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Comparison studies on sucrose metabolism and phenolic content during fruit growth and maturation in pear cultivars

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ABSTRACT

'Patharnakh' (*Pyrus pyrifolia* Burm.) (PN), a hard pear and 'Punjab Beauty' (*Pyrus communis* L. × *Pyrus pyrifolia* Burm.) (PB), a soft pear are dominant low-chill pear cultivars of subtropics of India. Present investigation reports the changes in sugar metabolism and related enzymatic activities in fruits of 'PN' and 'PB' cultivars harvested at different developmental stages from 45 to 150 days after fruit set. Total soluble sugars, fructose, and sucrose contents were higher in 'PB' as compared to 'PN' during fruit growth and maturation stages. Total phenols and flavanols increased initially and then showed a decreasing trend towards maturity. Sucrose synthase (SS) and sucrose phosphate synthase (SPS) activities strongly correlated to sucrose content in 'PN' but SPS was weakly related in 'PB' fruits. Acid and neutral invertases showed a negative correlation with sucrose content in 'PN', and a reverse trend in 'PB' cultivar was observed. It is concluded that SS and SPS are crucial for sucrose accumulation in 'PN', but invertase enzymes are also important for sucrose accumulation in 'PB' fruits.

KEYWORDS

low chill pear, 'Patharnakh', 'Punjab Beauty', development stages, soluble sugars, metabolism, enzymes

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1. INTRODUCTION

Pear belongs to the genus *Pyrus*, sub family *Amygdaloideae,* and family *Rosaceae*. It is the most important deciduous temperate fruit crop of the world with annual production to the tune of 23.9 m MT from an area of 1.38 m ha (FAO, 2019). Fruit is highly nutritive and contains about 15.4% carbohydrates, 0.69% protein; and also rich in K, Ca, Mg, P, vitamins C and B; and amino acids viz. glutamic acid, aspartic acid, tyrosine, and methionine (Mahammad et al., 2010). It also contains secondary metabolites, which possess anti-oxidant, anti-inflammatory, hypolipidemic, hepatoprotective, anti-bacterial, anti-diabetic, antipyretic activities that are beneficial for human health (Parle and Arzoo, 2016). In the plains of north India, pear cultivation is dominated by Oriental group (*P. pyrifolia* Burm.) cv. 'Patharnakh' and semi-soft pear (*P. pyrifolia* Burm. × *P. communis*) cv. 'Punjab Beauty' in selective regions due to the ideal climatic factors, particularly availability of low chilling units and optimum diurnal temperature for better flowering and fruit set. The fruit is usually consumed fresh and generally value-added products *viz*. jams, juice, puree, ready-to-serve, and jellies are also prepared. Fruit stone cell content, particularly sclerenchyma cells substantially influence the quality of pear fruit and hard or Asian pears possess higher amounts of fruit stone or gritty cells (Xu et al., 2021).

Fruit ripening occurs as a result of the changes in physiological and biochemical attributes. The variability among fruit characteristics like peel colour, desirable flavour, quality, and textural properties has been observed due to softening of fruits, change in peel colour, degradation of chlorophyll, synthesis of anthocyanins and carotenoids, ethylene production, glycolysis, and ATP formation as well as breakdown of polysaccharides into simple sugars (Giovannoni et al., 2017). Fruit softening is attributed to the pectin modifications in the cell wall that diminishes the binding force between pectin chains by ions and reduces the integrity of the cell wall and fruit firmness (de Jesús Ornelas-Paz et al., 2018).

Variability in bioactive compounds in the pear varieties during different harvesting stages is valuable for the utilisation of fruits for nutraceutical and processing industries. Soluble sugars like fructose, glucose, sucrose, and sorbitol are accountable for sweetness of fruit, and organic acids viz. maleic and citric acids depict the taste and sensory quality parameters (Oikawa et al., 2015). Fructose is the sweetest amongst all available sugars in fruits and presence of higher fructose and lower glucose and sorbitol contents effectively depicts fruit eminence.

In the family *Rosaceae*, sorbitol is the primary photosynthetic product that is translocated from leaves to fruits and converted to fructose by sorbitol dehydrogenase (SDH), which provides carbon during fruit development period (Choi et al., 2009). Sucrose synthase (SS) and sucrose phosphate synthase (SPS) catalyze the reversible reaction for sucrose synthesis and it is degraded in the fruit. The presence of SS and SPS involved in sucrose accumulation has been reported in Chinese (*P. bretschneideri* Rehd. cv. 'Yali') and Japanese (*P. pyrifolia* Nakai cv. 'Aikansui') pears (Zhang et al., 2014). However, systematic studies on chemical attributes of Asian pears during fruit growth and maturation have not been fully undertaken in northwest sub tropics of India. The present study was aimed to quantify proportion of carbohydrates and to characterise enzymes involved for their metabolism processes, physiological and biochemical parameters during fruit growth and maturation in pear cultivars, which are commercially grown in northwest India.

2. MATERIALS AND METHODS

2.1. Plant material

Present investigation was carried out at the Fruit Research Farm, Department of Fruit Science, Punjab Agricultural University (PAU), Ludhiana (longitude, 75.86°E and latitude, 30.90°N), Punjab, India, on 29-year-old uniform and healthy plants of 'Patharnakh' and 'Punjab Beauty' (Fig. 1) grafted on Kainth rootstock (*Pyrus pashia*) planted at spacings of 7.5 m × 7.5 m and 6 m × 6 m, respectively. The plants were maintained including application of fertilisers, irrigation, anti insect-pests and diseases, etc., as per the *Package of Practices for Cultivation of Fruits*, PAU, Ludhiana. The orchard is situated in sub-tropical climatic conditions at an altitude of 244 m above mean sea level having loam sandy soil texture. The observation regarding fruit set at ovary swollen stage and falling of petals was observed between 3–4th March in 'PN' pear and 14–15th March in 'PB' cultivar. The fruits on all directions of the tree were tagged at pea stage and harvested at 15 days intervals from 45 to 150 days after fruit set (DAFS). Fruits of uniform size were harvested freshly at different developmental stages during early morning hours. Misshapen, distorted, and diseased fruits were discarded during sorting. Fruits were shifted directly to the laboratory after packing in polythene bags, and these were evaluated for various biochemical parameters and enzymatic activities.

2.2. Biochemical analysis

Fruit pulp was homogenised with 80% ethanol and refluxed twice for 20 min in a boiling water bath. The supernatant was collected to evaporate ethanol and volume was made up to 10 mL with deionised water. The extract was used for the estimation of total soluble sugars, fructose, and sucrose contents by the procedure followed by Kaur et al. (2018). Pellet collected after sugar extraction was dried and treated with perchloric acid to hydrolyse starch into simple sugars, which were estimated using 5% phenol and concentrated H_2SO_4. Pectin and ascorbic acid contents were determined by the method described by Okimasu (1956) and Jagota and Dani

Fig. 1. Pear cultivars 'Patharnakh' and 'Punjab Beauty'

(1982), respectively. Fruit pulp was refluxed with methanol for 1 h for the estimation of phenols and flavanols (Kaur et al., 2018).

Enzymes viz. SS (EC 2.4.1.13), SPS (EC 2.4.1.14) and invertases (EC 3.2.1.26) both acid (AI) and neutral (NI) were extracted from fruit pulp using HEPES-NaOH buffer (pH 7.5), homogenate was filtered through three layers of cheese cloth and centrifuged at 10,000 *g* for 20 min at 4 °C. The enzymatic analysis was carried out by methods described by Asthir and Singh (1995). Sorbitol dehydrogenase (SDH, EC 1.1.1.14) was assayed using method described by Gerlach and Hiby (1974).

2.3. Statistical analysis

The experiment was laid out as a complete randomised block design during the year 2020 with four replications. The data (mean ± SE) were analysed by ANOVA and the differences were considered statistically significant at $P \leq 0.05$ using software CPCS1 developed by Punjab Agricultural University, Ludhiana. Data were subjected to Pearson's correlation analysis to assess the relationship between various attributes. Principal component analysis was used to examine the interrelations between different parameters.

3. RESULTS AND DISCUSSION

3.1. Carbohydrate composition

Total soluble sugars (TSS) and fructose contents increased significantly during fruit maturation in both pear cultivars (Fig. 2); however, pulp sucrose content showed a reverse trend. Sucrose content enhanced significantly ($P \leq 0.05$) in both cultivars up to 60 DAFS but decreased gradually on 90 DAFS in 'PN' and between 105 and 120 DAFS in 'PB' cultivar (Fig. 2). At the physiological maturity stage, the higher sucrose content recorded in the 'PB' than 'PN' cultivar might be due to higher SS activity and availability of hexose precursors in 'PB' cultivar (Figs 2 and 4). Giovannoni et al. (2017) also observed a gradual improvement in the sugars content in pear juice with fruit maturity. During the final stages of cell division, sucrose content is not required to provide energy, and some of the remaining monosaccharides are composited temporarily to sucrose, so the sucrose content increases slightly (Tian et al., 2021). An enhancement in total soluble sugars and reducing sugars at different fruit harvesting stages is attributed to the hydrolysis of polysaccharides to monosaccharides and also to the higher juice concentration, which causes loss of moisture through transpiration (Mesa et al., 2016). Fructose, the main storage sugar in pear fruit, begins to accumulate predominantly during active fruit enlargement phase and also contributed for the half of fruit total sugars content. Oikawa et al. (2015) reported that TSS and fructose content in pear fruit accumulates mainly during immature fruit development stages and finally increases exponentially at physiological maturity stage. In 'Ya Li' and 'Aikansui' pear cultivars, fructose sugar increased gradually during fruit developmental period and contributed above 36.0% of the total sugars (Zhang et al., 2014). Starch content decreased significantly from 45 to 150 DAFS that may be attributed to the conversion of starch to soluble sugars with the progression of maturity in pear fruits (Bhat et al., 2012).

During pear fruit ripening, there is a progressive change in the fruit firmness and texture due to the conversion of insoluble protopectin to soluble pectin and pectic acid. Pectin content was lower at the outset; however, improved significantly from 90 to 120 DAFS, and thereafter, the

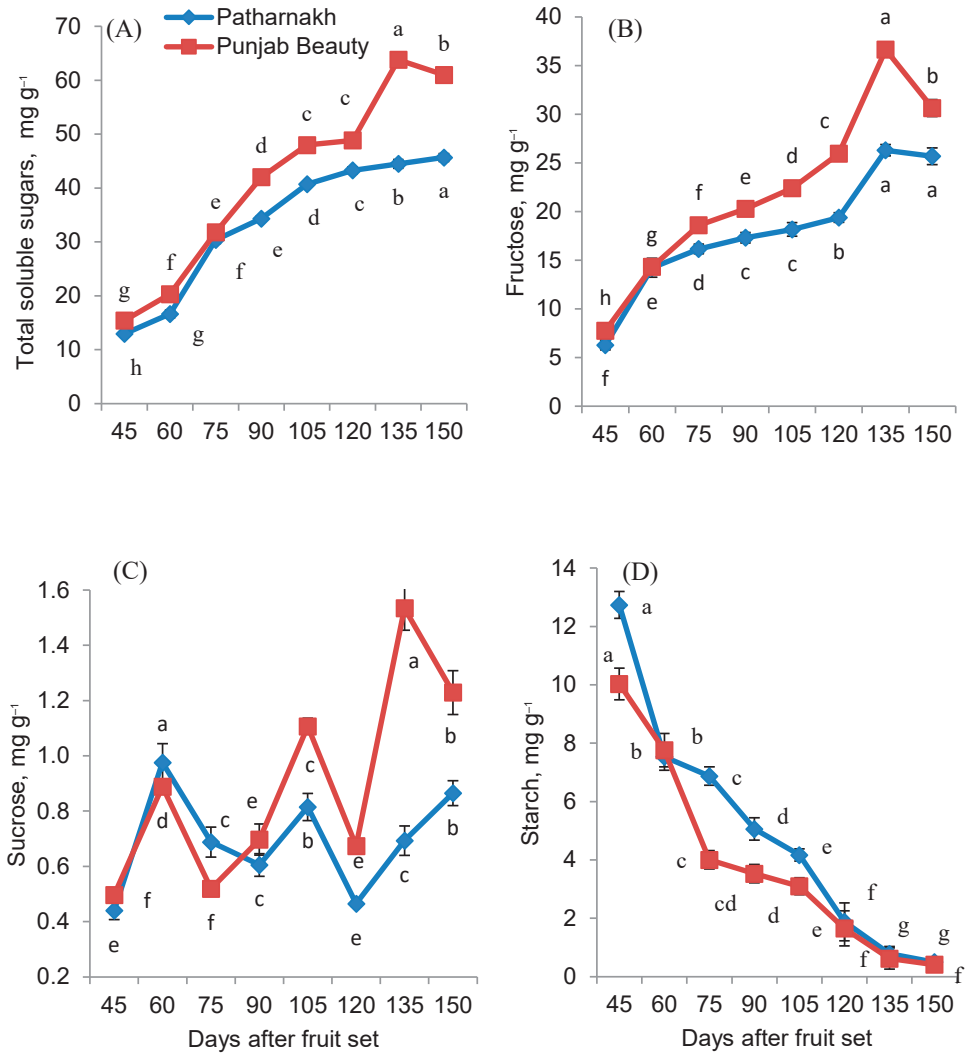

Fig. 2. Changes in (A) total soluble sugars, (B) fructose, (C) sucrose, and (D) starch contents in pear fruits during development stages

values declined non-significantly between 135 and 150 DAFS in both cultivars (Fig. 3). The increase in pectin content towards fruit maturity was due to cell enlargement and swelling of fruit and conversion of protopectin to pectin during ripening (Wang et al., 2018). The ascorbate levels were higher until 45 DAFS, and the values decreased considerably and maintained almost steady level at the final maturity stage (Fig. 3). Reduction in ascorbic acid levels occurred due to oxidation process and formation of dehydroascorbic acid as observed earlier also during ripening of 'Conference', 'Aikansui', and 'Gola' pears (Verma and Kushwaha, 2018) and 'YaLi' pear (Wang et al., 2021).

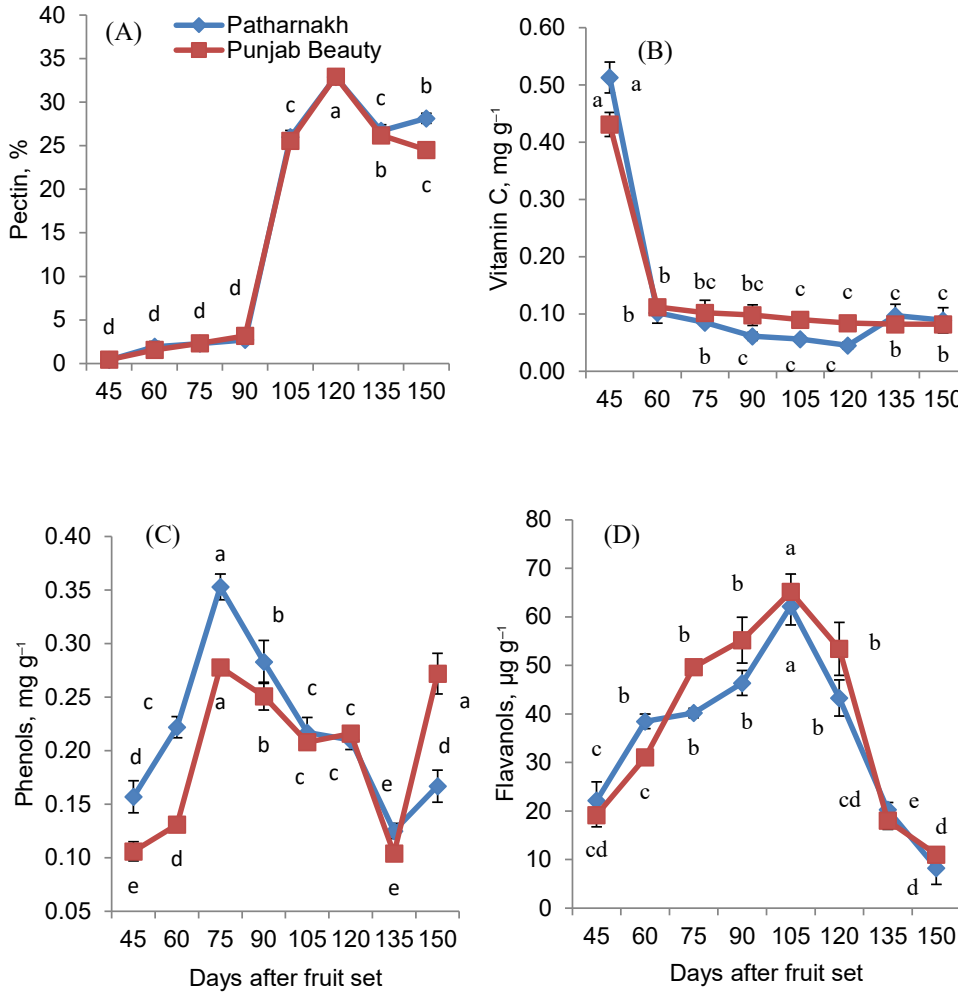

Fig. 3. Changes in (A) pectin, (B) ascorbic acid, (C) phenols, and (D) flavanols contents in pear fruits during development stages

3.2. Phenolic constituents

Phenol and flavanol compounds are the most vital bioactive compounds that exhibit the sensory quality attributes of the fruit. These contents showed a decreasing trend from immature to mature stages of the fruit (Fig. 3). These changes are related to the rapid growth and development of fruit tissues during initial stages, which in turn improved the fruit weight leading to dilution effect of polyphenolic compounds and reduced phenolic content (Sunila and Murugan, 2017). At final harvest maturity stage, the fruit tissue progressively exhibits slow growth pattern, and oxidation and transformation of phenolic compounds is attributed to the senescence of plant tissues (Sunila and Murugan, 2017).

3.3. Sugar metabolism

SS activity of the fruit increased up to 60 DAFS and subsequently, a decline in values was observed at 90 DAFS in both cultivars and then upsurge was registered until 105 DAFS in 'PN' cultivar. SS activity declined up to the final maturity stages in 'PN'; however, 'PB' cultivar showed an upward linear trend up to 120 DAFS and subsequently, values were lower at final harvest stage (Fig. 4). SS activity exhibited two peaks in both pear cultivars at 60 DAFS and concurrently at 105 DAFS in 'PN' and at 120 DAFS in 'PB' cultivar. SS activity was relatively lower in 'PB' in comparison to 'PN' during fruit growth and maturation. SPS activity increased at different fruit development stages up to 60 DAFS in both cultivars, and thereafter, showed a decreasing trend up to 120 DAFS. Furthermore, higher activity to some level was observed up to the final fruit maturity stage (Fig. 4). Higher sucrose content in 'PB' is related to SS activity for sucrose synthesis that significantly exhibited positive correlation between sucrose accumulation and SS activity (Table 1).

In 'PN', both SPS and SS enzymes showed positive correlation with sucrose accumulation and lower sucrose content owing to SS activity, which causes the breakdown of sucrose molecules rather than involved in its biosynthesis. Sucrose sugar is mainly translocated from source (leaf) to sink (fruit) and converted to hexoses by SS or invertase activity during the process of unloading from phloem to fruits. Sucrose accumulation during physiological maturity might be either due to the low breakdown of sucrose sugar due to low energy requirement or utilisation of these hexoses to synthesise sucrose temporarily as the result of the higher SPS as compared to SS activity. Zhang et al. (2014) also reported that SS enzyme is responsible for the sucrose cleavage rather than its synthesis in 'Ya Li' pear. However, SS played an important role in sucrose accumulation in 'Aikansui' pear as the results of the higher SS (synthesis) activity. The activity of SS (synthesis) in 'Aikansui' was lower in immature fruits but significantly higher sucrose accumulation was noted during fruit maturation. SS I and SS II isoforms in Japanese pears appeared mainly in young and mature fruits, respectively (Tanase and Yamaki, 2000), and the reaction of SS II inclined towards sucrose synthesis more than that of SS I. The activity of SPS was higher in immature fruits of 'La France', which further declined steadily during fruit growth and development; and an upsurge was negligible at physiological maturity (Yamada et al., 2006).

Acid invertase (AI) activity was enhanced during 45–75 DAFS in 'PN' cultivar and up to 90 DAFS in 'PB', and thereafter, exhibited a declining trend in both cultivars upon fruit maturity (Fig. 3). The AI was higher in 'PB' than 'PN' pear cultivar except during 75 DAFS. Neutral invertase (NI) showed maximum activity in 'PN' during fruit developmental stages up to 90 DAFS, subsequently, the values were statistically significant in both cultivars till the final fruit harvest stages (Fig. 4). At harvest maturity, NI activity declined to almost the half values on 120 DAFS in 'PN', and 'PB' exhibited 1.43-fold higher NI activity than 'PN' cultivar. Invertases are categorised into three forms as acid, alkaline, and neutral depending upon the optimum juice pH. The activity was highest in the immature fruits and substantially had lowest values during physiological maturity stages. AI plays an important role in hexose accumulation in pear fruit during fruit growth and maturation by regulating the osmotic pressure and also maintaining proportion of sucrose and hexose sugars ratio in cell vacuolar tissue (Yamada et al., 2006). In 'Yuzora' and 'Hokimomo' pears, AI activity slightly increased at the final mature stages, and the activity in 'Hokimomo' decreased again with further fruit growth and development. It is reported that NI may supply materials for growth and cell wall formation (Zhang et al., 2012).

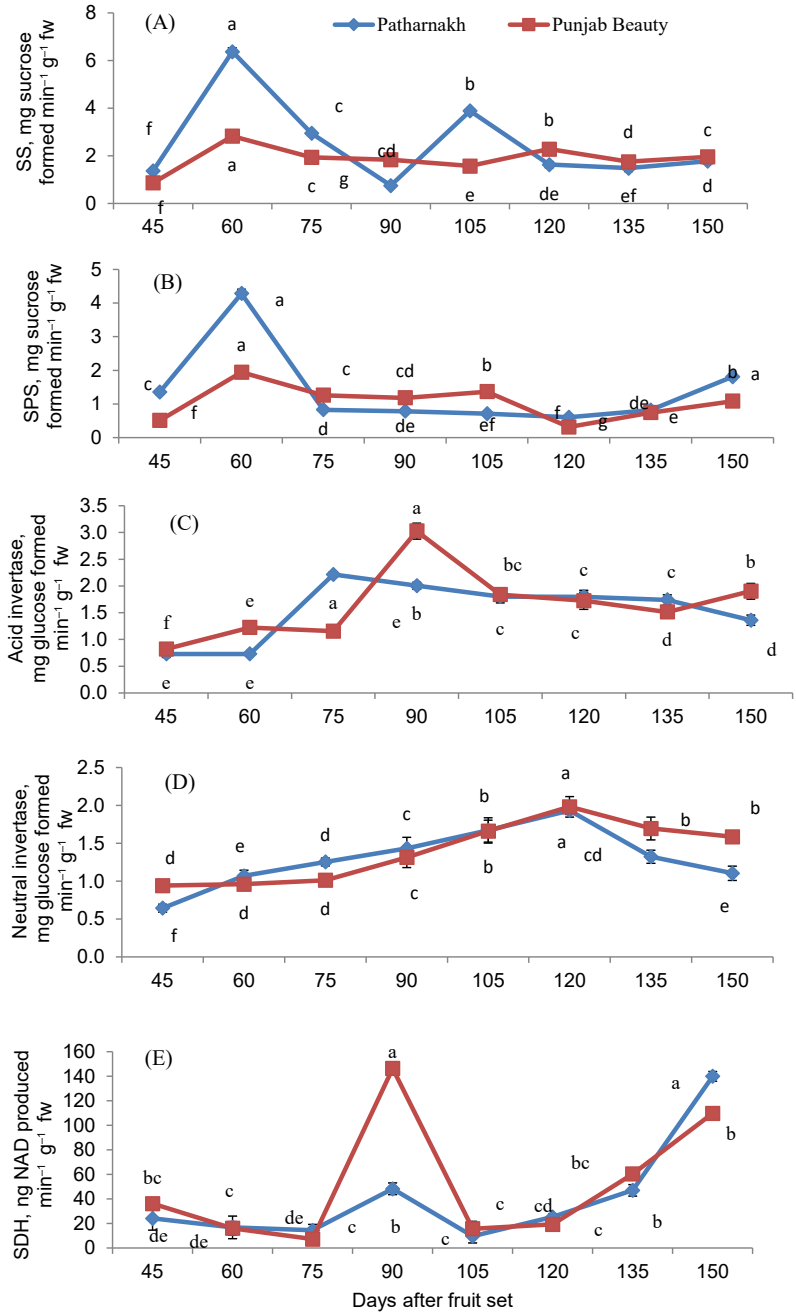

Fig. 4. Activities of (A) SS, (B) SPS, (C) AI, (D) NI, and (E) SDH enzymes in pear fruits during development stages

Table 1. Correlation coefficient values of sucrose and enzymes related to its metabolism in pear fruits at different developmental stages

	Patharnakh				Punjab Beauty			
	Sucrose	SS	SPS	AI	Sucrose	SS	SPS	AI
SS	0.714**				0.350*			
SPS	0.624**	0.758**			0.114	0.299		
AI	–0.144	–0.198	–0.549**		0.153	0.192	0.084	
NI	–0.095	–0.061	–0.438*	0.575**	0.506**	0.198	–0.455**	0.386*

**: Significant at $P \leq 0.05$.

Sorbitol dehydrogenase (SDH) activity decreased by two manifolds in fruits of 'PN' and four-folds in 'PB' up to 75 DAFS (Fig. 4). Thereafter, values increased linearly at 90 DAFS in both cultivars and then decreased significantly from 90 to 105 DAFS by 5.0 and 7.5 manifolds in 'PN' and 'PB' cultivars, respectively. Increasing trend was recorded till the final maturity stage. Zhang et al. (2014) reported that SDH slowed down the fructose biosynthesis pathway. Active fruit growth and development are accompanied by sorbitol and sucrose hydrolyses, which further implies that dominantly translocated sugars to the sources of hexose substrates are required for metabolism by fruits during their growth phases. In 'Niitaka' and 'Whangkeumbae' pears, NAD-dependent SDH activity increased at 110 DAFS up to 150 DAFB during progression in fruit growth (Choi et al., 2009).

3.4. Correlation analysis of sucrose content and related enzymes

In 'PN' cultivar, significant positive correlation between sucrose content and SS ($r = 0.714$) and SPS ($r = 0.624$) activities was observed (Table 1). In 'PB' cultivar, sucrose content positively correlated with SS ($r = 0.35$) but non-significantly correlated to SPS activity. Both acid and neutral invertase showed a negative correlation with sucrose content in 'PN' pear. The invertase activity exhibited a reverse correlation with 'PB' pear. Both SS and SPS played an important role in sucrose accumulation in 'PN' fruit as observed from correlations between enzymes and sucrose content. These results are in agreement with the results reported by Choi et al. (2009) and Zhang et al. (2012).

3.5. Principal component analysis

Principal component analysis (PCA) was conducted to understand the impact of the physiological parameters on fruit development and their relationship with different biochemical parameters in pear cultivars 'PN' and 'PB' (Fig. 5). In 'PN', two principal components (PCs) were able to explain 64.3% of the total variability. PC1 representing 41.3% of the total variance, was positively associated with total sugars, pectin, fructose, AI, and NI, while negatively related to starch, vitamin C, and SS. PC2 accounted for 23.0% of the total variance, and it was positively correlated with sucrose, SDH, and SPS, however, it was inversely correlated with phenol and flavanol.

In cultivar 'PB', total variance was explained by two principal components (PCs) contributing 62.1% of total variance. PC1 representing 44.3% of the total variance and was positively

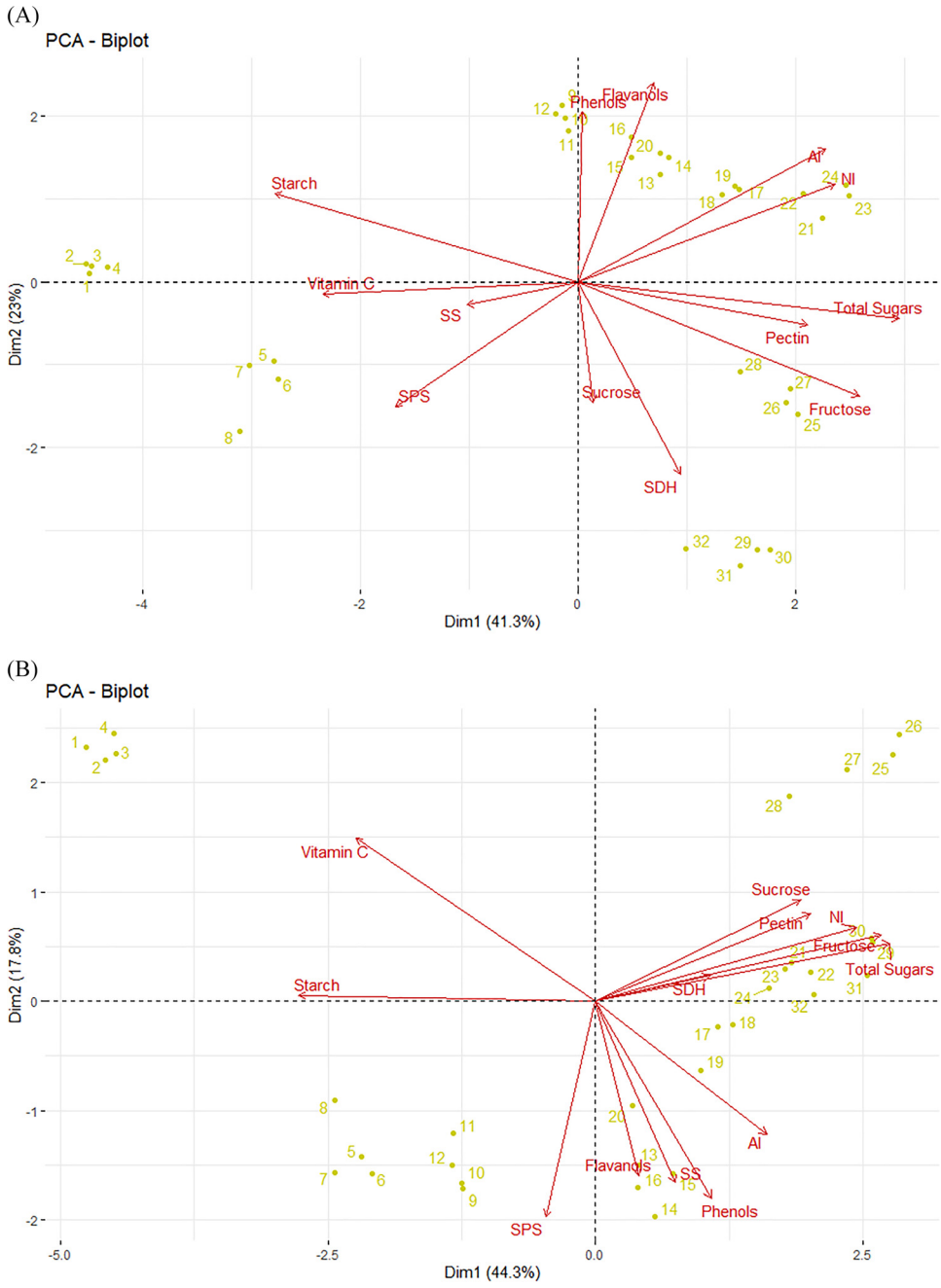

Fig. 5. PCA Biplot for qualitative parameters in 'Patharnakh' (top) and 'Punjab Beauty' (bottom) pear cultivars during fruit development

associated with total sugars, fructose, sucrose, pectin, NI, and SDH, however, negatively related to starch content. PC2 accounted for 17.8% of the total variance, and it was positively correlated with SPS, SS, AI, phenols, and flavanols, while it was inversely correlated with vitamin C.

This analysis highlights a clear metabolic and morphological shift from the early to final fruit developmental stages. Similarly, Jing and Malladi (2020) suggested that the initial development stages are metabolically separated from the later and final harvest stages due to changes in sucrose metabolism. Sucrose catabolism provides carbon molecules as backbones and energy for cell production during the early stage of development, but starch disintegration and recommencement of higher sucrose concentration occurred for cell expansion and ripening during final developmental stages.

4. CONCLUSIONS

It is concluded from the present studies that SS and SPS activities are critical for sucrose accumulation in Patharnakh 'PN', but invertases are also important for sucrose accumulation in Punjab Beauty 'PB' pear fruits.

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