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ORIGINAL RESEARCH ARTICLE

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# Assessment of phenological and physicochemical characteristics of peach (*Prunus persica* L.) varieties in Mustang, Nepal

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ARTICLE HISTORY	ABSTRACT				
Received: 30 October 2024 Revised received: 12 December 2024 Accepted: 17 December 2024	This research was conducted at the Temperate Horticulture Development Center (THDC), Mustang, Nepal, to study flowering phenology and evaluate the physicochemical characteris- tics of five peach varieties (Surprise, Benedict, Peregrine, Local Khallya and Early Red) from Eebruary to Sentember 2023. This research was conducted on a randomized complete block				
Keywords	design with four replications and five treatments (fruit trees of a particular variety were repli- cated four times). The flowering phonology of varieties was studied by dividing the develop				
Chemical characteristics Peach ( <i>Prunus</i> spp.) Phenological stages Total soluble solids Varieties	mental stages of a flower into eight different stages: first swelling, calyx green, calyx red, first pink, first bloom, full bloom, petal fall and fruit set. The completion of each phenological stage was marked from a reference date. Physicochemical analysis of fruits was performed following UPOV guidelines of peach. Physical characteristics were encompassed in individual fruit weight, fruit length, fruit volume, fruit size (length and width), stone weight, stone size (length and width) and mesocarp thickness and in chemical characteristics, pH, total soluble solids (TSS), titratable acidity (TA) and TSS/TA ratio were included. The study of flowering phenology revealed that Early Red was found to be the earliest (60.50 DARD) to reach the fruit-set stage. The highest and lowest fruit weights were measured in Benedict (153.85 g) and Early Red (46.5 g), respectively. Finally, chemical analysis revealed that TSS/TA was found to be maxi- mum in Benedict (12.38) and minimum in Early Red (6.03). It can be concluded that Early Red was the earliest maturing variety among the varieties. Also, Benedict was found to be the su- perior in terms of sweetness and size.				

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#### INTRODUCTION

Peaches (*Prunus persica* L.) are precocious, succulent fruits that grow on deciduous trees originally native to China (Hans *et al.*, 2020). Peaches are stone fruits characterized by red, pink, or orange skin covering yellow or white flesh that grow in a fairly limited range in dry, continental or temperate climates. They contain niacin, folate, iron, choline, magnesium, phosphorus, zinc and copper as well as a good source of antioxidants and beta-carotene (Ensle, 2015). In Nepal, it is mostly grown in hilly

regions, known by the name of *Aru*. Peach farming in Nepal has been done since immemorial times by the local farmers only for their use. In Nepal, the production, production area and productivity are 9252 Mt, 1378 ha and 6.72 Mt/ha (MoALD, 2023). Whereas in Mustang, the production, cultivated area and productivity are 8.0 Mt, 2.0 ha and 7.9 Mt/ha, respectively. In Nepal, the major peach-growing districts are Sankhuwasabha, Dhankuta, Sindhupalchowk, Rasuwa, Kavre, Nuwakot, and Manang (MoALD, 2023). Despite having higher productivity than the national average, the production area has been



decreased in recent years (MoALD, 2022). Farmers and commercial growers don't have exact idea for timing of pesticide treatment and carry out interculture operations due to lack of knowledge of phenology in local condition. Offseason flowering has also been observed in peach (Malla, 2008). Peach has been on cultivation at THDC farm since a long time ago yet research on phenological and pomological characteristics of peach have never been done for the maintenance of the phenological calendar (THDC, 2023). Some cultivars (local and novel) are not valued due to lack of information regarding their features (Szot et al., 2022). The flowering phenology is the fundamental characteristic of plants that enables them to adapt to environmental changes and progeny reproduction which is the beginning of plant reproductive growth and a significant stage of general phenology (Cortés -Flores et al., 2017). The phenological study of peaches has been described for the first time according to the BBCH scale (Biological Bundesanstalt, Bundessortenamt und Chemical Industry). Then, Hack et al. (1992) and Hess et al. (1997) proposed an extended BBCH scale to study the phenology of all monocotyledon and dicotyledon plant species. Phenology can show the influences of climate change on individual plant fitness and biodiversity through biological activities (Sun & Frelich, 2011). Study of phenological growth stages of peach tree can be used to control diseases and pests in distinct growth stages (Chapman & Catlin, 1976). Phenology is important to support peach growers in crop management practices such as orchard establishment, fertilization, irrigation, frost protection, and pollination under climate variability circumstances (Cifuentes-Carvajal et al., 2024). To obtain satisfactory production by determining suitable agronomic techniques, it is key to determine phenological behavior of crop (Ghrab et al., 2016). Phenology also serve as a basis for classification of early and late varieties grown at THDC as well as one of the indicator of climate change. Physical properties of peach are crucial for designing of equipment to handle, transport, process and store (Zohrabi et al., 2013). Also, by knowing physicochemical characteristics of peach, it becomes easier to understand product performance across value chain and ulility for intended purpose (Mosie et al., 2023). This research aims to provide detailed insights into assessment of phenological growth stages of different varieties of peach found at THDC and their physicochemical characteristics.

#### MATERIALS AND METHODS

#### Site and climatic observation

The study was carried out at the Temperate Horticulture Development Center at Gharapjhong Rural Municipality-2, Mustang district, coordinated at 28° 20' to 29°05' N and 83° 30' to 84° 15' E, which is 2,650 meters above sea level. The center is divided into four blocks (A, B, C and D). Our research was performed in block 'A' of THDC. Meteorological data (maximum and minimum mean daily air temperature, rainfall, and humidity) were taken from the automatic weather station (Meteorological Station, Thakmarpha-Index No. 0604) present at the THDC. The observed climatic variables during the research period are depicted in Figure 1.

#### Plant materials and research design

The research was conducted in 2023 at the peach orchard THDC for the single flowering season from February to September 2023. All the plants selected for the study were ten to fifteen years old. They were planted 6 ×6 m apart. The trees were grafted on 'chili' (local variety of apricot) rootstock. All of them were trained in an open-vase system. Different varieties of peach selected with their canopy parameters are shown in Table 1. The experiment was carried out in a Randomized Complete Block Design (RCBD). Five varieties of peach: Local Khallya, Surprise, Benedict, Peregrine and Early Red were replicated four times and each variety consisted of four trees. Similar orchard management practices (irrigation, fertilization, pruning, pest and disease management) were adopted in all selected plant materials as per the local conditions carried out in all the plants during the study.

#### Observation of phenological growth stages

The eight principal phenological stages of peach were described according to the BBCH general scale: first swelling, calyx green, calyx red, first pink, first bloom, full bloom, petal fall and fruit set. Cifuentes-Carvajal et al. (2024) and Mounzer et al. (2008). differentiated different floral stages of peach for a study similar to our procedure. Before observation of phenology, a reference date was first selected. February 15 2023, was selected as an arbitrary date for the study. Completion of a particular phenological stage was marked from the reference date. The beginning of dormancy occurs when more than 50% of leaves discolor or fall. The date of the first swelling stage was recorded when 75% of the floral bud stopped growing, resembling the resting stage. The first swelling was marked when light brown scales were visible, scales that were light-colored and hairy. Calyx green was confirmed when the calyx turned a light green color with hairs and calyx red was recorded when the calyx turned red color without bursting of bud. First pink was confirmed when the sepals opened: petal tips visible; single flowers with white or pink petals (still closed). The first bloom was recorded when the first flower opened from a single branch. Full flowering: at least 75% of flowers open. The petal fall stage occurred when the majority of petals fell. Similarly, the fruit set was recorded when more than 75% of the fruit was set in the trees.



**Figure 1.** Average daily maximum and minimum temperature (°C), relative humidity (%) and rainfall (mm) recorded at THDC, Mustang, 2023.

Table 1. Tree canopy parameters and thickness at THDC,Marpha, Mustang, 2023.

Variety	Tree canopy E-W (m)	Tree canopy N-S (m)	Tree height (m)	
Local Khallya	6.84	5.27	4.16	
Surprise	6.14	5.20	4.12	
Benedict	4.43	4.39	3.45	
Peregrine	3.99	4.11	2.85	
Early Red	4.74	4.33	3.13	

**Table 2.** Harvesting dates of different peach varieties at THDC,Marpha, Mustang, 2023.

S. No.	Variety	Harvesting date
1	Early red	18 June, 2023
2	Surprise	26 July, 2023
3	Peregrine	02 August, 2023
4	Benedict	09 August, 2023
5	Local Khallya	04 September, 2023

#### Harvesting and measurement of pomological traits

Harvesting of peach fruits was done manually on different dates (Table 2). Harvested fruits were kept in refrigeration at 0 °C until use for physicochemical analysis (Alhaj Alali *et al.*, 2023).

## Fruit length, fruit width, mesocarp thickness, stone length and stone width

After harvesting, random sampling was done for selecting fruits of different varieties (Omayio *et al.*, 2022). Physical characteristics of different varieties of peaches were determined using UPOV guidelines of peaches (UPOV, 2021). Twenty freshly harvested fruits were selected from each replication for the study. All the linear dimensions were measured using a vernier caliper with an accuracy of 0.01 mm (Neupane *et al.*, 2023).

#### Fruit weight, volume and stone weight

The average weight of fruit and stone was expressed in grams. For weighing individual fruit and stone weight, we used an electronic balance of 0.1 g accuracy (Singh *et al.*, 2016). Individual fruit volume was estimated following the water displacement method (Huynh *et al.*, 2022). It is a visual method to determine the volume of an object using a measuring cylinder of 300 mL capacity. A single fruit at a time was dipped into the cylinder, which was half full of distilled water. With the use of a metal sponge sinker, the fruit was permitted to completely submerge in water, and the height increase was noted. Fruit volume is expressed in milliliters (mL) as the difference between the measuring cylinder's initial and final water readings. Thus, volume of fruit was expressed in cc (1 mL= 1 cm<sup>3</sup>).

#### pН

pH was estimated by using a digital pH meter after calibration as

reported by Mehta *et al.* (2019). For calibration, the electrode of the pH meter was put in a buffer solution of pH 4 and 7 before measuring the pH of fruit juice. After calibration, the electrode was washed using distilled water. Finally, the pH meter was made ready for taking measurements.

#### **Total Soluble Solids (TSS)**

An ATC-1E automatic hand-held refractometer (Atago, Tokyo, Japan) with a 0-32° Brix scale at 20°C was used for measuring TSS (Muharfiza *et al.*, 2023; Ikinsi & Bolat, 2018). Before taking measurements, the refractometer was calibrated using distilled water to zero the reading. After obtaining fruit juice, 1-2 drops of juice were poured into a prismatic surface, which was covered with a daylight diffusion cover and observed through the eyepiece of the refractometer.

#### **Titratable Acidity (TA)**

TA was estimated using the titration principle (Paul *et al.*, 2010). 10 mL of juice was obtained by crushing fruits, which was poured in the conical flask. An equal volume (10 mL) of distilled water was added for dilution. Then, 1-2 drops of phenolphthalein (indicator) were added to the conical flask. The burette was filled with 0.1 N sodium hydroxide (NaOH), which was titrated with diluted fruit juice. The end point of titration is estimated by carefully observing the color change during titration. The volume of NaOH consumed is obtained by subtracting the initial and final readings of the burette. Finally, TA is calculated by using the following formula.

$$TA \% = \frac{V_b \times N \times M_{eq}}{V_a} * 100$$

Where:

TA %: titrable acidity % or g malic acid/100mL of juice  $V_b$ : total volume of NaOH consumed in the reaction, ml N: normality of NaOH used (0.1N)

 $M_{eq}$ : milliequivalent of malic acid (0.067 for malic acid)  $V_a$ : total volume of fruit juice used, mL (generally 10 mL is taken)

#### Ripening Index (RI)/ Maturity index

The RI was determined by using the following formula (Kwon *et al.*, 2015; Milosevic & Milosevic, 2012).

$$RI = \frac{TSS}{TA}$$

#### **Statistical analysis**

MS Excel was used for data entry and processing. The R Studio agricolae package was used to carry out all descriptive analyses and mean values were differentiated by using the Duncan Multiple Range Test (DMRT) at a 5% significance level (Dafaallah, 2019).

#### **RESULTS AND DISCUSSION**

#### Flowering phenology of different peach varieties

Different varieties of peach under the study varied significantly in their timing of phenological stages (Table 3). The phenological stages of different varieties of peach at THDC are shown in Figure 2. Local Khallya was only the variety that took the longest time to complete four phenological stages: first pink (36.00 DARD), full bloom (50.50 DARD), full bloom (60.25 DARD) and petal fall (84.00 DARD) from the reference date (February 15). Benedict showed an exactly similar figure with Local Khallya to complete the full bloom stage (60.25 DARD). Similarly, Surprise took the longest duration to complete the first swelling (8.25 DARD), whereas Benedict took the longest duration to complete the calyx green (17.00 DARD) and calyx red stage (27.75 DARD). On the contrary, Peregrine was only the variety that took the least time to complete four phenological growth stages: calyx green (13.25 DARD), calyx red (22.00 DARD), first pink (30.50 DARD) and full bloom (51.25 DARD). Similarly, the shortest duration to complete first swelling (6.25 DARD), first bloom (35.75 DARD) and petal fall (60.50 DARD) was observed in Early Red. Ikinsi & Bolat (2018) showed similar findings in the case of Early Red in phenological growth pattern from initiation of flowering to fruit set. Earliness of flowering in varieties of peach like Early Red and Peregrine is due to their low chilling requirement. Local Khallya and Peregrine (7.00 DARD) were found statistically similar to Benedict (7.75 DARD), Surprise and Early Red to complete the first swelling stage. Local Khallya (15.25 DARD) and Early Red (14.25 DARD) were found statistically similar, whereas Benedict (17.00 DARD) and Surprise (16.75 DARD) were found statistically similar to complete the calyx green stage. Benedict (27.75 DARD), Surprise (25.25 DARD) and Early Red (27.00 DARD) were found statistically similar to complete the calyx red stage, whereas Local Khallya (36.00 DARD), Surprise (35.25 DARD) and Benedict (34.75 DARD) were found statistically similar to complete the first pink stage. To complete the first bloom stage, Surprise (42.50 DARD) and Benedict (44.25 DARD) were statistically similar and Peregrine (37.00 DARD) and Early Red were statistically similar. Surprise (69.00 DARD) was statistically similar to Benedict (71.50 DARD) to complete petal fall stage. Kwon et al. (2015) observed that Western cultivars bloomed earlier than Eastern cultivars, with no difference in ripening time from phenological traits, which was consistent in the case of our study. Western varieties (Early Red, Surprise and Peregrine) bloomed earlier than the local variety (Local Khallya). The possible causes for the variability of phenological growth stages are due to altitude (Bhardwaj & Chand, 2021) and Growth Degree Day (Sharma et al., 2019). Yan et al. (2024) reported that the rate of flower bud development in peach is affected by variety and chilling requirements. The results for the average blooming period (9-13 days) corroborated with the findings reported by Radovic et al. (2020). Major QTL is able to delay blooming in peach by increasing heat requirement (Cirilli et al., 2021).

Table 3. Phenological flowering stages of different varieties of peach at THDC, Marpha, Mustang, 2023.

Phenological flowering stages of peach, DARD							
Variety	First swelling	Calyx green	Calyx red	First pink	First bloom	Full bloom	Petal fall
Local Khallya	7.00 <sup>ab</sup>	15.25 <sup>ab</sup>	18.50 <sup>d</sup>	36.00ª	50.50 <sup>a</sup>	60.25ª	84.00 <sup>a</sup>
Benedict	7.75ª	17.00 <sup>a</sup>	27.75 <sup>ª</sup>	34.75°	44.25 <sup>b</sup>	60.25ª	71.50 <sup>b</sup>
Surprise	8.25ª	16.75 <sup>ª</sup>	25.25ª	35.25°	42.50 <sup>b</sup>	58.25ª	69.00 <sup>b</sup>
Peregrine	7.00 <sup>ab</sup>	13.25 <sup>b</sup>	22.00 <sup>c</sup>	30.50 <sup>b</sup>	37.00 <sup>c</sup>	50.75 <sup>b</sup>	63.25 <sup>°</sup>
Early Red	6.25 <sup>b</sup>	14.25 <sup>ab</sup>	27.00 <sup>a</sup>	32.50 <sup>ab</sup>	35.75°	51.25 <sup>b</sup>	60.50 <sup>c</sup>
LSD (0.05)	1.3	2.64	1.25	3.75	4.42	3.75	5.18
SE <sub>m</sub> (±)	1.19	0.38	0.18	0.54	0.64	0.54	0.75
F Test	***	***	***	***	***	***	***
CV (%)	11.6	11.19	3.36	7.2	4.34	4.83	4.83
Mean	7.25	15.3	24.1	33.8	42	56.15	69.65

Means in a column followed by different letters are significantly different, DMRT at P<0.05; LSD: Least Significance Difference, SE<sub>m</sub>: Standard Error of the mean, CV: Coefficient of Variation, DARD= Days after Reference Date.



3. Local Khallya



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		F	mysical Chara	cteristics of p	each varieties			
Variety	Fruit weight	Fruit volume	Fruit	Fruit width	Mesocarp	Stone	Stone	Stone width
	(g)	(cc)	length (cm)	(cm)	thickness (mm)	weight (g)	length (cm)	(cm)
Surprise	96.25 <sup>b</sup>	75 <sup>b</sup>	4.98 <sup>b</sup>	5.78 <sup>b</sup>	1.28 <sup>c</sup>	5.62 <sup>b</sup>	2.98 <sup>b</sup>	2.07 <sup>ab</sup>
Benedict	153.85°	89.75°	6.37ª	6.67 <sup>a</sup>	2.2 <sup>a</sup>	8.20 <sup>a</sup>	3.17ª	2.28ª
Peregrine	75.95 <sup>bc</sup>	60.75 <sup>c</sup>	4.85 <sup>b</sup>	5.04 <sup>c</sup>	1.65 <sup>b</sup>	5.00 <sup>b</sup>	2.53 <sup>d</sup>	1.93 <sup>b</sup>
Local Khallya	65.75 <sup>cd</sup>	41.5 <sup>d</sup>	4.17 <sup>c</sup>	4.16 <sup>d</sup>	1.23 <sup>c</sup>	4.30 <sup>b</sup>	2.28 <sup>e</sup>	1.27 <sup>c</sup>
Early Red	46.5 <sup>d</sup>	41.75 <sup>d</sup>	4.02 <sup>c</sup>	4.36 <sup>d</sup>	1.10 <sup>c</sup>	4.50 <sup>b</sup>	2.78 <sup>c</sup>	1.9 <sup>b</sup>
LSD (0.05)	23.75	11.45	0.67	0.39	0.319	1.33	0.16	0.28
SEm (±)	3.45	1.66	0.097	0.057	0.046	0.19	0.023	0.04
F Probability	***	***	***	***	***	***	***	***
CV (%)	17.58	12.04	8.93	4.96	13.92	15.67	3.68	9.66
Grand Mean	87.66	61.75	4.88	5.2	1.49	5.53	2.75	1.89
CV (%) Grand Mean	17.58 87.66	12.04 61.75	8.93 4.88	4.96 5.2	13.92 1.49	15.67 5.53	3.68 2.75	9.66 1.89

Means in a column followed by the different letter(s) are significantly different by DMRT at P<0.05; LSD: Least Significance Difference, SE<sub>m</sub>: Standard Error of the mean, CV: Coefficient of Variance.

Table 5. Chemical characteristics of different peach varieties	s at THDC, Marpha, Mustang, 2023.
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Table 4. Physical characteristics of different varieties of peach at Marpha, Mustang, 2023.

Chemical characteristics of peach varieties						
Varieties	рН	TSS (°Brix)	TA (%)	TSS/TA		
Surprise	5.06ª	9.07 <sup>b</sup>	1.23 <sup>c</sup>	7.42 <sup>b</sup>		
Benedict	3.5°	13.175 <sup>a</sup>	1.06 <sup>d</sup>	12.38°		
Perigreen	3.55 <sup>°</sup>	8.75 <sup>b</sup>	1.38 <sup>b</sup>	6.34 <sup>b</sup>		
Local Khallya	4.93 <sup>a</sup>	9.6 <sup>b</sup>	1.38 <sup>b</sup>	6.97 <sup>b</sup>		
Early Red	4.25 <sup>b</sup>	9.4 <sup>b</sup>	1.56ª	6.03 <sup>b</sup>		
LSD (0.05)	0.41	2.38	0.138	2.085		
SEm (±)	0.059	0.34	0.02	0.3		
F Test	***	***	***	***		
CV (%)	6.26	15.48	6.77	17.28		
Mean	4.257	10	1.33	7.83		

Means in a column followed by the different letter(s) are significantly different by DMRT at P<0.05; LSD: Least Significance Difference, SE<sub>m</sub>: Standard Error of the mean, CV: Coefficient of Variance.

#### Physical characteristics of fruits

Physical properties of different varieties of peach varied significantly as displayed in Table 4. Benedict was found to have the highest individual fruit weight (153.85 g), fruit volume (89.75 cc), fruit length (6.37 cm), fruit width (6.67 cm), mesocarp thickness (2.2 cm), stone weight (8.2 g), stone length (3.17 cm) and stone width (2.28 cm). Shahkoomahally et al. (2020) reported that the right union of the rootstock and cultivar is important for optimizing fruit quality parameters. Probably chili rootstock was more favourable to Benedict than other varieties of peach. On the contrary, the lowest figures for fruit weight (46.5 g), fruit length (4.02 cm), stone width (1.27 cm) and mesocarp thickness (1.10 cm) were observed in Early Red, whereas the lowest figures for fruit volume (41.5 cc), fruit width (4.16 cm), stone weight (4.30 g) and stone length (2.28 cm) were observed in Local Khallya. For fruit volume (41.5 cc and 41.75 cc) and fruit length (4.17 cm and 4.02 cm) and fruit width (4.16 cm and 4.36 cm), Local Khallya and Early Red were statistically similar. The stone mass was not directly proportional with the fruit weight, and could be assumed as specific for the variety, as already reported by Nowicka et al. (2019). The stone mass for Surprise (5.62 g), Peregrine (5.00 g), Local Khallya (4.30 g) and Early Red (4.50 g) were not statistically different. Surprise (1.28 cm) and Local Khallya (1.23 cm) were found statistically similar for mesocarp thickness. Peregrine (1.93 cm) and Local Khallya (1.90 cm) were found statistically similar for stone width. Our results for physical parameters of different varieties of peach are supported by Singh et al. (2016) and Ikinsi & Bolat (2018). Physicochemical characteristics of peach are highly influenced by variety (Mosie *et al.*, 2023). Fruit quality, such as individual fruit weight and size, increases with a decrease in fruit load and vice versa (Wang *et al.*, 2022).

#### **Chemical characteristics of fruits**

Chemical characteristics of different varieties of peach varied significantly as shown in Table 5. The highest value of pH was measured in Surprise (5.06) and the lowest value of pH was in Benedict (3.5). Local Khallya (4.93) was statistically similar to Surprise, having a pH of 4.93, whereas Peregrine (3.55) was statistically similar to Benedict (3.5) for pH. TSS was highest in Benedict (13.175° Brix). The rest of the varieties were similar in terms of TSS: Surprise (9.07° Brix), Local Khallya (9.6° Brix), Early Red (9.4° Brix), and Peregrine (8.75° Brix). The highest value of TA was in Early Red (1.56 %) and the lowest was observed in Benedict (1.06%). Peregrine (1.38%) and Local Khallya (1.38%) were statistically similar for TA. The higher TA in Early Red compared to other varieties was due to high accumulation of malate and citrate, accompanied by quinate (Zheng et al., 2021). Late- harvested variety (Local Khallya) has lower TA than early-harvested variety (Early Red) due to the inverse relationship between TA and degree of ripening (Cascales et al., 2005). The highest TSS/TA ratio was measured in Benedict (12.38) and others showed statistically similar results: Local Khallya (6.97), Peregrine (6.34), Surprise (7.42), and Early Red (6.03) for TSS/ TA. The results of the current study are in agreement with the statement that TA and TSS are determined by maturity stages and cultivars (Frecon et al., 2002).

Hajilou & Fakhimrezaei (2011) observed that the variety that has the highest TSS/TA and TSS has the lowest acidity, similar to our findings in the Benedict variety. Mazzoni *et al.* (2022) reported that the fruit TSS significantly elevates with the diminution of the crop load level, while TA is not influenced by crop load and number of branches.

#### Conclusion

This study concluded that Early Red took the shortest time to reach fruit set and fruit maturity. However, the fruit weight was measured lowest in Early Red. Among different factors, variety was key for differences in phenology and physicochemical properties. The sweetest variety was found to be Benedict and the least sweet was Early Red. Thus, the Benedict found superior peach variety in terms of both sweetness and size among different varieties. Moreover, multiyear and multilocation studies of phenology and physicochemical characteristics of peach varieties are recommended.

#### DECLARATIONS

#### **Authors Contribution Statement**

Conceptualization: NB and SS, Methodology: NB and SS, Software and Validation: NB and SS, Original draft preparation: NB and SS, Review and Editing: SS and AKB and Supervision and Advising: PNA and SD. All the authors have read and approved the final manuscript.

#### **Conflict of Interest**

The authors declare that there is no conflict of interest regarding the publication of manuscript.

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**Consent for publication:** All co-authors gave their consent to publish.

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