days, respectively; physiological fruit beginning (BBCH73 and BBCH74) stage were advanced by 24-29, 12-15 and 10 days, respectively. Interestingly, towards the ripening of fruit for picking stage (BBCH85 and BBCH89) stage under TGT-3 elevated conditions, the advancement from the open control was more in the case of genotype Kinnow (12-14 days) followed by W Murcott (11-14 days) and least in Daisy (8-11 days).

#### 3.2. Effect on horticultural traits

Citrus cultivars vary in their propensity for their horticultural traits under different temperature conditions. The fruit set, fruit drop, fruit maturity, ultimate fruit yield and quality were influenced significantly by elevation in temperature (Table 2). The high temperature (TGT-3) led to a negative influence on fruit set in different citrus genotypes, i.e. Kinnow (28.98%), Daisy (21.63%) and W Murcott (30.70%). Likewise, the degree of fruit drop under TGT-3 conditions was maximum in W Murcott (78.42%) and minimum (30.58%) in Kinnow. The fruiting density of Daisy mandarin is 4.60 fruits/m<sup>3</sup> in TGT-3 (54.30%) which was significantly lower than that observed in TGT-1 (7.80 fruits/m<sup>3</sup>) and TGT-2 (6.39 fruits/m<sup>3</sup>). The reduction in fruiting density in the Kinnow mandarin and W Murcott ranged from 28.08-36.27% and 4.76-12.56%, respectively. Under elevated temperature (TGT-3) conditions, the fruit yield reduction was maximum (60.20%) in Daisy mandarin

TABLE 2

Yield parameters of three mandarin genotypes under open field and elevated temperature conditions

Genotypes	Temperature conditions	Fruit Set (%)	RP* (%)	Fruit Drop (%)	EP** (%)	Fruiting Density (no/ m <sup>3</sup> )	RP* (%)	Fruit Yield (Kg/ plant)	RP * (%)	Yield Efficiency (Kg/ m <sup>3</sup> )	RP *
Kinnow	Control	33.33 <sup>c</sup>	-	13.03 <sup>b</sup>	-	20.30 <sup>a</sup>	-	2.51 <sup>a</sup>	-	2.18 <sup>a</sup>	-
	TGT-1	27.33 <sup>e</sup>	18.00	12.93 <sup>b</sup>	0.77	14.40 <sup>c</sup>	29.06	1.71 <sup>c</sup>	31.80	1.51 <sup>b</sup>	30.57
	TGT-2	26.50 <sup>e</sup>	20.46	12.82 <sup>b</sup>	1.61	13.68 <sup>c</sup>	32.61	1.53 <sup>d</sup>	39.22	1.46 <sup>b</sup>	32.83
	TGT-3	23.67 <sup>f</sup>	28.99	12.71 <sup>b</sup>	2.46	10.96 <sup>d</sup>	46.01	1.25 <sup>e</sup>	50.03	1.28 <sup>c</sup>	41.23
Daisy	Control	21.84 <sup>g</sup>	-	8.14 <sup>d</sup>	-	13.70 <sup>c</sup>	-	1.85 <sup>b</sup>	-	1.36 <sup>bc</sup>	-
	TGT-1	19.04 <sup>h</sup>	12.82	8.02 <sup>d</sup>	1.47	11.02 <sup>d</sup>	19.56	1.42 <sup>d</sup>	23.44	1.00 <sup>de</sup>	26.15
	TGT-2	17.20 <sup>i</sup>	21.25	8.00 <sup>d</sup>	1.72	9.20 <sup>e</sup>	32.85	1.17 <sup>e</sup>	36.55	0.80 <sup>f</sup>	40.93
	TGT-3	16.24 <sup>i</sup>	25.64	7.70 <sup>d</sup>	5.41	8.54 <sup>e</sup>	37.66	0.74 <sup>f</sup>	60.20	0.56 <sup>g</sup>	58.52
W Murcott	Control	38.00 <sup>a</sup>	-	21.96 <sup>a</sup>	-	16.04 <sup>b</sup>	-	1.77 <sup>bc</sup>	-	1.07 <sup>d</sup>	-
	TGT-1	29.40 <sup>b</sup>	22.63	13.99 <sup>b</sup>	36.29	15.41 <sup>b</sup>	3.93	1.67 <sup>c</sup>	5.81	0.97 <sup>de</sup>	9.26
	TGT-2	27.00 <sup>d</sup>	28.95	13.12 <sup>b</sup>	40.25	13.88 <sup>c</sup>	13.47	1.48 <sup>d</sup>	16.38	0.93 <sup>e</sup>	13.52
	TGT-3	26.33 <sup>d</sup>	30.71	12.51 <sup>c</sup>	43.03	13.82 <sup>c</sup>	13.84	1.46 <sup>d</sup>	17.51	0.90 <sup>e</sup>	15.51
CD	-	2.03	-	1.15	-	2.15	-	0.13	-	0.08	-

\*RP= reduction percentage, \*\*EP= elevated percentage, no/ m<sup>3</sup>= number per cubic meter, Kg/ m<sup>3</sup>= Kilogram per cubic meter, %= percentage, CD= critical difference. Superscript alphabetic letters represent significant differences determined by DMRT (at P < 0.01). Same letters are not significantly different at P≤0.05.

TABLE 3

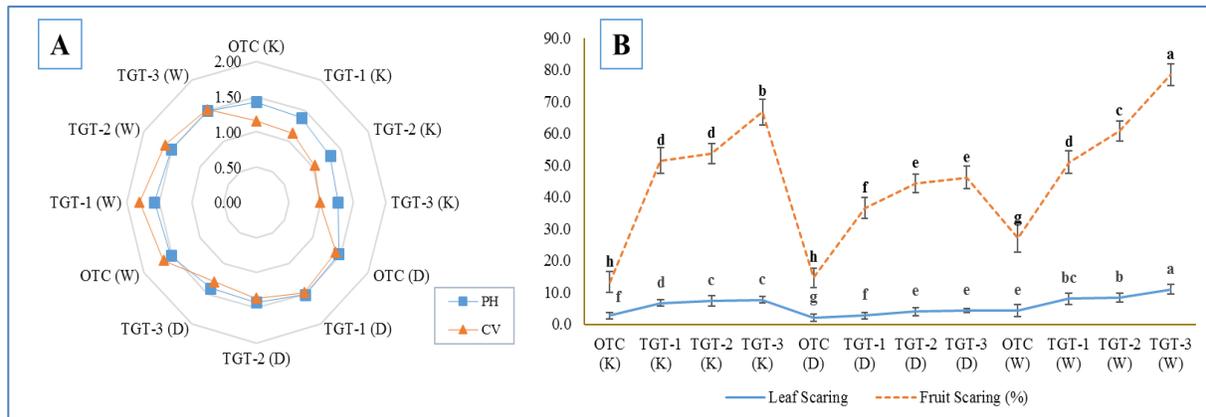
Fruit physical and physicochemical characteristics of three mandarin genotypes under open field and elevated temperature conditions

Genotypes	Temperature conditions	Fruit Weight (gm)	RP* (%)	Fruit Length (mm)	RP * (%)	Fruit Breadth (mm)	RP * (%)	TSS (°B)	RP * (%)	TA (%)	EP ** (%)
Kinnow	Control	123.63 <sup>c</sup>	-	5.41 <sup>c</sup>	-	6.05 <sup>b</sup>	-	10.17 <sup>b</sup>	-	0.55 <sup>c</sup>	-
	TGT-1	119.30 <sup>d</sup>	3.50	5.34 <sup>c</sup>	1.36	5.93 <sup>cd</sup>	1.93	9.83 <sup>c</sup>	3.31	0.58 <sup>c</sup>	6.06
	TGT-2	117.40 <sup>d</sup>	5.04	5.28 <sup>c</sup>	2.40	5.88 <sup>cd</sup>	2.75	9.72 <sup>d</sup>	4.46	0.63 <sup>b</sup>	15.15
	TGT-3	114.10 <sup>e</sup>	7.71	5.22 <sup>c</sup>	3.51	5.77 <sup>d</sup>	4.68	9.52 <sup>f</sup>	6.42	0.67 <sup>a</sup>	21.21
Daisy	Control	135.03 <sup>a</sup>	-	5.82 <sup>a</sup>	-	6.15 <sup>a</sup>	-	10.27 <sup>a</sup>	-	0.56 <sup>c</sup>	-
	TGT-1	128.87 <sup>b</sup>	4.56	5.72 <sup>ab</sup>	1.72	6.02 <sup>bc</sup>	2.17	9.82 <sup>c</sup>	4.41	0.57 <sup>c</sup>	2.38
	TGT-2	126.00 <sup>b</sup>	6.69	5.63 <sup>b</sup>	3.26	5.95 <sup>c</sup>	3.25	9.67 <sup>de</sup>	5.87	0.58 <sup>c</sup>	4.17
	TGT-3	122.47 <sup>c</sup>	9.30	5.52 <sup>bc</sup>	5.21	5.85 <sup>d</sup>	4.88	9.50 <sup>f</sup>	7.50	0.62 <sup>b</sup>	10.12
W Murcott	Control	110.33 <sup>f</sup>	-	5.35 <sup>c</sup>	-	5.42 <sup>e</sup>	-	9.83 <sup>c</sup>	-	0.69 <sup>a</sup>	-
	TGT-1	106.40 <sup>h</sup>	3.56	5.30 <sup>c</sup>	0.93	5.40 <sup>e</sup>	0.37	9.63 <sup>e</sup>	2.03	0.69 <sup>a</sup>	0.00
	TGT-2	107.07 <sup>g</sup>	2.96	5.25 <sup>c</sup>	1.87	5.38 <sup>e</sup>	0.74	9.58 <sup>ef</sup>	2.54	0.69 <sup>a</sup>	0.00
	TGT-3	105.47 <sup>h</sup>	4.41	5.22 <sup>c</sup>	2.49	5.33 <sup>e</sup>	1.66	9.50 <sup>f</sup>	3.36	0.71 <sup>a</sup>	2.89
CD	-	2.15	-	0.19	-	0.09	-	0.08	-	0.04	-

\*RP= reduction percentage, \*\*EP= elevated percentage, gm= grams, mm= millimeter, %= percentage, °B= degree brix, CD= critical difference. Superscript alphabetic letters represent significant differences determined by DMRT (at P < 0.01). Same letters are not significantly different at P≤0.05.

followed by intermediate (50.03%) in Kinnow and lowest (17.51%) in W Murcott. The fruit yield efficiency of citrus genotypes, *i.e.* Daisy, Kinnow and W Murcott was reduced in decreasing order by 26-58, 30-41 and 9-15%, respectively.

Temperature significantly affects the fruit weight and TSS/acid ratio of mature fruits (Table 3). The high temperature (TGT-3) led to the reduction of fruit characteristics from the control conditions of citrus genotypes W Murcott, Kinnow and Daisy in increasing order, *i.e.* fruit weight by 4.4, 7.7 and 9.3%, respectively;



**Figs. 4(A&B).** A) Plant height, plant canopy volume characteristics and B) Fruit scarring and leaf burning (%) of mandarin cultivars under open field and elevated temperature conditions. PH= plant height (cm), CV= canopy volume ( $m^3$ ), K= Kinnow, D= Daisy, W= W Murcott, TGT = Temperature Gradient Tunnel, OTC= Open field conditions

fruit length by 2.5, 3.5 and 5.2%, respectively and fruit breadth by 1.7, 4.7 and 4.9%, respectively. Under elevated temperature conditions, the TSS (%) was reduced in citrus genotypes, i.e. by 4.4-7.5% of the Daisy mandarin, by 3.3-6.4% of Kinnow and by 2.0-3.3% of W Murcott.

In general, the elevated temperature has a depressing effect on the plant height and canopy volume of citrus genotypes. However, under the TGT-1 elevated temperature conditions in the case of genotype Daisy and W Murcott mandarin, an increase in plant height response by 3.26 and 3.61%, respectively and an increase in canopy volume response by 4.69 and 9.17%, respectively, were observed (Fig. 4A). The leaf burning and fruit scarring were increased under elevated temperature regimes (Fig. 4B). Amongst the three genotypes, maximum leaf scarring ranging from 8-11% was observed in genotype W Murcott, followed by 7-8 % in Kinnow and at least 3-4% in Daisy. The fruit scarring under ambient control temperature was observed to be 13% in Kinnow, 14% in Daisy and 27% in W Murcott. However, with an increase in mean elevated temperature by 1.54, 2.04, and 2.31 °C during the two growing seasons, an increase in the incidence of fruit scarring was recorded maximum in the range of 51-78% in genotype W Murcott, followed by 51-67% in Kinnow and 36-46% in Daisy.

### 3.3. Correlation analysis

The correlation analysis of the horticultural traits depicts the significant effect of the different temperature regimes on the association between the pairs of different variables (Fig. 5). The fruit drop (%) has non-significant correlations with FY (Fruit Yield) and YE (Yield Efficiency) under ambient conditions, while there was a strong positive correlation between FD vs. FY under elevated temperature regimes. Contrastingly, fruiting density (FDT) has a strong positive correlation with FY (r

= 1.00) and YE (r = 0.98) with open control (O) conditions, while the degree of association between FDT vs FY (r = 0.81) and FDT vs YE (r = 0.94) is somewhat less under TGT-1 (T1) conditions. A similar magnitude of correlation was observed between FDT, FY and YE for TGT-2 (T2) and TGT-3 (T3) growing conditions. Amongst the fruit traits, FW (Fruit Weight) depicted a high positive correlation with FL (Fruit Length; r = 0.90) and FB (Fruit Breadth; r = 0.94) under O growing conditions as compared to elevated temperature regimes.

Similarly, TSS (Total Soluble Solids) depicted differential degrees of correlation coefficients with fruit yield traits, i.e. for O growing conditions wherein it was positively correlated with FY (r = 0.39), YE (r = 0.53) and FW (r = 0.97). Under T1 conditions it was negatively correlated with FY (r = -0.34), but positively with YE (r = 0.58) and FW (r = 0.89); under T2 growing conditions, the TSS was positively correlated with YE (r = 0.64) and FW (r = 0.67) but had a non-significant correlation with FY. However, under the T3 growing conditions, TSS was positively correlated with YE (r = 0.88) and had a non-significant correlation with FW. However, TA (Titratable Acidity) varied in the opposite direction to fruit physical traits.

### 3.4. PCA and heatmap analysis

The PCA-biplot analysis revealed a prominent difference in the horticultural traits of different mandarin genotypes at elevated temperature regimes and ambient conditions. The first two axes (PC1 and PC2) explain a total variation of 88.9% (Fig. 6). PC1 alone explains 51.5% of the variation and is loaded for FD, FW, FL, FB, TSS and TA. While the PC2 is related to FS, FDT, FY and YE. Among the horticultural traits, traits like FDT, FY and YE remained in proximity and did not illustrate a significant variation. Furthermore, amongst the genotypes

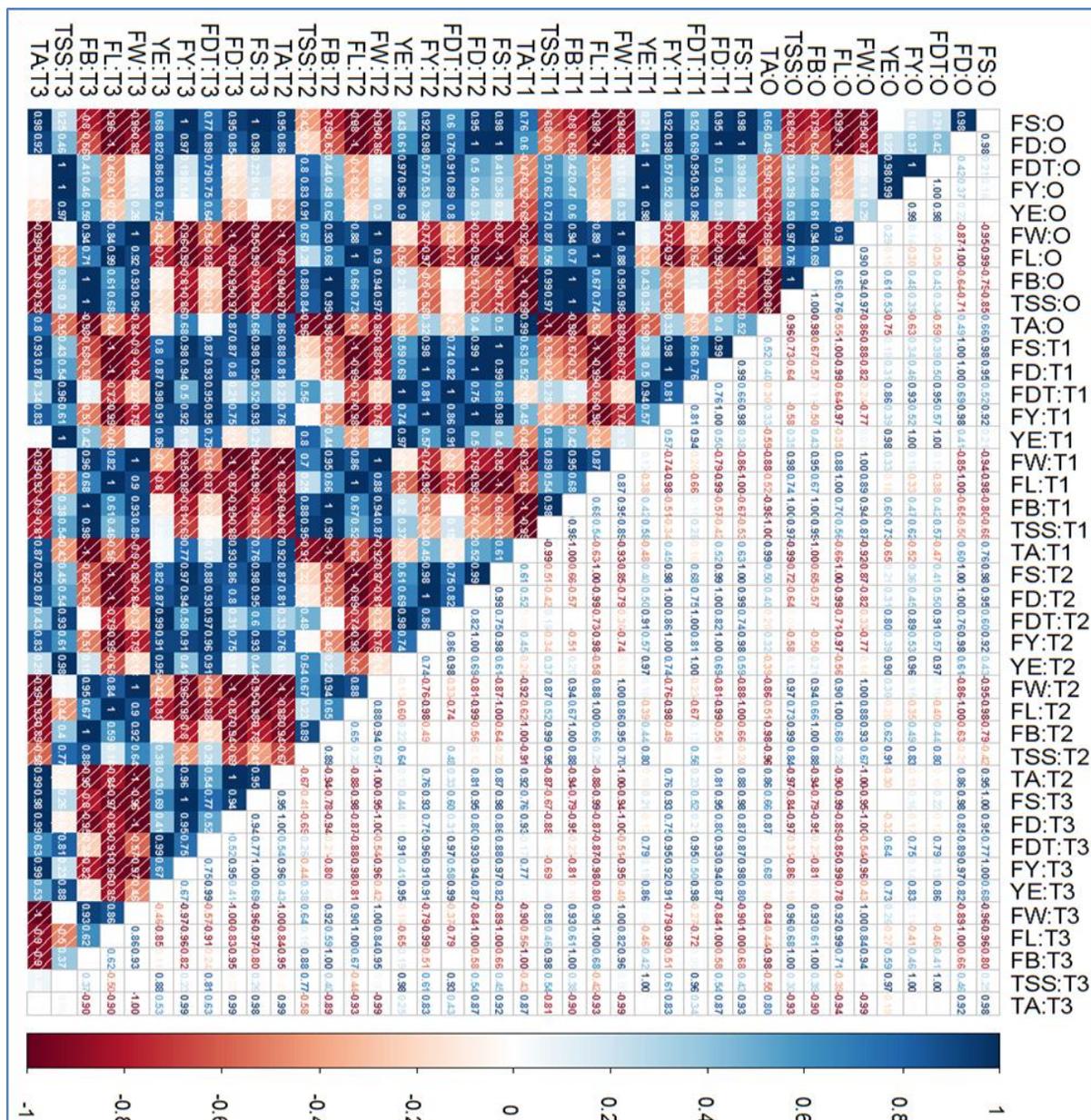
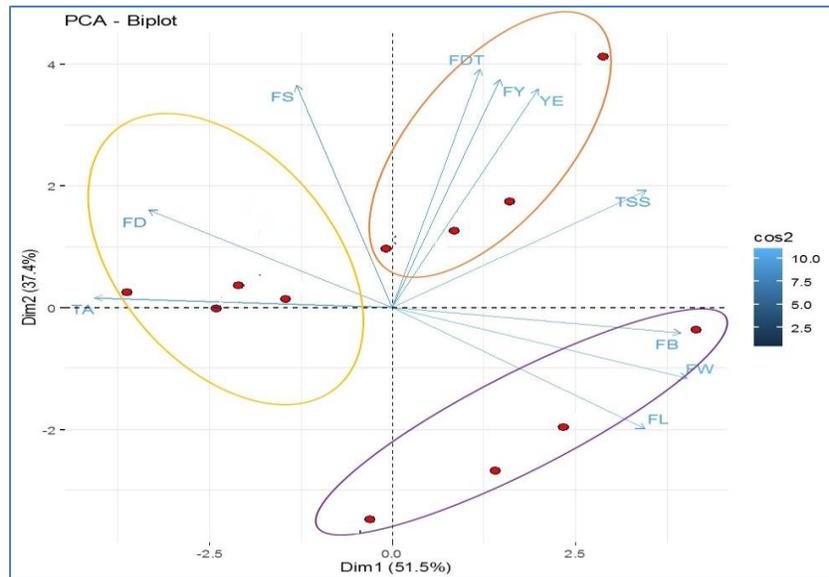


Fig. 5. Significance of the correlation among horticultural traits at ambient and elevated temperature of citrus mandarin genotypes. The intensity of color is proportional to the correlation coefficient, while the red and blue color indicates negative and positive correlation respectively

(Kinnow, Daisy, and W Murcott), Kinnow at TGT-1 had comparatively the lowest dispersion, while they demonstrated a maximum divergence as compared to the other treatment combinations.

The cluster heat map analysis summarised the horticultural response of different mandarins under ambient and elevated TGT conditions. In the context of trait association, the heat map grouped the genotypes into two main dendrograms concerning the regime of temperature conditions. One cluster contained Kinnow

and Daisy at ambient (OTC) conditions, while the rest of the genotypic treatments marked another separate cluster, in terms of the trait association and expression. Thus, the genotypes depicted an analogous trend of dendrogram formation at the elevated temperature conditions within the TGT. However, the horticultural traits illustrated differential associations varying from positive to negative extremes in studied mandarin genotypes under the ambient and high-temperature regimes (Fig. 7) and were, therefore, grouped into two major clusters.



**Fig. 6.** PCA-biplot graphic scattered plot of horticultural trait clustered according to their similarity and dissimilarity among three different mandarin genotypes. The vectors derived from biplot origin represent positive and negative associations among traits

### 3.5. Effect of climate change on citrus

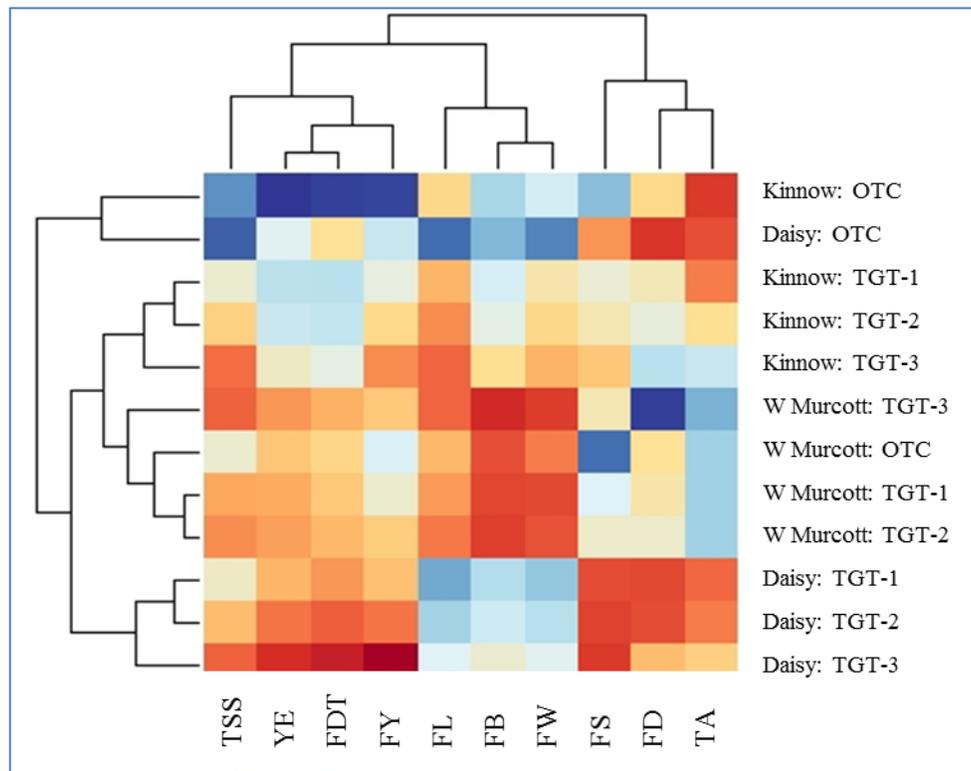
An increase in temperature due to the emission of greenhouse gases (GHG) is one of the major detrimental effects of climate change on woody perennials (Fraga *et al.*, 2021). Climate change is expected to have more serious consequences in subtropical regions (Gornall *et al.*, 2010). The north Indian regions were identified to be affected by future warming due to climate change (Mitra, 2021). Within the citrus growing regions in India, the northern regions are major citrus production areas. Citrus cultivars exemplify the climate shift scenario, as they may experience physiological challenges resulting in reduced productivity and compromised fruit quality. These environmental stressors are becoming more frequent and severe, and hence, this increase in suffering from plants is leading to a loss of citrus productivity and fruit quality (Balfagón *et al.*, 2022). A Temperature Gradient Tunnel (TGT) is a very economical facility to study the response of field (Brar *et al.*, 2022) and horticulture (Brar *et al.*, 2024) crops to increased temperature environments. Therefore, the present study aimed to understand and study the impact of elevated temperature conditions within a Temperature Gradient Tunnel (TGT) on phenological and horticultural variations among the three different mandarin cultivars.

### 3.6. Effect of temperature increase on the phenology of citrus mandarin

Flowering time is one of the most important phenological phases, regulated by the climatic conditions

of the locality. An increase in temperature can cause advancement in the shifts of different phenological stages, *i.e.* from bud burst (BBCH1) stage to winter dormancy period (BBCH97) at varied intensity. In the present study, the three genotypes behaved differently w.r.t. the timing of their phenological growth stages. Oliveira *et al.*, (2017) observed that some of the phenological stages are highly affected by a slight elevation in temperature and are therefore called “Critical stages”. The results of the present study provided better insights into the variability of the flowering phase that was observed in the elevated temperature regimes as compared to controlled ambient conditions. The mean temperature increased by 1.54, 2.04, and 2.31 °C during the two growing seasons, advancing the flowering stages (BBCH60, BBCH65 and BBCH69) maximum by 14-17 days in genotype W Murcott, followed closely by 13–17 days in Daisy and at least by 11-13 days in Kinnow.

Earlier studies have also reported that the bud burst and flowering in citrus are controlled by environmental factors rather than intrinsic cues (Tan and Swain, 2006; Connellan *et al.*, 2010) and hence the climatic variations at the regional level must be considered when assessing the phenological shifts and framing of orchard plantations of citrus. However, citrus trees do not experience strong “endo-dormancy” relative to temperate deciduous trees, so they are potentially at risk due to an increase in temperatures, especially during winter and early spring. Further, the advancement in bud burst and flowering exposes the buds and flowers to frost damage for a long period ( Rigby and Porporato, 2008 ). Dalal *et al.* (2017)



**Fig.7.** Cluster heat map analysis summarizing the responses of ambient and elevated temperature on horticultural performance of mandarin genotypes. The colour bands reveal the differential association of traits. The color intensity represents the degree of change. Rows represent genotypes and columns the studied traits expressed as the difference between the individual genotypes of each treatment (within a Column). FS= Fruit set (%), FD=Fruit drop (%), FDT= Fruiting density (no/ m<sup>3</sup>), FY= Fruit yield (Kg/ tree), YE= Yield efficiency (Kg/ m<sup>3</sup>), FW= Fruit weight (gm), FL= Fruit length (mm), FB= Fruit breadth (mm), TSS= Total soluble solids (°B), TA= Titratable acidity (%)

reported the advancement in dates of occurrence of the bud burst and other phenological stages in north Indian citrus belts. The effects of increasing temperature will vary according to the phenological stage of the crop and may anticipate or even shorten the events that require degree-day accumulation (De Ollas *et al.*, 2019). Discordance in the degree of shift and direction can also occur in response to disease and insect pests, which increases the difficulty of determining the point at which pesticides should be applied (Gordo and Sanz, 2005). Moreover, the current warming conditions might be more amenable to outbreaks of disease and insect pests, which can damage buds, fruit and quality yield.

### 3.7. Effect of temperature increase on fruit characteristics of citrus mandarin

Atmospheric warming causes a reduction in fruit yield and quality of fruit crops, especially if changes in short-term temperature extremes coincide with key stages of crop

development (Romero *et al.*, 2021). In citrus plants, the fruit drop is greatly affected by an increase in temperature conditions. In the present study, under the three elevated temperature regimes, there was a reduction in fruit setting in the range of 17-21, 18-29 and 25-31% in genotypes Daisy, Kinnow and W Murcott, respectively, in increasing order as compared to open control environments. Furthermore, the high-temperature conditions significantly altered horticultural traits of mandarin genotypes by affecting fruit weight, fruit length, fruit breadth and TSS. The fruit weight of citrus cultivars decreased along with an increase in temperature inside the TGT. The reduction of TSS of different citrus genotypes also varied under elevated temperature conditions. The total soluble sugar contents of Kinnow mandarin displayed significant differences among the TGT treatments, while W Murcott and Daisy showed non-significant differences among the temperature treatments. The leaf scaring (%) and fruit scaring (%) were significantly higher under elevated temperature regimes as compared to the ambient environment.

In a recent study, Balfagon *et al.* (2022) reported that the increase of temperatures above the critical range adversely affects the citrus fruit characteristics, thereby causing fruit abscission, reduction in size of fruit and degradation of organoleptic fruit quality. Under elevated temperature conditions in the study, the fruit traits such as FDT, FY, YE and TSS showed a strong negative association with the elevated temperature, while the rest of the parameters also depicted somewhat similar behavior. Overall, the differential temperature conditions significantly altered the degree and intensity of correlation among horticultural traits, *i.e.*, FD has a non-significant relationship with FY at OTC and a strong degree of correlation between FD vs. FY within TGT conditions (Fig. 5). Similarly, the heatmap analysis further authenticated the tolerance behavior of genotype Kinnow under the elevated temperature conditions for the response of FY, YE and TSS. In the case of the other two genotypes (Daisy and W Murcott) examined under elevated conditions, they formed separate clusters (Fig. 7). Besides, all three genotypes under open (O) control conditions and Kinnow under TGT-1 (T1) environment illustrated the maximum degree of association for the FDT, FY, YE and TSS as compared to other treatments (Fig. 6).

The effect of elevated temperatures on citrus production trends is still not clear (Rasera *et al.*, 2023) because of the interactions between crop management, environmental factors and genotypic specificity. Little scientific information regarding the effect of increased temperatures is available on woody perennial crops (Medda *et al.*, 2022). However, a strong concentration of the research efforts still seems focused on measuring the consistency of the direct effect of temperature increases on fruit crops and testing tools for response to the adaptation needs (Ettinger *et al.*, 2020). From this perspective, a more intense elaboration of prevision models for crop behaviour and a wide application of agricultural practices like TGT may be useful futuristic tools for woody crops (Serrano *et al.*, 2020). The severity of damage produced by temperature rise in perennial cropping systems is dependent on the phenological stage (De Ollas *et al.*, 2019).

Rising atmospheric warming may indirectly impinge on fruit crops through their effects on the resistance of crops to specific insect pests and diseases, on the pathogenicity of organisms, and the amplitude of outbreaks (Gregory *et al.*, 2009). Whereas, shifts in floral phenophases can affect plant-pollinator interactions, leading to pollination depression, thus compromising yield and quality of crops (Morton *et al.*, 2017). Phenotypic plasticity is a key plant strategy in response to abiotic stress (Pájaro-Esquivia *et al.*, 2021; Stotz *et al.*, 2021). Therefore, the differential expression of diverse citrus genotypes to extreme temperature conditions helps the citrus growers

and/or scientists as adaptation strategies to climatic buffering and assists in futuristic citrus orchard management strategies for a warming climate.

#### 4. Conclusions

A climatic scenario with high atmospheric warming as a future perspective makes it necessary to study the elevated temperature effects on phenological behavior and horticultural performance of citrus. This information about the phenological response of different mandarin genotypes to varied elevated temperatures provides a framework for future predictions of flowering intensity and transitional shifts of phenophases. The elevated temperature mediates a reduction in horticulture performance and organoleptic quality and ultimately leads to the degradation of consumerable production. Thus, studying citrus tolerance behavior to artificially increased temperature changes may hold the key to the development of new citrus varieties which are capable of withstanding future environmental conditions and help to better decision-making and orchard management, and it would help to more sustainable and profitable crop production.

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#### Data availability

The datasets generated for this study are available to the corresponding author upon reasonable request.

#### Authors' Contributions

Jaswinder Singh Brar carried out the experiment, analyzed the data, and wrote the manuscript (*email: jsbrar74@pau.edu*).

Sukhjinder Singh Mann analyzed the data and wrote the manuscript (*email: Sukhjinder-fs@pau.edu*).

Prabhjyot-Kaur provided the study material and edited the manuscript (*email: prabhksidhu@gmail.com*).

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