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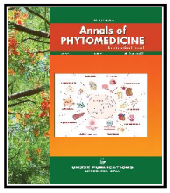

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Profiling of antioxidants and metabolite pigments in diverse tomato (*Solanum lycopersicum* L.) genotypes

P. Sudheer Kumar Reddy◆, Suresh Reddy Yerasu*, P. Syam Sundar Reddy**, Jagesh Kumar Tiwari* and Nagendra Rai***

** Division of Vegetable Improvement, ICAR-Indian Institute of Vegetable Research, Varanasi-221305, Uttar Pradesh, India*

*** Department of Vegetable Science, College of Horticulture, Dr. YSR Horticultural University, Anantharajupeta-516105, Andhra Pradesh, India*

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Abstract

Worldwide, malnutrition is an epidemic typically impacting children under the age of five and the elderly as well. As one of the most consumed vegetable crops globally, tomato is a vital food source for preventing malnutrition. Therefore, finding genotypes that possess powerful antioxidant capabilities and increased dietary content is critical in the fight against malnutrition. The current study explored metabolite pigments, antioxidants, and nutrient composition in thirty different tomato genotypes, revealing considerable variation across the genotypes. Among the pigments, the maximum amount of chlorophyll A (23.21 mg/g), total chlorophyll (27.96 mg/g) recorded in AVTO1314, maximum chlorophyll B (4.95 mg/g), and total carotenoids (5.03 mg/g) recorded in Punjab Ratta, maximum lycopene (5.44 mg/100g) found in Kashi Chayan and LA4026, and maximum β-carotene (0.62 mg/100 g) was found in Pusa Gaurav. The highest amount of ascorbic acid 24.83 mg/100 g was recorded in LA4025 and Kashi Amul, TSS (4.97 ^{0}Brix), and proteins (2.37 g/100 g) in Kashi Chayan, phenols (62.77 mg GAE/100 g) in Kashi Aman, titratable acidity (0.78%) in Pusa Gaurav, and sugars (5.29 g/100 g) in VRT78-2. The multi-trait genotype ideotype distance index identified Kashi Chayan, Kashi Amul, Kashi Adarsh, and Arka Vikas as superior genotypes for antioxidants, nutritional and metabolite pigments, and it also revealed strengths and weaknesses of genotypes for each trait. The tomato genotypes identified in the study can be used in breeding programmes to increase the nutritional content of tomatoes.

1. Introduction

Over two billion people globally are estimated to be plagued by "hidden hunger" (deficiency in minerals and vitamins) (Lowe, 2021). When it comes to fighting hidden hunger, horticultural crops like tomatoes (*Solanum lycopersicum* L.), which are known to be protective foods, are more important. Tomatoes and tomato-based products provide numerous health benefits and have a vital role in human nutrition (Saran *et al*., 2021). Tomatoes encompass several nutrients and phytochemicals, notably lycopene, potassium, iron, folate, and vitamin C, that possess bioactive properties such as antibacterial, antimutagenic, anti-inflammatory, and anti-carcinogenic impacts (Uc'an and Ugur, 2021) and gives immunity against viral infections (Indhuleka *et al.,* 2020). Tomatoes also contain antioxidants including β-carotene, and phenolic compounds like flavonoids, hydroxycinnamic acid, chlorogenic, homo vanillic acid, and ferulic acid, in addition to metabolite pigments like carotenoids and chlorophyll (Tamasi *et al*., 2019). These natural antioxidants hamper reactive oxygen species (ROS) by scavenging free radicals that can prevent cellular proliferation, apoptosis, alter enzymatic activities and signal transduction pathways (Hossen *et al*., 2017). Lycopene comprises 80-90% of the carotenoid amount found in tomato fruit, while β-carotene contributes about 7-10% (Frusciante *et al*., 2007). Lycopene encompasses a high singlet oxygen quenching rate and strong antioxidant abilities, whereas β-carotene has been associated with provitamin A activity (Sies, 1991), skeletal muscle metabolism (Liu *et al*., 2021), spleen damage prevention (Dai *et al*., 2021), neuroprotection, and hypocholesterolemic activities (Liu *et al*., 2021). These compounds may have a greater impact in mitigating the risk of numerous fatal diseases, particularly cancer and coronary artery disease. Elevated lycopene concentrations within the blood are believed to be tied to a lower risk of prostate and various other cancers (Assar *et al*. 2016; Shamna and Poyil, 2023). Tomato consumption is associated with higher levels of lycopene in plasma and serum (Rao *et al,* 2018). Tomatoes also serve as an adequate supplier of phenols, total soluble solids, and pH (Raffo *et al*., 2002). Titratable acidity, also known as total acidity level, determines the entire quantity of acid in the food and is a more reliable indicator of impact of acid on flavour. However, overall acidity does not provide complete information about a product as the ability of a microbe to grow in a particular food source is dictated by the concentration of free hydronium ions, H_3O^+, rather than titratable acidity. These nutrients assist with a variety of physiological functions, including lipid metabolism preservation, blood circulation stimulation, and bone structure maintenance (Vats *et al*., 2020). Owing to the perceived value of nutrients, crop quality traits, and increased consumer awareness, generating crop varieties with greater nutritive and antioxidant qualities has become one of the key objectives of crop enhancement.

Corresponding author: Dr. Suresh Reddy Yerasu
Division of Vegetable Improvement, ICAR-Indian Institute of Vegetable Research, Varanasi-221305, Uttar Pradesh, India
E-mail: yerasusureshreddy@gmail.com
Tel.: +91-7376635332

The current propensity in the food industry is to examine, and find more suitable uses for all by products, especially peel, seeds, stems, and leaves. Tomato crop by products embrace bioactive chemicals that have the potential to be sources of antibacterial, antiviral, and antioxidant compounds, rendering them economically valuable in the agricultural sector. Tomatoes dietary and physiochemical attributes vary according to cultivar and weather factors (Ali *et al*., 2021). In this context, identifying cultivars with high nutritional value is an effective approach for selecting tomato varieties that offer enhanced nutritional benefits and health-promoting properties. The multi-trait genotype ideotype distance index (MGIDI) is a statistical approach to identify the most suitable genotypes by considering the intended values associated with every selected characteristic (Olivoto and Nardino, 2021). In this study, thirty tomato genotypes were analyzed for their antioxidant levels, nutrient content, and metabolite pigments compounds to identify superior varieties. These genotypes can be utilized in breeding programs focused on enhancing nutritional quality and may also aid in selecting varieties for the extraction of bioactive compounds and nutraceuticals.

2. Materials and Methods

2.1 Plant materials and field experiment

A set of thirty tomato genotypes (Table 1) was used to estimate metabolite pigments, antioxidants and nutritional characteristics. Among the thirty genotypes, LA3473, LA4025 and LA4026 were imported from to Tomato Genetics Resource Center (TGRC), UC Davis, USA, and AVTO1314 and AVTO1219 were imported from World Vegetable Centre (WVC), Taiwan. The remaining genotypes were taken from the gene bank, ICAR-IIVR, Varanasi. Tournefort (1694) based on multi-lobular number of the cultivated tomato fruit, separated it from genus *Solanum* and considered under the genus name *Lycopersicon*. Recently, based on phylogenetic studies utilizing DNA sequences and more thorough analyses of plant shape and distribution. Peralta *et al.* (2005) included tomato under the genus *Solanum* and this inclusion gained widespread acceptance. The accepted scientific name of cultivated tomato is *Solanum lycopersicum* L. The present research was conducted at the Division of Vegetable Improvement, ICAR-Indian Institute of Vegetable Research, Varanasi, Uttar Pradesh in the rabi season of 2023-2024. The institute is located at an elevation of 80.71 m above mean sea level and the coordinates of the campus are 25°10'N latitude and 82°52'E longitude under subtropical climate conditions. The seeds were sown during September and 25 days old seedlings were transplanted during October (first week) of 2023. In each replication, twenty seedlings of each genotype were transplanted and grown on raised beds in paired rows at 60 × 45 cm^2 spacing by following standard cultural practices. The experimental design was Randomised block design (rBD) with three replications.

2.2 Tomato fruit sample preparation

Uniformly ripened healthy fruits at red-ripe stage were harvested and immediately analysed for antioxidant, biochemical, nutrients and metabolite pigments. Further, chlorophyll and carotenoid contents in different genotypes in leaves were estimated. Three uniform fruits from each replication were cut into small pieces and sequentially homogenized. The homogeneous suspension was used for analyses.

2.3 Assessment of metabolite pigments

2.3.1 Lycopene and β-carotene

A sample of 0.5 g of tomato fruit was crushed in 5 ml of acetone and transferred to a separation funnel. Subsequently, 5 ml of both petroleum ether and distilled water was added into the separation funnel and mixed well. Aqueous phase (bottom liquid) was discarded and top liquid phase was collected (Top liquid). The absorbance of lycopene and β-carotene was measured at 503 nm and 452 nm wavelengths, respectively, by using a spectrophotometer and quantified with petroleum ether used as a blank (Ranganna *et al*., 1976):

Lycopene (mg /100 g) =

$$\frac{3.1206 \times \text{O.D of sample} \times \text{volume made up} \times \text{dilution} \times 100}{\text{Weight of the sample} \times 1000}$$

Beta carotene (mg/100 g) =

$$\frac{3.857 \times \text{O.D of sample} \times \text{volume made up} \times \text{dilution} \times 100}{\text{Weight of the sample} \times 1000}$$

Chlorophyll and carotenoids

Chlorophyll and carotenoids were estimated by dimethyl sulfoxide (DMSO) method (Hiscox and Israelstam, 1979). A fresh 500 mg leaf sample was collected from 45 days old seedlings and added in 10 ml of DMSO. Tubes were covered with aluminium foil and kept in a hot air oven for 4 h at 72^0C. One ml pure solution from the above sample was collected and diluted to 5 ml by using DMSO. The samples were read at 645, 663 and 480 nm wavelength in UV-Spectrophotometer using clear DMSO as a blank:

Chlorophyll a (mg/g) =

$$\frac{(12.7 \times A_{663nm}) - (2.69 \times A_{645nm}) \times \text{volume} \times \text{dilution}}{\text{Weight of the sample (g)} \times 1000}$$

Chlorophyll b (mg/g) =

$$\frac{(22.7 \times A_{645nm}) - (4.68 \times A_{663nm}) \times \text{volume} \times \text{dilution}}{\text{Weight of the sample (g)} \times 1000}$$

Total chlorophyll (mg/g) =

$$\frac{(22.7 \times A_{645nm}) - (4.68 \times A_{663nm}) \times \text{volume} \times \text{dilution}}{\text{Weight of the sample (g)} \times 1000}$$

Total carotenoids (mg/g) = $\dfrac{(1000A_{480} - 1.29C_a - 53.78C_b)}{220}$

2.4 Assessment of antioxidant activities

2.4.1 Ascorbic acid

The ascorbic acid content of tomato fruits was determined according to the method described by Ranganna (1986). The tomato fruit juice of 10 g was taken and made up to a volume of 100 ml with 3% metaphosphoric acid (HPO_3) solution. The suspension was filtered

by using Whatman No.1 filter paper. The 2, 6-dichlorophenol indophenols dye solution was standardized by titrating against standard ascorbic acid solution and the dye factor was calculated before actual titration. The sample juice of 5 ml was taken from the filtrate and titrated against a standardized dye solution through a burette till the pink colour appeared as an endpoint. Ascorbic acid content was calculated by the following formula and the results were expressed as mg/100 g of pulp:

$$\text{Ascorbic acid (mg/100 g)} = \frac{\text{Dye factor} \times V_2 \times 100 \times 100}{V_1 \times W}$$

where,

V_1 = Volume of sample extract taken for dye titration

V_2 = Volume of dye required (titre)

W = Weight of sample

2.4.2 Titratable acidity (TA)

One g of fruit sample was crushed in 15 ml of distilled water and centrifuged at 10,000 rpm for 10 min. Ten ml of the supernatant was collected and 2 drops of phenolphthalein solution was added to the supernatant. The acidity was estimated by titrating it using 0.1N NaOH solution to appear light pink colour (Suh *et al.,* 2018):

$$\text{Titratable acidity (\%)} = \frac{\text{Volume / Reading} \times 0.1 \times 0.064}{\text{Weight of sample}} \times 100$$

2.4.3 Total phenols

The total phenolic content was determined by using the spectroscopic method described by Ainsworth *et al*. (2007). The reaction mixture was prepared by mixing 1 mg plant extracts, 1 ml of 10 % Folin-Ciocalteu's reagent dissolved in 13 ml of deionized water followed by the addition of 5 ml of 7 % Na_2CO_3 solution. The mixture was mixed thoroughly and kept in the dark at room temperature for 2 h. The blank solution was also prepared without plant extracts. The absorbance was recorded using a spectrophotometer (at 760 nm).

Total phenols (mg GAE/100 g) =

$$\frac{\text{Absorbance reading} \times 110 \times \text{final volume of assay}}{\text{Weight of sample} \times \text{volume of supernant}}$$

2.5 Processing and nutritional traits

2.5.1 pH and total soluble solids (TSS)

Fruit pH was determined from 15 ml of fruit juice by using a pH meter (Model: LMPH-9). To estimate the total soluble solids, fresh tomato fruits were squeezed to obtain the fresh tomato juice. The TSS is a refractometric index that indicates the proportion (%) of dissolved solids in a solution. The TSS contents in 100 µl of fresh juice extract were measured using a digital refractometer.

2.5.2 Total sugars

The phenol sulphuric acid reagent method was used to estimate total sugar content (Dubois *et al*., 1951). The reaction mixture included 250 µl of reagent 1 (5% phenol solution), 1.25 ml of reagent 2 (96% sulphuric acid) and 500 µl of tomato fruit extract. The reaction mixture was placed in a water bath for 20 min at 30°C. Later absorbance of the reaction mixture was measured at 490 nm using a spectrophotometer. The results were reported as g/100 g of fresh weight (FW) after plotting the standard curve between absorbance at 490 nm versus micrograms of protein concentration.

2.5.3 Total protein

The tomato extract was used for the estimation of total protein content by the Bradford method (1976). One gram of fruit sample was crushed in 1 ml of protein extraction buffer in a cold mortar and pestle. The collected fruit sample paste was transferred into a 1.5 ml microcentrifuge tube and centrifuged for 15 min at 8000 rpm at 4°C and 100 µl of supernatant was collected. Diluted two different concentrations of the extract, *i.e*., 20 µl and 5 µl made up the volume to 100 µl with protein extraction buffer, later 5 ml of Bradford dye reagent was added and mixed well. After 5 min and before 1 h, read the absorbance at 595 nm against a reagent blank (100 µl of extraction buffer with 1 ml of dye reagent). Bovine serum albumin is used as the standard. The results were reported as mg/100 g of fresh weight (FW) after the preparation of the standard curve between absorbance versus micrograms of protein.

2.6 Statistical analysis

All the selected individual variables of genotypes were statistically analysed by using R software and Duncan's Multiple Range Test (DMRT) analysis was carried out by using the GRAPES software, version 1.0.0. (Gopinath *et al*., 2020). The 'metan' package in the R software, version 4.4.1 was used to analyze and select the best performing genotypes for rich metabolites and antioxidants (R Studio, 2020).

3. Results

The estimates of tomato genotypes in terms of antioxidants (ascorbic acid, titratable acidity, and total phenols), metabolite pigments (chlorophyll, carotenoids, lycopene, and β-carotene), along with processing and nutritional characteristics such as total soluble solids, total pH, total proteins, and total sugars indicated presence of significant level of diversity among the genotypes. The mean values for each variable exhibited substantial differences among the tomato genotypes, as shown in Table 1.

3.1 Profiling of tomato varieties for metabolite pigments

Significant heterogeneity was identified across the tomato genotypes in different metabolite pigments (chlorophyll-A, chlorophyll-B, total chlorophyll and carotenoids in leaves, and lycopene, and -carotene in fruits) (Table 1). Chlorophyll-A is a prominent pigment in the process of photosynthesis enabling plants to turn light energy into chemical energy and contributing to carbohydrate production. Chlorophyll-A content among the genotypes ranged from 23.21 to 14.75 mg/g. The highest chlorophyll-A content (23.21 mg/g) was found in AVTO1314, followed by Kashi Amrit (21.84 mg/g) and Punjab Ratta (21.82 mg/g), and the lowest chlorophyll-A value (14.75 mg/g) was noticed in VRT2-2-3-1 genotype, followed by Punjab Kesari (15.56 mg/g) and Punjab Varkha Bahar-1 (15.84 mg/g). Chlorophyll-B levels ranged from 4.95 mg/g to 2.15 mg/g. The Punjab Ratta genotype possessed the highest amount of chlorophyll-B at 4.95 mg/g, while comparable content was found in Punjab Chhuhara (4.93 mg/g), EC538441 (4.92 mg/g), and the lowest amount of chlorophyll-B was recorded in Punjab Kesari (2.15 mg/g), followed by VRT2-2-3-1 (2.89 mg/g) and LA4026 (2.96 mg/g). Total chlorophyll

concentrations ranged from 17.68 to 27.96 mg/g. The AVTO1314 genotype had the highest total chlorophyll content (27.96 mg/g), which was statically on par with Punjab Ratta (27.33), Kashi Chayan (26.64 mg/g), and Kashi Amrit (26.47 mg/g). The VRT2-2-3-1 genotype had the lowest total chlorophyll content (17.68 mg/g), followed by LA4026 (19.21 mg/g) and Punjab Kesari (19.36 mg/g).

In terms of carotenoids, Punjab Ratta had the highest quantity (5.03 mg/g), followed by Kashi Chayan (4.86 mg/g), Kashi Amrit (4.85 mg/g), Punjab Upma (4.78 mg/g), and Punjab Chhuhara (4.71 mg/g) genotypes, which were statistically on par, and LA4026 had the lowest (2.28 mg/g) amount of carotenoids content, followed by LA3473 (2.52 mg/g) and VRT78-2 (2.72 mg/g). Lycopene and β-carotene are the crucial metabolite pigments in tomatoes, imparting red and orange colours, respectively. The highest amount of lycopene 5.44 mg/100 g was found in Kashi Chayan and LA4026, followed by EC538441 (5.12 mg/100 g), and Punjab Upma possessed the lowest amount of lycopene 3.15 mg/100 g, which was nearly identical to the Moneymaker genotype (3.17 mg/100 g). Carotenoids are produced by tomato plants in their leaves, flowers, and fruits. β-carotene, an essential carotenoid prevalent in tomatoes, is of special significance owing to its provitamin action (Table 1).

Table 1: Mean performance of tomato genotypes for different metabolite pigments

S. No.	Genotypes	Metabolite pigments					
		Chlorophyll A (mg/g)	Chlorophyll B (mg/g)	Total chlorophyll (mg/g)	Carotenoids (mg/g)	Lycopene (mg/100 g)	β-carotene (mg/100 g)
1	Punjab Upma	20.8^{cd}	4.42^{cd}	25.42^{def}	4.78^{bc}	3.15^{n}	0.35^{k}
2	Punjab Chhuhara	19.6^{ef}	4.93^{a}	25.81^{cdef}	4.71^{bcd}	4.36^{ghi}	0.39^{ij}
3	VRT78-4	18.4^{hij}	4.14^{e}	23.60^{h}	4.51^{ef}	3.77^{m}	0.21^{o}
4	AVTO1314	23.21^{a}	3.71^{ghi}	27.96^{a}	3.99^{ij}	4.36^{ghi}	0.38^{j}
5	VRT8-6-1	18.3^{hij}	3.68^{hi}	22.12^{jk}	4.13^{hi}	4.22^{ij}	0.42^{gh}
6	LA3473	16.5^{nop}	3.11^{kl}	20.42^{mno}	2.52^{q}	4.44^{efgh}	0.29^{l}
7	Vaibhav	16.14^{opq}	3.21^{k}	20.60^{mn}	3.63^{l}	4.56^{de}	0.49^{de}
8	VRT78-2	17.33^{klm}	4.91^{ab}	22.42^{ij}	2.72^{o}	3.72^{m}	0.54^{b}
9	Pusa Gaurav	20.12^{de}	4.52^{cd}	25.34^{ef}	4.56^{de}	3.24^{n}	0.61^{a}
10	EC538441	21.51^{bc}	4.92^{a}	26.35^{bcde}	4.84^{b}	5.12^{b}	0.25^{n}
11	Kashi Aman	18.75^{gh}	3.57^{ij}	23.56^{h}	4.29^{gh}	4.69^{cd}	0.54^{b}
12	Kashi Adarsh	21.75^{b}	4.37^{d}	26.19^{cde}	4.62^{cde}	4.57^{de}	0.38^{j}
13	Kashi Amrit	21.84^{b}	4.54^{c}	26.47^{bcd}	4.85^{ab}	4.37^{fghi}	0.33^{k}
14	VRT2-2-3-1	14.75^{r}	2.89^{m}	17.68^{q}	3.36^{m}	3.98^{k}	0.48^{e}
15	Kashi Chayan	20.85^{cd}	4.75^{b}	26.64^{bc}	4.86^{ab}	5.44^{a}	0.56^{b}
16	Arka Vikas	19.47^{efg}	3.93^{f}	23.45^{hi}	4.34^{fg}	5.10^{b}	0.51^{cd}
17	Punjab Varkha Bahar-1	15.84^{pq}	3.07^{kl}	19.93^{nop}	3.05^{n}	3.96^{kl}	0.35^{k}
18	EC538411	17.98^{ijk}	3.78^{fgh}	21.84^{jkl}	3.81^{jk}	3.25^{n}	0.29^{l}
19	LA4026	16.42^{op}	2.96^{lm}	19.21^{p}	2.28^{p}	5.44^{a}	0.49^{e}
20	VRT18-1	18.95^{fgh}	4.82^{ab}	23.93^{gh}	4.07^{i}	4.77^{c}	0.41^{gh}
21	Pusa Ruby	16.21^{opq}	3.43^{j}	21.13^{klm}	3.66^{kl}	4.35^{ghi}	0.43^{g}
22	Selection-12	17.19^{lmn}	3.64^{hi}	20.59^{mn}	3.78^{kl}	4.38^{fghi}	0.45^{f}
23	Punjab Varkha Bahar-2	16.63^{mno}	3.62^{hi}	$20.92l^{mn}$	3.65^{kl}	4.28^{hij}	0.43^{g}
24	LA4025	17.86^{ijkl}	3.86^{fg}	22.11^{jk}	4.05^{i}	4.18^{j}	0.28^{lm}
25	Punjab Ratta	21.82^{b}	4.95^{a}	27.33^{a}	5.03^{a}	3.81^{lm}	0.39^{ij}
26	Moneymaker	16.24^{opq}	3.19^{k}	$20.76l^{mn}$	3.77^{kl}	3.17^{n}	0.26^{mn}
27	Kashi Vishesh	18.71^{hi}	3.94^{f}	23.54^{h}	3.11^{n}	4.53^{def}	0.47^{ef}
28	Punjab Kesari	15.56^{q}	2.15^{n}	19.36^{op}	4.28^{gh}	4.43^{efgh}	0.51^{c}
29	Kashi Amul	18.27^{hij}	4.57^{c}	24.91^{fg}	4.30^{gh}	4.51^{efg}	0.48^{e}
30	AVTO1219	19.76^{e}	2.85^{m}	23.3^{hi}	3.69^{kl}	4.35^{ghi}	0.41^{hi}
	C.D (5%)	0.74	0.17	1.09	0.18	0.16	0.04
	$SE_m(\pm)$	0.26	0.06	0.39	0.06	0.06	0.01

Note: Treatments with same letters are not significantly different.

3.2 Profiling of tomato varieties for antioxidants

Antioxidant levels were estimated in 30 distinct tomato genotypes and their findings are summarised in Table 2. The results showed statistically significant variations across the genotypes in terms of antioxidant attributes. Tomatoes are an inexpensive source of antioxidants and are widely available to consumers. In terms of antioxidants, the range among the genotypes was 24.83 to 14.68 mg/100 g, which is considered moderate when contrasted to other vegetable crops. LA4025 genotype possessed 24.83 mg/100 g of dietary ascorbic acid, which was higher than all other genotypes. Kashi Amul, Punjab Varkha Bahar-2, and VRT8-6-1 genotypes had statistically equal amounts of ascorbic acid values of 23.04, 22.98, and 22.56 mg/100 g, respectively. Punjab Chhuhara has the least quantity of ascorbic acid at 14.68 mg/100 g.

Moreover, the Pusa Gaurav variety exhibited the highest level of acidity (0.78 mg/100 g), followed by Moneymaker (0.67 mg/ 100 g), and AVTO-1314 had the lowest acidity (0.25 mg/100 g), which is statistically on par to Kashi Aman (0.26 mg/100 g) and LA4026 (0.28 mg/100 g). Regarding the amount of total phenols, LA3473 was estimated to have 62.88 mg GAE/100 g, which is higher compared to other genotypes and statistically similar to the levels found in Kashi Aman, VRT78-2, and Punjab Varkha Bahar-1, which had 62.77, 62.75, and 62.67 mg GAE/100 g, respectively. Kashi Adarsh had the least quantity of phenols (38.89 mg GAE/100 g), followed by LA4025 at 9.77 mg GAE/100 g.

Table 2: Mean performance of tomato genotypes for antioxidants and processing traits

S. No.	Genotypes	Ascorbic acid (mg/100 g)	Titratable acidity (%)	Total phenols (mg GAE/ 100 g)	TSS (^{0}Brix)
1	Punjab Upma	15.64^{kl}	0.63^{c}	55.54^{ef}	3.89^{klm}
2	Punjab Chhuhara	14.68^{m}	0.37^{k}	49.23^{h}	4.56^{de}
3	VRT78-4	16.76^{ij}	0.38^{jk}	44.98^{ijk}	3.96^{jkl}
4	AVTO1314	19.36^{fg}	0.25^{o}	43.09^{kl}	4.22^{ghi}
5	VRT8-6-1	22.56^{b}	0.32^{m}	56.91^{cde}	3.75^{mno}
6	LA3473	14.87^{lm}	0.39^{j}	62.88^{a}	4.77^{b}
7	Vaibhav	17.48^{hi}	0.32^{m}	54.05^{f}	4.37^{fg}
8	VRT78-2	15.82^{k}	0.43^{i}	62.75^{a}	4.82^{ab}
9	Pusa Gaurav	18.97^{g}	0.78^{a}	39.86^{m}	4.56^{cde}
10	EC538441	16.91^{ij}	0.51^{g}	44.99^{ijk}	3.98^{jkl}
11	Kashi Aman	16.66^{j}	0.26^{o}	62.77^{a}	4.45^{ef}
12	Kashi Adarsh	19.64^{fg}	0.51^{g}	38.89^{m}	4.69^{bcd}
13	Kashi Amrit	20.54^{de}	0.56^{f}	46.45^{i}	3.63^{op}
14	VRT2-2-3-1	21.55^{c}	0.31^{m}	55.85^{def}	4.11^{hij}
15	Kashi Chayan	15.87^{k}	0.44^{i}	51.55^{g}	4.97^{a}
16	Arka Vikas	17.99^{h}	0.61^{d}	57.98^{bcd}	4.26^{gh}
17	Punjab Varkha Bahar-1	19.99^{ef}	0.59^{e}	58.22^{bc}	4.49^{ef}
18	EC538411	19.77^{ef}	0.32^{m}	43.73^{jkl}	3.47^{pq}
19	LA4026	15.27^{klm}	0.28^{n}	58.62^{bc}	4.25^{gh}
20	VRT18-1	17.99^{h}	0.44^{i}	49.03^{h}	4.33^{fg}
21	Pusa Ruby	17.32^{hij}	0.57^{f}	44.85^{ijk}	4.06^{ijk}
22	Selection-12	19.98^{ef}	0.47^{h}	56.92^{bcde}	4.77^{b}
23	Punjab Varkha Bahar-2	22.98^{b}	0.51^{g}	62.67^{a}	3.69^{no}
24	LA4025	24.83^{a}	0.34^{l}	39.77^{m}	4.73^{bc}
25	Punjab Ratta	15.04^{lm}	0.28^{n}	59.07^{b}	3.84^{lmn}
26	Moneymaker	16.92^{ij}	0.67^{b}	45.88^{ij}	4.04^{jk}
27	Kashi Vishesh	20.87^{cd}	0.57^{f}	49.73^{gh}	3.45^{q}
28	Punjab Kesari	17.48^{hi}	0.56^{f}	50.56^{gh}	3.78^{mno}
29	Kashi Amul	23.04^{b}	0.39^{j}	42.67^{l}	4.36^{fgc}
30	AVTO1219	21.03^{cd}	0.47^{h}	49.11^{h}	3.83^{lmn}
	C.D (5%)	0.78	0.04	2.16	0.18
	SE_m (±)	0.27	0.02	0.76	0.06

Note: Treatments with the same letters are not significantly different.

3.3 Profiling of tomato varieties for processing and nutritional-related traits

Figures 1-3 show the estimates for processing and nutritional traits of different tomato genotypes. The pH, total sugar, and total protein levels differed substantially among the tomato genotypes explored. The genotypes EC538441 and Kashi Chayan had the highest mean pH (4.87 and 4.56, respectively), followed by LA3473 (4.45), while Kashi Amrit and Kashi Amul had the lowest mean value (3.49 and 3.69). In addition, the VRT78-2, Kashi Adarsh, and Kashi Chayan genotypes had greater sugar levels (5.29, 5.23, and 5.19 g/100 g), but the VRT8-6-1 genotype had less sugars (2.54 g/100 g), which was comparable to the Punjab Upma (2.56 g/100 g). Kashi Chayan, Punjab Upma, and Kashi Aman genotypes exhibited the highest protein levels (2.37, 2.36, and 2.33 g/100 g, respectively), while EC538411 had the lowest protein levels (0.42 g/100 g). Total soluble solids (TSS) are an essential fruit quality indicator that represents the sugar content in tomato juice. There was statistically significant variation among genotypes in terms of biochemical and nutritional parameters. Kashi Chayan had the highest TSS value (4.97 ^{0}Brix) than other genotypes, preceding VRT78-2 (4.82 ^{0}Brix), whereas Kashi Vishesh had a lower TSS value (3.45 ^{0}Brix), which is comparable to EC538411 (Table 2).

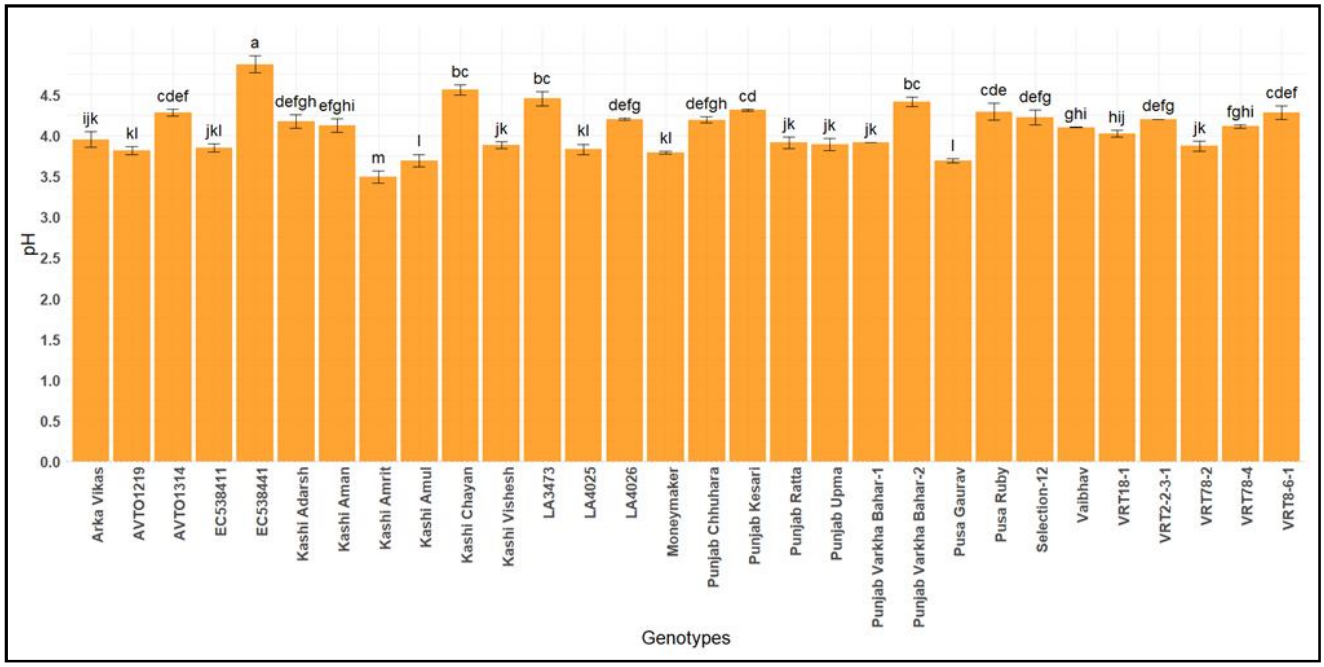

Figure 1: pH variability among the tomato genotypes.

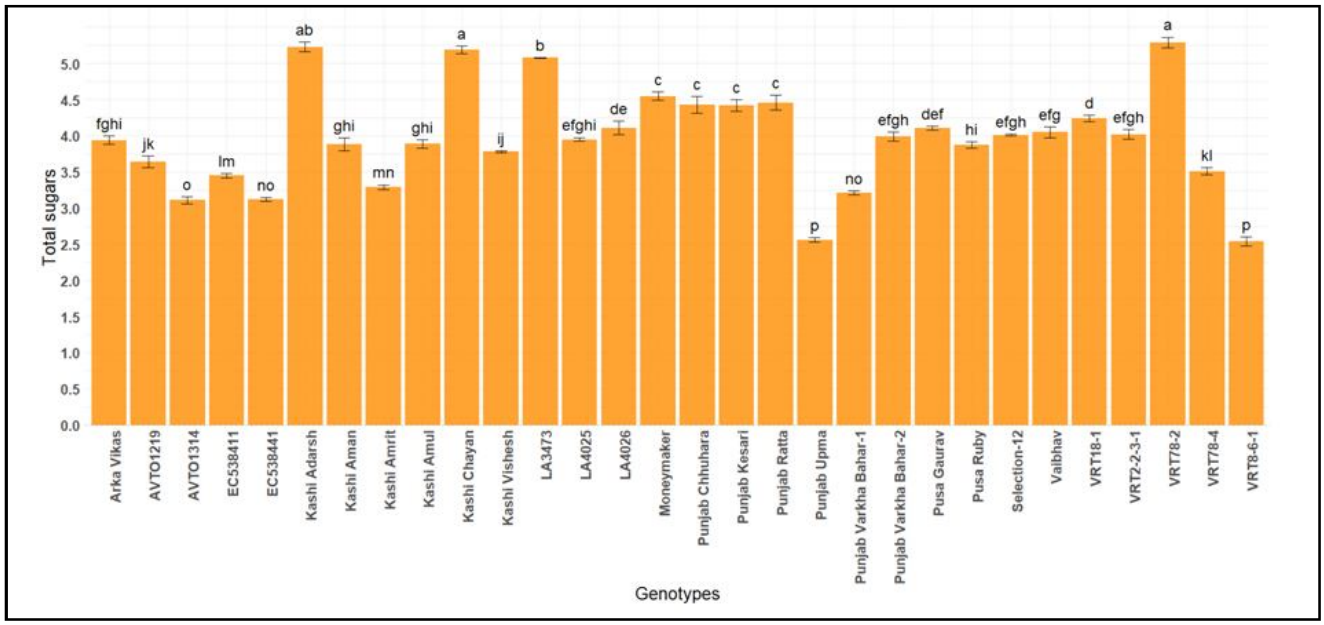

Figure 2: Total sugars content among the tomato genotypes.

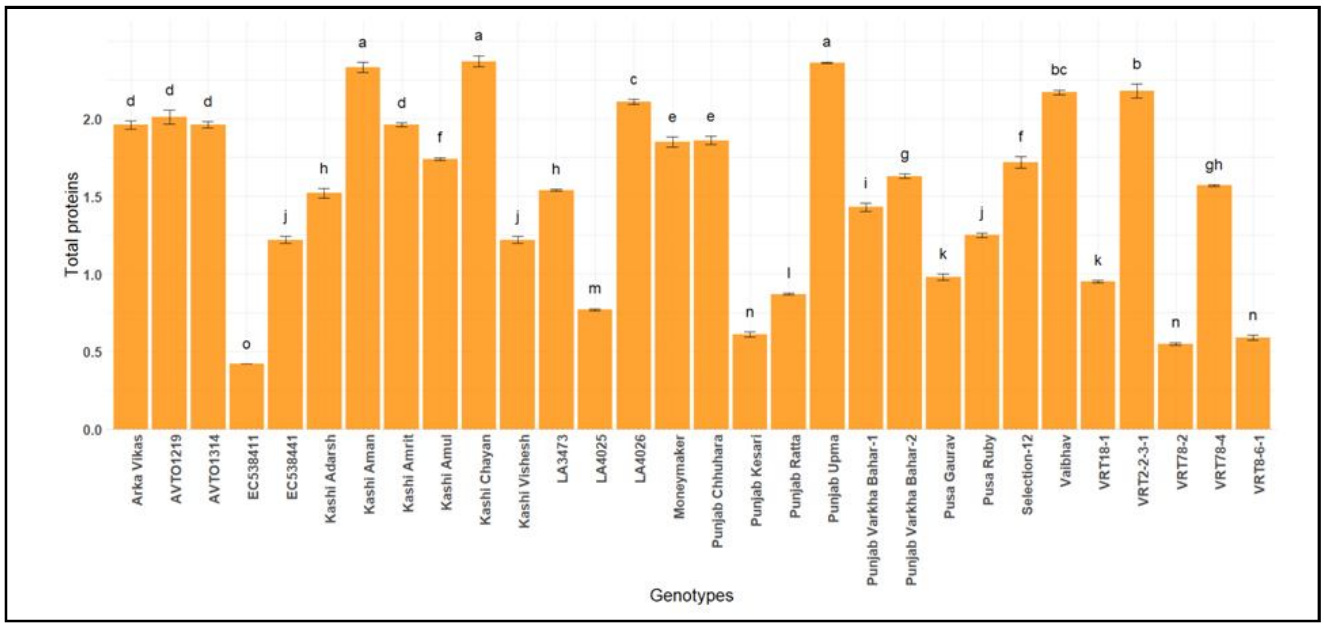

Figure 3: Total protein content among the tomato genotypes.

3.4 Selection of superior genotypes based on the MGIDI index

The Multi-trait Genotype Ideotype Distance Index (MGIDI) is an effective and versatile method for finding superior genotypes of varied crops based on multiple traits. It is a multimodal selection indicator which brings together several traits and information into a single value and ranks genotypes according to their distance from the ideal genotype. Figures 4 and 5 show the ranking of the thirty tomato genotypes determined by antioxidants, metabolite pigments, and nutritional properties using the MGIDI index. In Figure 4, these genotypes are listed in descending order of MGIDI index values, with the greatest value in the middle and the lowest in the outside circle. The MGIDI selection index determines the selection threshold, which is represented by the red circle. Genotypes were chosen based on their MGIDI index, represented by the red dots. The MGIDI index highlighted Kashi Chayan as the most preferred genotype, followed by Arka Vikas, Kashi Amul, and Kashi Adarsh.

3.5 The strengths and weaknesses view of the factors

Predicted genetic gain for the effective traits in the MGIDI index is given in Table 3. Kashi Chayan, Kashi Adarsh, and Kashi Amul had prominent strengths by delivering above average to FA1, indicating that these genotypes were superior performance for most FA1 related traits namely chlorophyll A, chlorophyll B, total chlorophyll and carotenoids but Arka Vikas provided below average, indicating its weakness to these specific traits. In contrast, Kashi Chayan and Kashi Adarsh stated strengths by providing above average to FA2 traits including total sugars and total soluble solids, while Arka Vikas and Kashi Amul contributed below average, which indicates weakness. In FA3, four genotypes contributed superior performance for titratable acidity, lycopene and pH. Arka Vikas is one genotype that contributes above average performance, but Kashi Amul, Kashi Adarsh, and Kashi Chayan contribute poor results compared to FA4's average performance for β-carotene and proteins. The Kashi Adarsh and Kashi Amul genotypes exhibited positive performance for ascorbic acid and phenols in FA5 factor, whereas the remaining two genotypes contributed negatively to these two specific traits. Figure 5 and Table 4 depicts the strengths and weaknesses of the previously selected genotypes, which are delineated by the percentage of each trait to the genotypes MGIDI score. Factors are included relative to genotypes in various colours to highlight their effect, while dotted lines denote average factor contribution.

Table 3: Predicted genetic gain for the effective traits in the MGIDI index

Traits	Factors	Xo	Xs	SD	SD (%)	h^2	SG	SG (%)	Indicators
Chlorophyll A	FA1	18.6	20.1	1.51	8.12	0.98	1.49	8.00	Increase
Chlorophyll B	FA1	3.88	4.40	0.52	13.4	0.99	0.51	13.3	Increase
Total chlorophyll	FA1	23.1	25.3	2.16	9.34	0.98	2.11	9.15	Increase
Carotenoids	FA1	3.94	4.53	0.58	14.8	0.99	0.58	14.7	Increase
Total sugars	FA2	3.96	4.56	0.59	15.0	0.99	0.59	14.9	Increase
TSS	FA2	4.20	4.56	0.36	8.54	0.97	0.35	8.36	Increase
Acidity	FA3	0.45	0.49	0.03	8.47	0.99	0.03	8.46	Increase
Lycopene	FA3	4.28	4.90	0.61	14.4	0.99	0.61	14.3	Increase

pH	FA3	4.08	4.09	0.01	0.34	0.95	0.01	0.32	Increase
β-carotene	FA4	0.41	0.48	0.07	16.8	0.99	0.07	16.7	Increase
Proteins	FA4	1.52	1.90	0.37	24.5	1.00	0.37	24.50	Increase
Ascorbic acid	FA5	18.6	19.1	0.53	2.87	0.99	0.53	2.84	Increase
Total phenols	FA5	51.3	47.8	-3.48	-6.78	0.99	-3.44	-6.71	Decrease

Xo = overall mean, Xs = mean of selected genotypes, SD = selection differential, h^2 = broad sense heritability, SG = selection gain

Table 4: Selected genotypes for multi-traits by using MGIDI and its factors

Factors	Traits considered	Genotypes
FA1	Chlorophyll A, chlorophyll B, total chlorophyll A and carotenoids	Kashi Chayan, Kashi Adarsh and Kashi Amul
FA2	Total sugars and total soluble solids	Kashi Chayan and Kashi Adarsh
FA3	Titratable acidity, lycopene and pH	Kashi Adarsh, Arka Vikas, Kashi Amul and Kashi Chayan
FA4	β-carotene and proteines	Arka Vikas
FA5	Ascorbic acid and total phenols	Kashi Adarsh and Kashi Amul

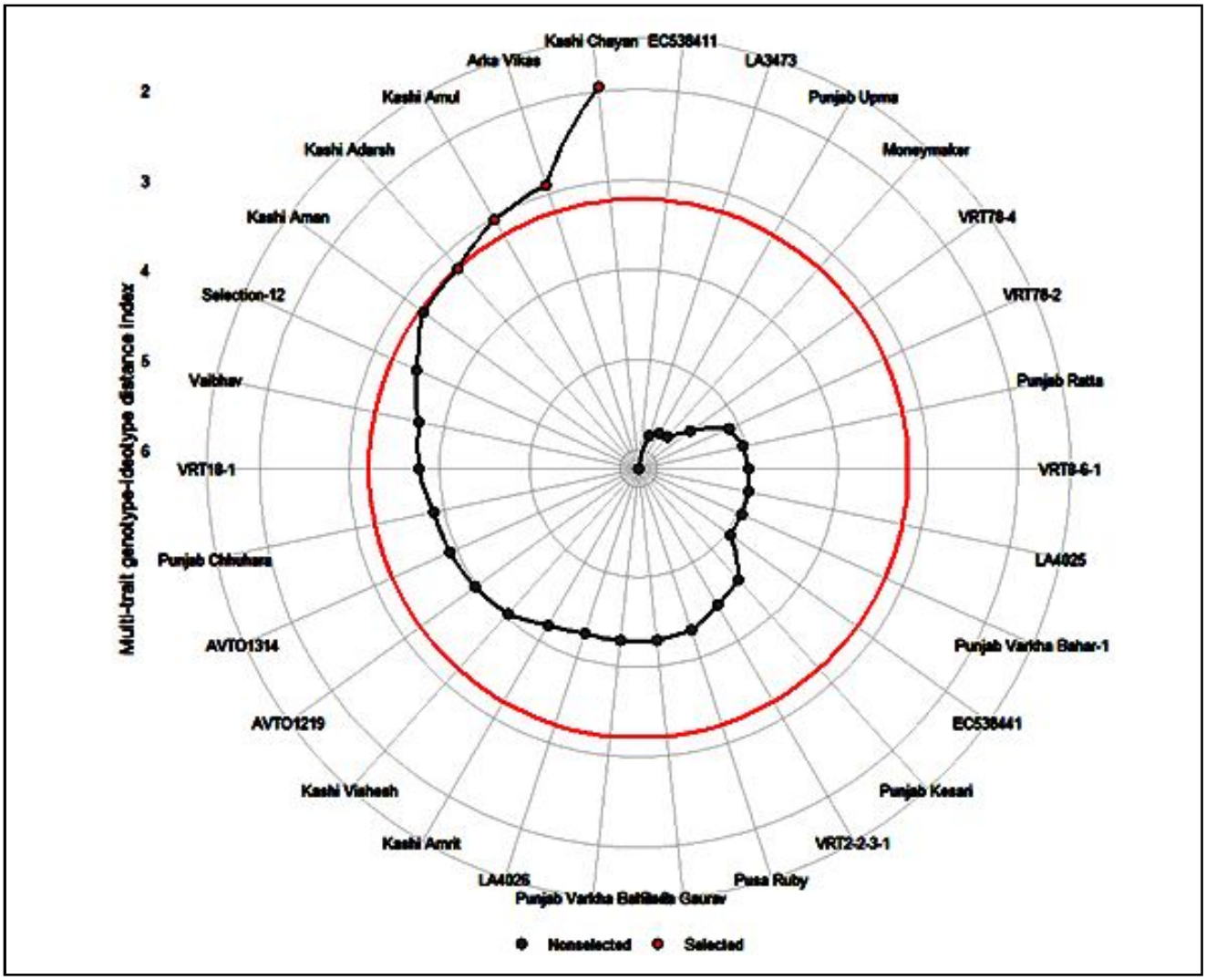

Figure 4: Genotype ranking of selected genotypes based on MGIDI. The red dots indicate selected accessions and the red circle represents the cut point based on the selection pressure.

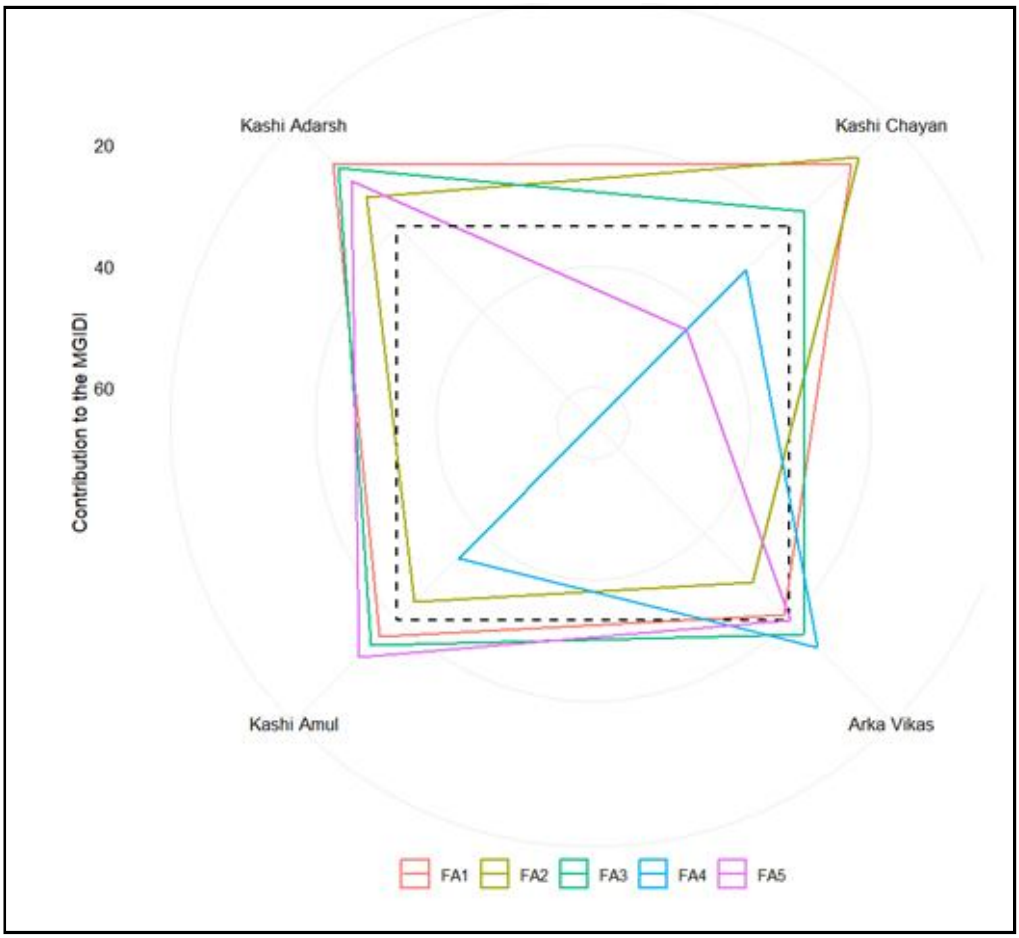

Figure 5: Strengths and weaknesses view of superior genotypes for each factor.

4. Discussion

Tomato (*Solanum lycopersicum* L.) is an excellent source of nutrients, antioxidants, and pigments (Salehi *et al*., 2019; Ali *et al*., 2021). These nutrients contribute to a variety of physiological functions, including lipid profile maintenance, blood circulation stimulation, and bone structure maintenance. A direct association was discovered between tomato fruit consumption and anti-cancerous activities (Wargovich, 2000). Tomato fruit contains high concentrations of natural antioxidants that inhibit reactive oxygen species (ROS) through free radical scavenging, prevent cellular proliferation and apoptosis, and regulate enzymatic activities and signal transduction pathways (Agarwal and Rao, 2000; Hossen *et al*., 2017; Navarro-Gonzaìlez *et al*., 2018). The current study was carried out to profile and select superior genotypes in terms of nutritional, pigment, and antioxidant potential in tomato fruits. Various biochemical analyses and MGIDI was used to identify nutritionally rich genotypes that may be further utilised in crop improvement programs.

Tomato plants produce different pigments such as chlorophyll-A and B, total chlorophyll, and carotenoids, which have greater human nutritional value. Phytochemical contents vary from organ to organ and depend on physiological maturity (Tiwari *et al*., 2013). The AVTO1314 genotype had the highest levels of chlorophyll-A and total chlorophyll content, and Punjab Ratta exhibited the highest amounts of chlorophyll-B (4.9 mg/g) and total carotenoids (5.03 mg/g). Laayouni *et al*. (2023) witnessed similar outcomes in potential tomato breeding lines. The incidence of light has a significant effect on the contents of chlorophyll and carotenoids in tomato plants, and exposure to light increases the concentrations of these metabolites (Lumpkin, 2005). Lycopene is a potent lipophilic antioxidant found in tomatoes and is the most effective free radical scavenger among all the carotenoids (Shi and Maguer, 2000). It has been shown to increase glutathione levels and the overall activity of antioxidant enzymes. Lycopene makes up about 80-95% of the total carotenoid content in tomatoes (Karniel *et al*., 2020). Lycopene antioxidant action protects lipids, DNA, and other macromolecules from damage (Anlar and Bacanli, 2020). In this investigation, lycopene levels in fresh tomato fruit varied from 3.15 to 5.44 mg/100 g, which was consistent with the findings of Frusciante *et al*. (2007). It has been established that the largest concentration of lycopene accumulates in the tomato skin, resulting in a reddish colour (Chattopadhyay *et al*., 2021). β-carotene is a prominent colourant with antioxidant properties which promote human health. The number of constituents removed during the extraction procedure determines the production of β-carotene extract from vegetables (Rifqi *et al*., 2023). β-carotene levels varied significantly between 0.21 and 0.61 mg/100 g, with the highest values found in the Pusa Ruby (0.61 mg/100 g), Kashi Chayan (0.56 mg/100 g), Kashi Aman (0.54 mg/100 g) and VRT78-2 (0.54 mg/100 g). Similar estimates were reported by Kondratieva and Golubkina (2016) in tomato and Mitra *et al.* (2021) in sweet potato.

Ascorbic acid is a major non-enzymatic antioxidant. High ascorbic acid content in tomato has an immense effect on plant life and human health (Di Matteo *et al*., 2010). Furthermore, ascorbic acid functions as a plant growth regulator through hormone signalling (Khalid and Hameed, 2017). The human body cannot produce ascorbic acid on its own because biosynthesis is inhibited at the last stage (Mittu *et al*., 2022). Ascorbic acid is the major hydrophilic antioxidant found in tomatoes. In the current study, ascorbic acid concentration varied from 24.83 to 14.68 mg/100 g, with LA4025 and Kashi Amul having higher values (24.83 and 23.04 mg/100 g, respectively). These results are strongly consistent with Debnath *et al*. (2021) findings. The degree of titratable acidity in tomato fruits is an essential factor in sensory qualities such as flavour and astringency. Titratable acidity ranged significantly between 0.25 and 0.78 per cent. The findings are consistent with Manna and Paul (2012), who observed acidity levels ranging from 0.30 to 0.73 per cent. Plants have a wide array of phenolic compounds. These chemicals are necessary for plant development and reproduction. Furthermore, phenolic compounds are natural antioxidants that may be found throughout the plant and act as antibiotics and insecticides (Gupta and Sharma, 2014). The total phenol levels in tomato fruit ranged from 38.89 to 62.88 mg GAE 100/g FW and these findings are consistent with Athinodorou *et al*. (2021) in tomato and Saiharini and Padmaja (2022) in watermelon. Similarly, Sumalan *et al*. (2020) found more diversity in tomato landraces from local farmers, with total phenol levels ranging from 51.49 to 123.3 mg/GAE 100 g FW. TSS is an essential indicator of a crop's shelf-life and quality, both in its fresh and processed forms. Furthermore, TSS levels exhibit a significant impact on tomato taste and consistency (Stevens *et al*., 1997). In the present study, TSS varied between 3.45 (Kashi Vishesh) and 4.97 °Brix (Kashi Chayan). The findings are consistent with those of Saimbhi *et al*. (1995), Kaur *et al*. (2005), George *et al*. (2004); Hammed *et al*. (2012). Tomato TSS predominantly consists of reducing sugars (Beckles, 2012). Thus, any factor that modifies the synthesis of sucrose (photosynthetic activity) will influence glucose and fructose deposits in the fruits, thus affecting TSS. Sugars are a vital component of tomato fruit, as they govern sweetness and taste. The greatest tastes require higher sugar content. Tomato fruit is mostly made up of glucose and fructose, with tiny quantities of sucrose (Tadesse *et al*., 2012). In this investigation, total sugar accumulation varied from 2.54 (VRT8-6-1) to 5.29 g/100 g (VRT78-2). These findings were in line with findings of Ibrahim *et al*. (2017).

The acquired total protein content data usually fell within the range commonly reported in tomato genotypes (Ibrahim *et al*. 2017), ranging from 0.42 g/100 g (Kashi Chayan) to 2.37 g/100 g (EC538411). The highest pH level recorded in EC538441 with a value of 4.87, indicating that EC538441 had less acidity than other genotypes, perhaps owing to the lower number of free hydronium ions in its fruit juice. The pH levels among the thirty genotypes tested varied between 3.49 to 4.87, consistent with the findings of Ibrahim *et al*. (2017) and Laayouni *et al*. (2023).

Plant breeders often seek to combine numerous relevant antioxidants, metabolite pigments, and nutritional properties in one outstanding genotype, resulting in higher nutritional quality. In this context, numerous multivariate techniques are often used, such as PCA (principal component analysis), factor analysis, cluster analysis, and different samples to categorise tangible traits or choose test genotypes (Bhandari *et al*., 2017). In this respect, Olivoto and Nardino (2021) created a selection index for detecting genotypes and recommending treatments based on multiple trait data. MGIDI is the most efficient index for choosing genotypes with desirable features, demonstrating its relevance and efficacy in crop improvement programmes (Olivoto and Lucio, 2020). The MGIDI view on strengths and weaknesses makes it easier to identify genetic strengths and weaknesses of genotypes based on a multiple-trait framework. As a result, the genotypes namely, Kashi Chayan, Kashi Amul, Kashi Adarsh, and Arka Vikas may be deemed the best genotypes in terms of antioxidants, nutritional content, and pigments. These selected genotypes can be used in future breeding programmes for developing advanced breeding lines for antioxidants, nutrition, and pigments. The MGIDI model's versatility is demonstrated by its successful application to analyse suitable attributes in crops such as Bush Yam (Adewumi *et al*., 2023), Maize (Palaniyappan *et al*., 2023), Eggplant (Uddin *et al*., 2021), Soybean (Volpato *et al*., 2019); *Cymopsis tetragonoloba* (Benakanahalli *et al*., 2021). These different experiments demonstrated the effectiveness of multivariate selection indices for simultaneous trait selection.

5. Conclusion

Enhancing the nutritional and processing qualities of tomatoes has become an important objective in tomato breeding programs. In this study, the genotypes Kashi Chayan, Kashi Amul, Kashi Adarsh, and Arka Vikas emerged as superior in terms of antioxidants, nutritional content, and metabolite pigments, making them excellent candidates for future breeding initiatives. The application of the Multi-Trait Genotype-Ideotype Distance Index (MGIDI) effectively identified the strengths and weaknesses of each genotype across various traits, providing valuable insights for targeted improvement strategies. Additionally, the genotypes LA4026, and Pusa Gaurav exhibited the highest levels of lycopene and carotenoids, highlighting their potential for developing tomato varieties with enhanced nutritional benefits. The comprehensive data generated from this study can be used to address the identified weaknesses and further optimize tomato cultivars for both human dietary intake and industrial processing.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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