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Biophysical and biochemical changes in tomato (*Solanum lycopersicum*) genotypes at different fruit developmental stages under protected cultivation

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ABSTRACT

An experiment was conducted during 2020–21 and 2021–22 to evaluate the biophysical and biochemical characteristics of tomato [*Solanum lycopersicum* (L.)] genotypes (Pusa Rohini, Pusa Cherry-1, S-22, PKM-1 and Pusa Protected-1) grown at Centre for Protected Cultivation Technology (CPCT), ICAR-Indian Agricultural Research Institute, New Delhi. Tomato fruits were harvested at 14 different fruit developmental stages (DS01-DS14) starting from 4 days after post anthesis to the final red riped stage. Fruit size (length and width), biomass, firmness, total soluble solids (TSS), titratable acids (T.A), β-carotene, ascorbic acid and lycopene content were analysed for all the developmental stages. The highest value of fruit length, breadth, biomass, TSS, β-carotene and lycopene content was observed at full ripe tomatoes and the lowest in the early immature green stage. However, parameters like fruit firmness, T.A and ascorbic acid content were higher in early immature to mature green stage and decreased once the fruit entered the ripening stage. Thus, irrespective of the genotypes considered in this study, all the above parameters except fruit firmness, ascorbic acid and T.A were found to increase gradually with the advancement of ripening process. This clearly showed a significant relationship between the biochemical and biophysical parameters of the tomatoes with their fruit developmental stages, hence could be used as an indicator to characterize the fruit developmental stages.

Keywords: β-carotene, Fruit developmental stages, Lycopene content, Tomato, Total Soluble Solids, Titratable acids

Tomato [*Solanum lycopersicum* (L.)] is one of the most popular cultivated and versatile garden vegetable crops grown in the world (Kumar *et al.* 2013). Nutritional value, colour and flavour of their fruits as well as quality of the processed products depend mainly on lycopene, β-carotene, ascorbic acid and sugar contents. The two most important carotenoids found in fruits of tomato are lycopene, which determine the fruit's red colour and β-carotene, which accounts for approximately 7% of the tomato carotenoids. Therefore, tomato products and their quality can be well characterized by their biophysical and biochemical parameters (Coelho *et al.* 2023). Most of the research work describes biochemical composition of tomato in fully ripen stage (in red tomato), as in technical fruit maturity stage. To understand the synthesis and changes in the carotenoids and other biochemical compounds, their concentration should be compared not only in red fruits, at the last stage of maturity, but also at all the fruit developmental stages (from immature green to the technical fruit maturity stage) in different genotypes. During tomato fruit ripening process, carotenoids concentrations are changing constantly, which is related with chlorophyll degradation and carotenoids synthesis processes, when chloroplasts are synthesized in to the chromoplasts (Hadley *et al.* 2003). Maturity at harvest is very important, as it determines the composition and quality of tomatoes. This is especially a problem with tomatoes, when picked green since it is difficult to differentiate between mature and immature-green fruits (Radzevicius *et al.* 2016). Tomatoes accumulate acids, sugars and ascorbic acid during ripening on the vine. Fruit firmness is related to the susceptibility of tomato fruit to physical damage during harvest and storage. Also, it can be the most important consumer preference characteristic (Bobinaite *et al.* 2009) that changes with the developmental stages. Hence there is a need to characterise the changes in the biochemical and biophysical parameters of the tomato at different developmental stages to understand their differences in different genotypes.

MATERIALS AND METHODS

Field experiments were conducted with the 4 genotypes of tomato (Table 1), differing in their shape, size and morphological appearance, grown under protected condition, at Centre for Protected Cultivation Technology (CPCT), ICAR-Indian Agricultural Research Institute (28°38′23″ N and 77°09′27″ E and 228.6 metres amsl), New Delhi during

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September-April, 2020–21 and 2021–22. The climate of the research area was subtropical to semiarid, with hot, dry summers and chilly winters. The soil at the test location was sandy loam, non-calcareous, slightly alkaline, and belongs to the primary group of Indo-Gangetic alluvium.

Crop management: Tomato crop cultivation as described by Singh *et al.* (2022) was followed. Seeds of the tomato genotypes (Pusa Rohini, Pusa Cherry-1, S-22, PKM-1 and Pusa Protected-1) were grown in the same polyhouse that was used for experimentation in plastic pro/plug trays. A 25-day-old seedlings at the 3–4 leaf stage were transplanted in the month of Septembers at a spacing of 75 cm × 60 cm during both the years of experimentation. At the time of land preparation, about 25–30 metric tonnes per hectare of well decomposed farmyard manure was applied. For proper crop growth, fertigation with N:P:K 18:18:18 water soluble through drip irrigation was applied. First irrigation was given two days after transplanting and subsequent fertigation were applied twice a week. Crop was maintained pest and disease free and kept weed free with frequent hoeing. Staking was done at 20 to 25 days after transplanting. Regular pruning of the side shoots was done during the entire crop duration. To determine the biochemical and biophysical parameters, 10 fruits in triplicate were collected during each of the 14 developmental stages considered in this study.

Biophysical and biochemical parameters: According to the methods outlined in Table 2, different biophysical and biochemical parameters, including fruit size (length and breadth), biomass (g/fruit), total soluble solids (°Brix), firmness (kg/cm^2), titratable acids (%) and β-carotene (ppm), ascorbic acid (ppm), and lycopene content (ppm), were measured. All parameters were examined in this study for all 5 genotypes and for all fruit developmental stages, commencing at 4 days after post anthesis (DS01) and ending at red ripe stage (DS14, 56 days after post anthesis).

The data obtained were analysed statistically by adopting factorial CRD using R-Software (v4.3.0) and SPSS 20. The differences among means were tested for significance using critical difference tests (CD) and the results obtained from the analysis are presented in pictorial form as spider plot.

Table 1 Tomato genotypes investigated in this study and their characteristics

Genotype	Special feature
Pusa Rohini	Highly suitable for long distance transport, and for spring-summer season
Pusa Cherry-1	Red colour fruit. Fruit wt. 8–11 g. High yielder. Fruits are borne in clusters
S-22	Semi determinate. Fruit wt. 80–90 g. Vigorous growth and excellent yield potential.
PKM-1	Flattish round fruit dark red with green shoulder. Fruit wt. 70–80 g. Highly suitable for long distance marketing
Pusa Protected-1	Indeterminate, tolerant to high temperature. Fruit weight 80–82 g

RESULTS AND DISCUSSION

Biophysical and biochemical parameters:

Fruit Size (Length and width): Length and width increased with the stage of development. Highest length was observed in PKM-1 (56.51 mm), followed by S-22 (52.01 mm), Pusa Rohini (50.83 mm), Pusa Protected-1 (49.57 mm) and the lowest was in the Pusa Cherry-1 (23.65 mm) in fully riped stage (DS14). Maximum fruit width was observed in Pusa Rohini (51.18 mm) followed by S-22 (50.88 mm), PKM-1 (49.97 mm), Pusa Protected-1 (47.16 mm), and lowest in Pusa Cherry-1 (24.99 mm) in the red riped stage (DS14) (Supplementary Fig 1 a–b). In general, among the different genotype, fruit length was 14.27 mm in early immature stage (DS01) and it increased to a maximum up to 46.51 mm in the red riped stage (DS14). Fruit width was the minimum (15.35 mm) in early immature stage (DS01) and increased with the advancement in the fruit developmental stages with the maximum (44.84 mm) in red ripened stage (DS14) (Supplementary Fig 2 a–b). These changes are in accordance to the fact, that the fruit development from anthesis to full maturation is in general, regulated by changes in endogenous and external environmental signals whose perception is relayed by hormonal and sugar signalling (Mounet *et al.* 2009, Wang *et al.* 2009). There are 5 recognizable phases, anthesis, fertilization, cell division, cell expansion and ripening, with some overlap between stages. Cell division occurs in the newly-formed fruit for 7–10 days or in large-fruited cultivars for 20 days (Bertin *et al.* 2007), after which the final fruit cell number is set. The cells then expand from 10–40 days post-anthesis (DPA) due to the vacuolar storage of photosynthate and water, leading to a more than 10-fold increase in fruit size (Huang *et al.* 2021). During the final stage of ripening, the fruit undergoes several metabolic transformations brought on by climacteric ethylene, including the rapid import and accumulation of sugars, degradation of starch and synthesis of lycopene and

Table 2 Measured crop biophysical and biochemical parameters

Biophysical/ Biochemical parameters	Unit	Method/ Instrument	Reference
Fruit size (Length, width)	mm	Digital vernier caliper	Mahmoud *et al.* (2022)
Biomass	g/fruit	Constant weight method	-
Total Soluble solids	°Brix	Refractometer	Javanmardi and Kubota (2006)
Firmness	kg/cm^2	Penetrometer	Kim *et al.* (2021)
Titratable acids	%	Titrimetric method	Sawant (2019)
β Carotene	ppm	Spectrophotometer	Nagata and Yamshita (1992)
Ascorbic acid	ppm	Titrimetric method	Albrecht (1993)
Lycopene content	ppm	Spectrophotometer	Davis *et al.* (2003)

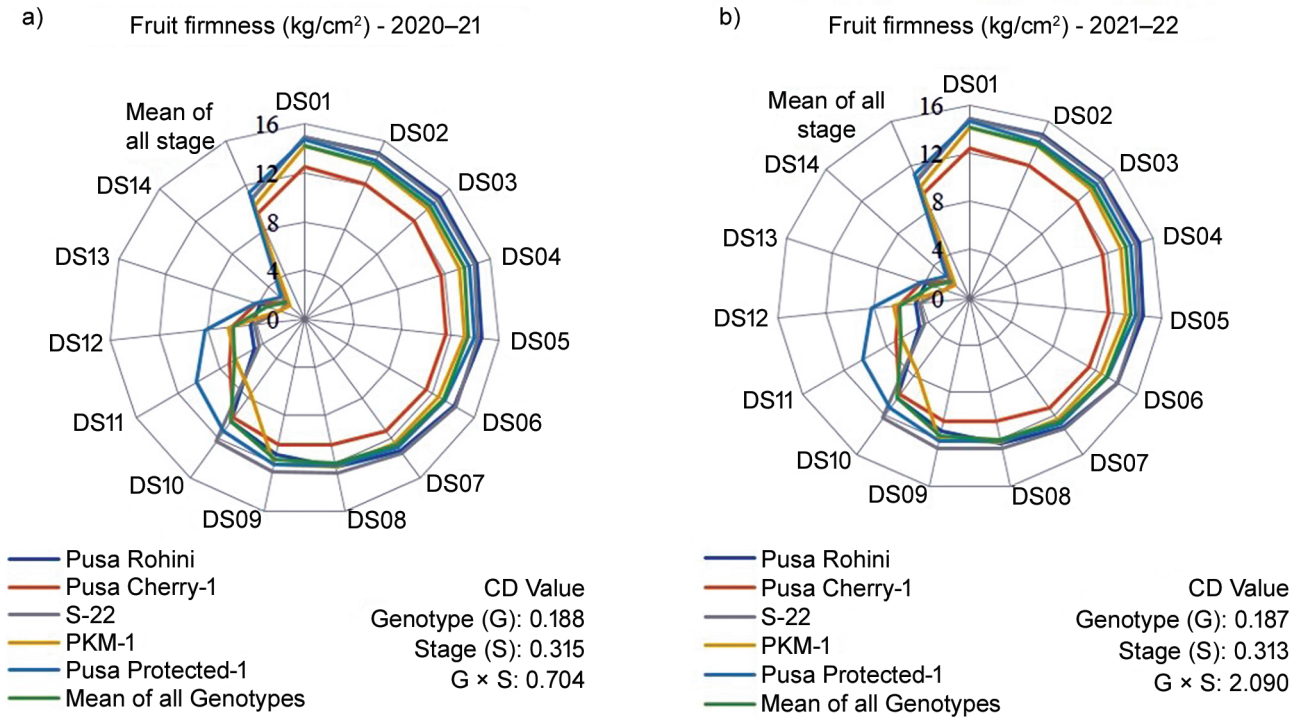

Fig 1 Variation in fruit firmness at different developmental stage in tomato genotypes during the year 2020–21 and 2021–22 along with their CD value at P=0.001.

carotenoids, the degradation of chlorophyll and the softening of the cell wall (Quinet *et al.* 2019).

Biomass: Fruit biomass increased with the fruit developmental stage i.e. from the early immature stage to the red riped stage. Irrespective of the developmental stage the maximum fruit biomass was observed in the genotype PKM-1 (3.19 g/fruit) followed by S-22 (2.45 g/fruit), Pusa Protected-1 (2.15 g/fruit), Pusa Rohini (1.97 g/fruit) and the lowest biomass in Pusa Cherry-1 (0.93 g/fruit). Similarly, among the different fruit developmental stages considered in this study, irrespective of the genotype, the minimum (0.29 g/fruit) fruit biomass was observed in early immature stage (DS01) and it increased to a maximum (4.08 g/fruit) in red riped stage (DS14) (Supplementary Fig 3 a–b).

Firmness: Among all the genotypes, fruit firmness was observed to be the maximum in Pusa Protected-1 (11.32 kg/cm^2), followed by S-22 (10.82 kg/cm^2), Pusa Rohini (10.76 kg/cm^2), PKM-1 (10.12 kg/cm^2), and the minimum in Pusa Cherry-1 (9.50 kg/cm^2). With respect to fruit developmental stages, firmness was the highest (14.25 kg/cm^2) in the early immature stage (DS01) and then it followed a decreasing trend to the red riped stage (DS14) and reached a minimum (2.09 kg/cm^2) fimness (Fig 1 a–b). Similar results were also reported by Bui *et al.* (2010).

Total Soluble Solids: Total soluble solids (TSS) was observed to be the highest in the genotype Pusa Cherry-1 (4.15 ^{o}Brix) followed by Pusa Protected-1 (4.10 ^{o}Brix), S-22 (4.04 ^{o}Brix), PKM-1 (3.96 ^{o}Brix) and Pusa Rohini (3.74 ^{o}Brix). Among the different fruit developmental stages considered in this study, TSS was observed to be the lowest i.e. 3.67 ^{o}Brix during the early immature stage (DS01) and then increased to its maximum (5.69 ^{o}Brix) in red riped stage (DS14) (Fig 2 a–b). Similar trends were also reported by other researchers (Karki 2005, Majidi *et al.* 2011), which supports our observation, that the amount of soluble sugars tends to rise as tomato fruits ripen. The increase in soluble solids percentage with the fruit developmental stage might be explained by the degradation of polysaccharides during ripening process and degradation of pectin substances into oligosaccharides (simple sugar) (Young *et al.* 1993).

Titratable Acids: Among the genotype, maximum titratable acid as citric acid equivalent was observed in the genotype Pusa Rohini (0.58%) followed by Pusa Cherry-1 (0.50.%), S-22 (0.48%), PKM-1 (0.46%) and Pusa Protected-1 (0.35%). Under different fruit developmental stages, titratable acidity was the minimum during the earliest stage (DS01) i.e. 0.39% and it started increasing till it reached the mature green stage (DS09) with its maximum value of 0.59% and again it started decreasing as fruit started ripening with the minimum at ripening stage (Supplementary Fig 4 a–b). In fully ripened stage the titratable acidity was 0.48% and similar results were obtained for both the years as it was grown under the same protected condition. The combined effect of growth process and ripening had a considerable impact on the total titratable acidity content which was in accordance with the result obtained by Moneruzzaman *et al.* (2008). This may be due to the metabolic activities of the living tissues of the tomato where organic acid depletion takes place during ripening. Also, due to higher ripening and respiration rate

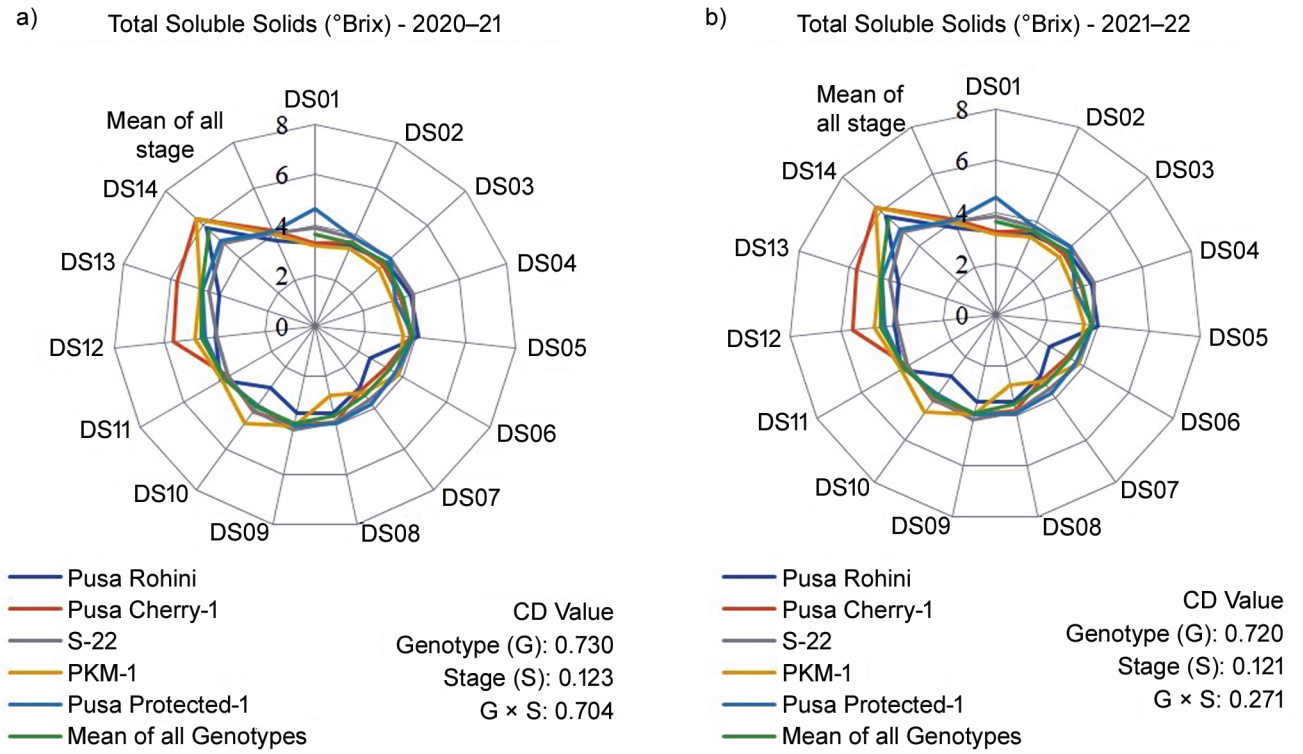

Fig 2 Variation in total soluble solids at different developmental stage in tomato genotypes during the year 2020–21 and 2021–22 along with their CD value at P=0.001.

the organic acids may be consumed as a substrate during the respiration process or owing to the conversion to other sugars (Al-Dairi *et al.* 2021).

β-carotene: β-carotene content varied among the different genotypes of tomato considered in this study. It was observed that β-carotene content was the highest in Pusa Protected-1 (6.31 ppm) followed by, Pusa Cherry-1 (5.69 ppm), S-22 (3.61 ppm), PKM-1 (1.99 ppm) and Pusa Rohini (0.77 ppm). Different fruit developmental stages showed an increasing pattern of β-carotene content varying from 1.29 ppm in the first developmental stage (DS01) to 8.59 ppm in the red riped stage (DS14) (Supplementary Fig 5 a–b). Similar trends were reported by Radzevicius *et al.* (2012) with the highest amount of β-carotene found in fully ripen stage and ranged from 1.40 to 1.69 mg/100 g (1.40–16.9 ppm). The least amount of β-carotene was detected in the green tomato fruits and ranged from 0.20 to 0.47 mg/100 g (2.0–4.7 ppm). Due to the advancement of the ripening and maturity processes, there is rapid accumulation of carotenoids as chloroplasts are converted to chromoplasts (Abiso *et al.* 2015).

Ascorbic acid: Ascorbic acid content varied in different genotypes of tomatoes. It was observed that ascorbic acid content was the highest in the genotype Pusa Rohini (541.5 ppm), followed by S-22 (459.56 ppm), PKM-1 (160.06 ppm), Pusa Protected-1 (108.67 ppm) and Pusa Cherry-1 (105.02 ppm). With respect to fruit developmental stages, it was around 221.34 ppm during early immature stage (DS01) and increased to 351.20 ppm during the mature green stage (DS07–DS10) thereafter, it again started decreasing and reached its minimum of 187.43 ppm during the red riped stage (DS14) (Supplementary Fig 6 a–b). In general tomato has only moderate ascorbic acid content than other fruit species; in fact, commercial cultivars have significantly lower ascorbic acid than wild accessions. Ascorbic acid content was high during the breakage stage (where fruit changes from green to yellow) and during ripening process the ascorbic acid concentration was less, due to the high cell expansion in the developing tissue or high demand by the developing and expanding cells (Mellidou *et al.* 2012).

Lycopene: Lycopene content is quite variable among the different genotypes of tomato. In the present study, it was the highest in the Pusa Cherry-1 (8.99 ppm) followed by PKM-1 (7.38 ppm), Pusa Rohini (7.27 ppm), Pusa Protected-1 (5.26 ppm) and S-22 (1.89 ppm). Under the different fruit developmental stages, lycopene content was negligible in green stage starting from early immature green to mature green stage (0.42-1.46 ppm) once the ripening starts, lycopene contents increased and reached its maximum value i.e. 43.25 ppm in red ripped stage (Fig 3 a–b). These results are consistent with the trends and values reported by Mladenovic *et al.* (2014), ranging from 0.031 mg/100 g to 4.330 mg/100 g (3.1–43.3 ppm). Hammed *et al.* (2012), observed that fresh tomato fruits had lycopene content ranging from 0.29 to 3.31 mg/100 g (2.9–33.1 ppm) and was the maximum during ripening stage.

Thus, the present study revealed significant variation

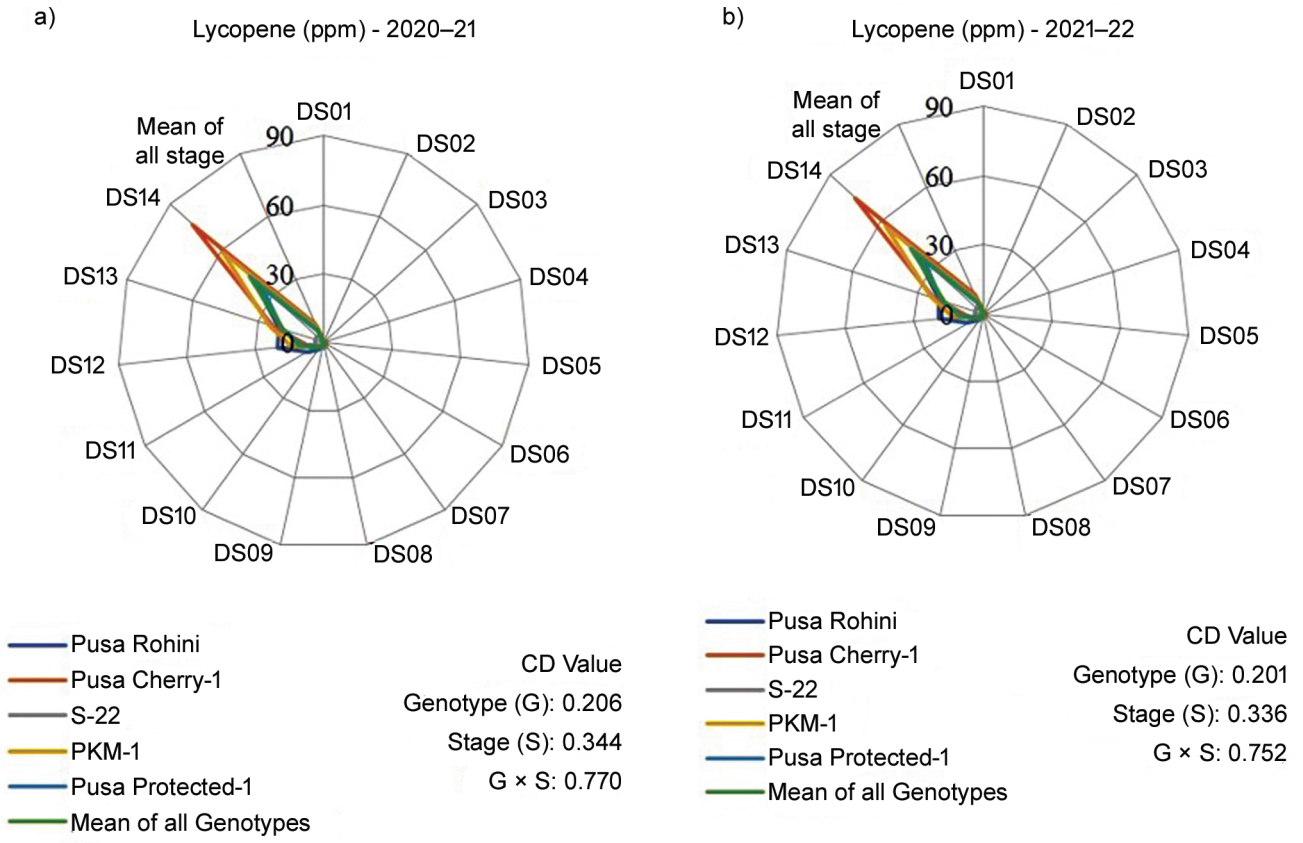

Fig 3 Variation in lycopene content at different developmental stage in tomato genotypes during the year 2020–21 and 2021–22 along with their CD value at P=0.001.

in biophysical and biochemical characters like length, width, biomass, firmness, titratable acid, β-carotene, ascorbic acid and lycopene content during different fruit developmental stage of tomato genotypes. The highest values of fruit length, breadth, biomass, TSS, β-carotene and lycopene content were observed under full ripe stage of the tomatoes with their lowest in the early immature green stage. However, parameters like the fruit firmness, titratable acidity and ascorbic acid content were higher during early immature to mature green stage and decreased once the fruit entered into ripening stage and the ripening process starts. Thus, irrespective of the genotype the values of all the above parameters except fruit firmness, ascorbic acid, and lycopene content were found to increase gradually with the advancement of ripening process. This clearly showed a significant relationship between the biochemical and biophysical parameters of the tomatoes and their fruit developmental stages hence, could be used as an indicator to characterise their fruit growth stages. Further studies are required to develop useful indices to characterise the fruit developmental stages of the tomatoes using these parameters, which could be useful to the growers in the future era of high-tech agriculture.

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