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Research Article

Functional quality and antioxidant properties of tomato genotypes for breeding better quality varieties

R. S. Pal*, N. K. Hedau, Lakshmi Kant and A. Pattanayak
ICAR-Vivekananda Institute of Hill Agriculture, Almora, Uttarakhand, INDIA
E-mail: ramesh.pal@icar.gov.in

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Abstract
Twenty-two tomato advanced lines were investigated for the functional and antioxidant properties as well as their contents. Wide variations were observed for most of the traits, e.g., total soluble solids (3.50-6.03%), pH (3.90-5.08), pericarp thickness (5.40-8.70mm), fruit firmness (0.418 to 0.959 kg), lycopene content (2.84-9.83 mg/100g fw) and β-Carotene (3.37-8.16 mg/100g FW), indicative of considerable levels of genetic diversity. Highest concentration of ascorbic acid was found in the cultivar VT 1320 (305.59 mg/ kg). Total phenolic content ranged from 0.60 to 1.14 mgGAE/g FW whereas total flavonoids content ranged from 0.99 to 1.75 mgCE/g FW. Total flavonoids, total antioxidant activity, ABTS and DPPH free radical inhibition, total polyphenols and lycopene are having strong positive correlation among themselves. The results of present study are potentially useful for tomato breeders working on the development of new varieties with better quality.

Keywords
Tomato, antioxidant activity, ascorbic acid, carotenoids, phenolics

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is a crop of great interest, being widely consumed either as fresh or processed. Among the most prominent phytochemicals in tomatoes are the carotenoids, of which lycopene is the most abundant in the ripened fruit, accounting for approximately 80–90% of the total pigments (Helyes *et al.,* 2009). Tomatoes are also a concentrated source of phenolic compounds, such as flavonoids and hydroxycinnamic acid derivatives, containing 98% of the total flavonols in tomato skin as conjugated forms of quercetin and kaempferol (Ray *et al.,* 2011). Many of these phytochemicals present in tomatoes have antioxidant properties and in combination with lycopene may contribute to the numerous health benefits (Ray *et al.,* 2011). These tomato antioxidants have an important role in chronic disease prevention, including cancer, neurodegenerative diseases, cardiovascular disease, asthma, cataract and also in improving the immune function (Ray *et al.,* 2011).

Pericarp thickness and fruit firmness are considered to be very important criteria among breeders for selecting cultivars for improved storage capacity (Hedau *et al.,* 2008). The nutritional importance of tomato suggests that it is imperative to formulate breeding programmes to develop cultivars not only with high yield but also rich in antioxidant compounds and processing traits (Dar and Sharma, 2011). Therefore, it is becoming increasingly important to assess nutritional value of breeding lines even before starting yield evaluation. The present investigation was carried out to evaluate twenty-two advanced lines of tomato developed from parents with higher total soluble solids, total phenolics, lycopene, β-carotene, ascorbic acid content as well as for the antioxidant properties.

Materials and Methods

Twenty two different round-type tomato (*Lycopersicon esculentum* Mill.) cultivars were used in this study. Plants were grown under a greenhouse at ICAR-VPKAS, experimental farm, Hawalbagh (29°56′ N, 79°40’ E and 1250 m above amsl) during the spring season (March-July). Collected sample were washed, blotted with a paper towel and stored at –18 °C until analysis.

Soluble solids content (%) was measured with a digital refractometer while pH was measured by pH meter. Pericarp thickness (PT) was measured by a digital slide calipers. Ascorbic acid was determined according to the volumetric method (Thimmaiah, 1999). The result was expressed as mg ascorbic acid /100 g FW. Fruit firmness (FF) was measured using Texture Analyser (TA-XT2i; Stable Micro Systems Ltd) as reported by Saha *et al.* (2010). Chlorophyll and carotenoids were estimated by

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spectrophotometric analysis following the method of Nagata and Yamashita (1992).

For the total polyphenols, total flavonoids and antioxidant activity evaluation, tomato fruit tissues were extracted by homogenizing 5.0 g of frozen fruit (5± 0.2 g) in 25 mL of 80% ethanol. Samples were transferred in 50 mL centrifuge tubes and were centrifuged at 12,000 × g for 15 min at 4 ^{0}C, filtered through Whatman No1 filter paper and diluted to a final volume of 50 ml. Total polyphenols (TP) content was determined spectrophotometrically by the Folin-Ciocalteu method (Singleton and Rossi 1965) and results were expressed as mg gallic acid equivalent (GAE)/g fresh weight (FW). Total flavonoids were estimated using the standard methodology of Sun *et al.* (1998) and results were expressed as mg catechins equivalent (CE)/100g FW. The DPPH and ABTS assay were done by Brand-Williams *et al.* (1995) and Arnao *et al.* (2001) respectively. The total antioxidant activity (THAA) of the methanolic extract of samples was measured using a phosphomolybdenum method (Prieto *et al.,* 1999) and total antioxidant activity was expressed as equivalents of gallic acid (mg GA equ/g FW).For determination of various parameters, three biological replicate and three technical replicate from each biological replicate was used. Analyses of variance (ANOVA) was performed using the SAS enterprise, version 4.3 and significance of each group was verified with one-way analysis of variance followed by Duncan's multiple range test ($P < 0.05$). Principal Component Analysis (PCA, Pearson 1901) and heat map analysis were performed using a demo version of XLSTAT–Pro (Addinsoft). PCA was based on pearsons correlation matrix. Correlation biplots of traits were generated on which genotypes (lines) were superimposed. Heat map analysis to analyze traits and genotype (line) clustering simultaneously was done using the same software. Non-specific filtering with an inter-quartile range <0.25 was done to remove traits with low variability. The colour scale used was 'red to green through black'.

Results and Discussion

The total soluble solids ranged between 3.50 to 6.03% in the 22 advanced lines studied (Table 1). Significantly ($P < 0.05$) higher amounts of total soluble solids was found in the fruits of lineVT 1328 (6.03 %), followed by VT 1316 (5.37%) whereas the lowest levels were found in line VT 1320 (3.50%) and 'VT 1323' (3.67%). Saha *et al.* (2010) reported 2.0% and 4.0% total soluble solids in the fruits of 53 tomato genotypes. pH ranged between 3.90 in the tomato genotypes. pH ranged between 3.90 in the line (VT 1326) and 5.08 in the line (VT 1319). Overall, non-significant ($P < 0.05$) differences in pH among lines was observed (Table 1). Titratable acidity has no significant effect on tomato flavour unless pH is low. For this reason, a pH below 4.5 and citric acid content of above 0.35 g/100 g of fruit fresh weight are desirable. Pericarp thickness in tomato fruits is an important parameter associated with physical quality like fruit firmness. The pericarp thickness was determined between 5.40mm and 8.70mm in the analyzed tomato lines. Significantly ($P < 0.05$) higher pericarp thickness was found in the fruits of VT 1322 (8.70mm), followed by 'VT 1319 and VT 1308-2 (8.50mm), whereas the lowest levels was found in VT 1317 (5.40mm) (Table 1). Fruit firmness varied significantly between 0.418 (VT 1308-2) to 0.959 Kg (VT 1328). Similar results were also reported by Olaiya *et al.* (2010) in studies of different tomatoes genotypes. The line VT 1312 tomatoes had the highest content of β-carotene (8.16 mg/100g fw) followed by the line VT 1311 (7.31 mg/100g fw). The rest of the genotypes evaluated in the present work had β-carotene contents between 3.37 and 7.14 mg/100g FW. These values are in good agreement with previous studies (Gupta *et al.* 2011). The lycopene content showed a significant variation among different genotypes (Table 2). The variation ranged from 2.84 (VT 1323) to 9.83 (VT 1325) mg/100g FW. Dar and Sharma (2011) studied 60 diverse genotypes of tomato, and reported lycopene content of 19.5 to 46.2 mg /kg fw. Significant differences ($P < 0.05$) were found among the average lycopene content of genotypes in the present study. There were significant differences in the amount of ascorbic acid in the different lines of tomatoes studied, and the highest concentration was found in VT 1320 (305.59 mg/ kg) followed by VT 1323 (292.08 mg/ kg). Ascorbic acid content of 97-378 mg/ kg have been reported by various workers (Dar and Sharma, 2011 and Pinela *et al.*, 2012) in different genotypes of tomato. Total phenolic content in the studied lines ranged from 0.60 (VT 1322) to 1.14 (VT 1309) mg GAE/g. The total phenolic content found in this study was similar to the report by Erge and Karadeniz (2011). Tomatoes can be considered as good sources of total flavonoids, having the antioxidant properties. Total flavonoids content showed significant genotypic effect and content ranged from 0.99 (VT 1325) to 1.75 (VT 1311) mgCE/100g FW in the present set of advanced lines. Similar values were earlier reported by Marinova *et al.* (2005). The free radical scavenging activities are presented in the form of percentage inhibition of free radical DPPH and ABTS. DPPH

free radical inhibition ranging from 54.44 % (VT 1322) to 70.26 % (VT 1309) (Table 2), is in agreement with the results reported by Erge and Karadeniz (2011). ABTS free radical inhibition were found between 49.23 % (VT 1322) and 77.12 % (VT 1309) (Table 2). These are in agreement with the results reported by Erge and Karadeniz (2011). The total antioxidant activity ranged from 12.82 (VT 1325) to 22.91 (VT1310) mg GAE/g FW). The antioxidant capacity of natural antioxidants is due to the termination of the free radical chain reaction (Shimada *et al.* 1992).

Principal component analysis indicated strong positive correlations among TA, TF, TPP, DPPH and ABTS as seen from the plot of Factor 1 (F1) and Factor 2 (F2) which described 61.52% of the total variation (Fig 1a). β-carotene, brix and fruit firmness (FF) were also positively correlated. On the other hand, Vitamin c, pH and PT were negatively correlated. This plot, however, failed to explain properly the status of pH and lycopene, which was viewed on F1:F3 (Fig. 1b) and F1:F4 (Fig. 1c) plots, respectively. The F1:F3 plot indicated that like Vitamin c, pH and PT were negatively correlated to other traits. Superimposing the genotypes (lines) on the three trait plots (Fig 1a-c, biplots on the left) showed that VT1309 is unique for TA, TF and DPPH combination while VT 1311 is also unique for DPPH. Similarly, VT 1328 is unique for FF. As may be seen from Table 1, VT 1309 showed high values for all the above traits, which is not seen in any other genotype. A perusal of Table 1 indicates that VT 1328 showed the highest fruit firmness (FF). Study of the F1:F3 plot indicated that VT 1319 was unique for high pH. The F1:F4 plot indicated that VT 1328 and VT 1317 were unique for ABTS. No genotype was found unique for lycopene and on Vitamin c. In the heat map analysis, non-specific filtering removed three traits *viz* pH, FF and TF. A perusal of the heat map (Fig. 2) indicates that traits related to or indicative of free radical scavenging were grouped together in one cluster (β carotene, DPPPH, ABTS, TF and TA) while the other two groups were of mixed nature (the left dendrogram). The genotypes were also grouped into three major clusters (top dendrogram). Cluster 1 showed intermediate to very high values for free radical scavenging related traits while cluster 3 showed low values for these traits. Cluster 2 was intermediate for these traits. Thus, heat map analysis was able to group genotypes based on the expression of trait combinations. PCA, on the other hand, helped to plot trait relations and identify genotypes on the basis of the trend of trait combinations.

Present study revealed that sufficient variability was found for the functional compounds and antioxidant properties in the tomato genotypes studied. From a breeding point of view, the high variability suggests that it would be possible to obtain appreciable responses to selection for these traits in a targeted breeding programme. The results of present study could help tomato breeders to identify interrelationship between important nutritive, functional and antioxidative properties and to find out the better tomato donors and breed these characteristics into improved varieties.

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Table 1. Variations of textural and functional attributes in advanced lines of tomato

S No	Advanced lines	Brix (%)	pH	Pericarp Thickness (mm)	Fruit Firmness (Kg)	β- Carotene (mg/100g FW)	Lycopene (mg/100g FW)	Vitamin C (mg/kg FW)
1	VT 1308 -1	4.83^{de}	4.12^{ab}	6.47^{efg}	0.579^{cd}	5.16^{efghi}	4.74^{gh}	186.32^{ef}
2	VT 1308 -2	4.37^{fgh}	4.03^{ab}	8.50^{ab}	0.418^{f}	4.32^{ij}	3.32j	165.48^{g}
3	VT 1309	4.47^{fg}	3.94^{ab}	6.83^{ef}	0.567^{cd}	6.23^{bcde}	4.13^{i}	222.54^{c}
4	VT1310	6.00^{a}	4.03^{ab}	6.77^{ef}	0.512^{def}	6.28^{bcde}	7.40^{c}	152.32^{g}
5	VT 1311	5.00^{d}	3.94^{ab}	6.77^{ef}	0.526^{cd}	7.31^{ab}	9.77^{a}	199.53^{de}
6	VT 1312	5.80^{a}	4.03^{ab}	7.27^{cdef}	0.584^{cd}	8.16^{a}	5.51^{e}	127.29^{h}
7	VT 1313	4.87^{de}	4.14^{ab}	7.03^{def}	0.511^{def}	6.81^{bcd}	4.86^{fg}	185.13 ^{ef}
8	VT 1314	5.03^{cd}	3.94^{ab}	6.73^{ef}	0.656^{bc}	7.14^{abc}	8.16^{b}	189.76^{de}
9	VT 1315	5.30^{bc}	3.98^{ab}	6.30^{fg}	0.574^{cd}	4.45^{hij}	4.92^{fg}	163.86^{g}
10	VT 1316	5.37^{b}	3.88^{b}	6.90^{ef}	0.731^{b}	6.15^{bcde}	6.16^{d}	136.37^{h}
11	VT 1317	4.93^{d}	3.99^{ab}	5.40^{g}	0.577^{cd}	5.78^{cdefgh}	4.66^{gh}	210.77^{cd}
12	VT 1318	3.80^{jk}	4.02^{ab}	8.17^{abcd}	0.577^{cd}	4.72^{fghij}	5.32^{ef}	275.94^{b}
13	VT 1319	4.13^{hi}	5.08^{a}	8.50^{ab}	0.609^{cd}	5.99^{bcdef}	6.14^{d}	194.93^{de}
14	VT 1320	3.50^{l}	4.12^{ab}	8.27^{abc}	0.442^{ef}	4.43^{hij}	4.26^{hi}	305.59^{a}
15	VT 1321	4.63^{ef}	4.09^{ab}	7.70^{abcde}	0.575^{cd}	3.37^{j}	4.56^{ghi}	188.23^{e}
16	VT 1322	4.03^{ij}	4.04^{ab}	8.70^{a}	0.544^{cd}	4.57^{ghij}	4.07^{i}	199.32^{de}
17	VT 1323	3.67^{kl}	4.12^{ab}	6.77^{ef}	0.571^{cd}	5.89^{bcdefg}	2.84^{j}	292.08^{ab}
18	VT 1324	4.23^{ghi}	4.08^{ab}	7.43^{bcdef}	0.549^{cd}	7.09^{abc}	7.99^{b}	167.35^{fg}
19	VT 1325	4.37^{fgh}	4.04^{ab}	7.63^{abcde}	0.511^{def}	5.91^{bcdefg}	9.83^{a}	190.69^{de}
20	VT 1326	5.10^{bc}	3.90^{b}	7.13^{cdef}	0.609^{cd}	5.60^{defghi}	3.05^{j}	125.05^{h}
21	VT 1327	5.00^{d}	4.04^{ab}	7.43^{bcdef}	0.587^{cd}	5.96^{bcdefg}	7.73^{bc}	120.60^{hi}
22	VT 1328	6.03^{a}	4.15^{ab}	7.23^{cdef}	0.959^{a}	6.41^{bcde}	3.34^{j}	102.64^{i}
CD (5%)		0.265	NS	1.040	0.093	1.209	0.481	18.417

Means in a column with the same letter are not significantly different (P≥0.05)

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Table 2. Variations of Antioxidant metabolites and properties in advanced lines of tomato

S No	Advanced lines	Total polyphenols (mgGAE/g FW)	Total Flavonoids (mgCE/100g FW)	DPPH free radical scavenging activity (% inhibition)	ABTS free radical scavenging activity (% inhibition)	Total Antioxidant (mg GAE/g FW)
1	VT 1308 -1	0.74^{fghij}	1.23^{ghi}	61.01^{fg}	59.22^{g}	15.40^{efghi}
2	VT 1308 -2	0.69^{ghij}	1.43^{def}	61.34^{efg}	53.04^{hi}	15.98^{efghi}
3	VT 1309	1.14^{a}	1.65^{ab}	70.26^{a}	77.12^{a}	22.00^{ab}
4	VT1310	1.09^{ab}	1.60^{abc}	69.98^{a}	68.47^{bcd}	22.91^{a}
5	VT 1311	0.95^{bcd}	1.75^{a}	70.23^{a}	66.85^{bcd}	20.69^{abcd}
6	VT 1312	0.95^{bcd}	1.51^{bcd}	66.03^{abcdef}	64.62^{def}	18.82^{abcdef}
7	VT 1313	0.84^{cdefg}	1.25^{ghi}	66.48^{abcdef}	66.67^{bcd}	$16.86^{cdefghi}$
8	VT 1314	0.98^{bcd}	1.49^{bcd}	66.70^{abcde}	70.12^{bcd}	$17.61^{bcdefgh}$
9	VT 1315	0.65^{ij}	1.10^{ijk}	57.46^{gh}	64.72^{def}	15.65^{efghi}
10	VT 1316	0.82^{defgh}	1.47^{bcd}	63.15^{cdef}	68.46^{bcd}	$17.28^{cdefghi}$
11	VT 1317	0.91^{cde}	1.32^{efg}	67.16^{abcd}	71.82^{bc}	19.58^{abcde}
12	VT 1318	0.78^{efghi}	1.31^{efg}	62.73^{defg}	66.94^{bcd}	$16.60^{cdefghi}$
13	VT 1319	0.72^{ghij}	1.35^{defg}	61.17^{efg}	70.65^{bc}	13.96^{ghi}
14	VT 1320	0.65^{hij}	1.10^{ijk}	57.65^{gh}	57.54^{gh}	13.35^{hi}
15	VT 1321	0.66^{hij}	1.13^{hijk}	55.08^{h}	59.24^{g}	13.54^{hi}
16	VT 1322	0.60^{j}	1.06^{jk}	54.44^{h}	49.23^{i}	14.73^{fghi}
17	VT 1323	1.07^{ab}	1.51^{bcd}	69.27^{ab}	73.07^{ab}	21.03^{abc}
18	VT 1324	0.98^{abc}	1.48^{bcd}	68.44^{abc}	69.12^{bcd}	$18.65^{abcdefg}$
19	VT 1325	0.74^{fghij}	0.99^{k}	68.90^{ab}	60.03^{fg}	12.82^{i}
20	VT 1326	0.77^{efghi}	1.13^{hijk}	67.30^{abcd}	62.12^{efg}	15.47^{efghi}
21	VT 1327	0.73^{fghij}	1.20^{ghij}	64.11^{bcdef}	58.50^{g}	16.23^{defghi}
22	VT 1328	0.89^{cdef}	1.29^{fgh}	65.19^{abcdef}	72.52^{ab}	15.33^{efghi}
CD (5%)		0.143	0.145	4.853	4.843	3.97

Means in a column with the same letter are not significantly different (P≥0.05)

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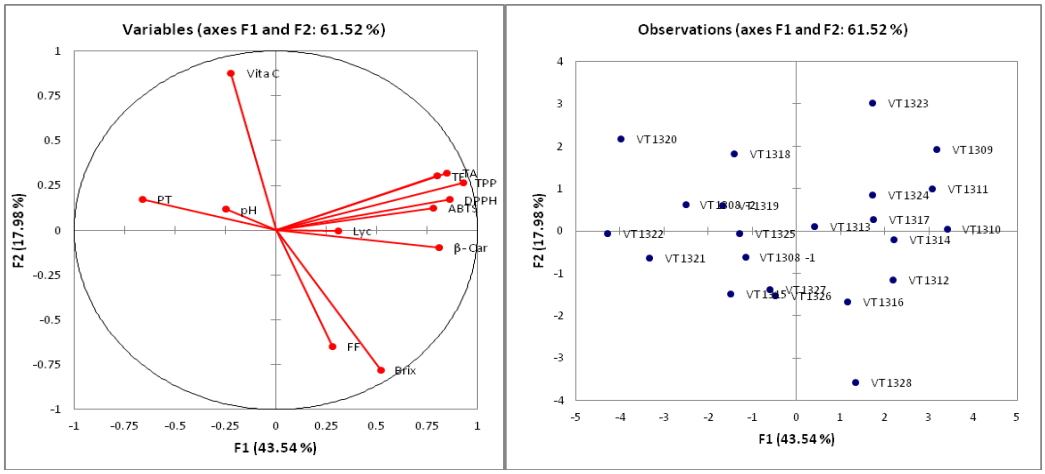

Fig. 1 a. F1:F2 biplot showing relationship among traits and lines

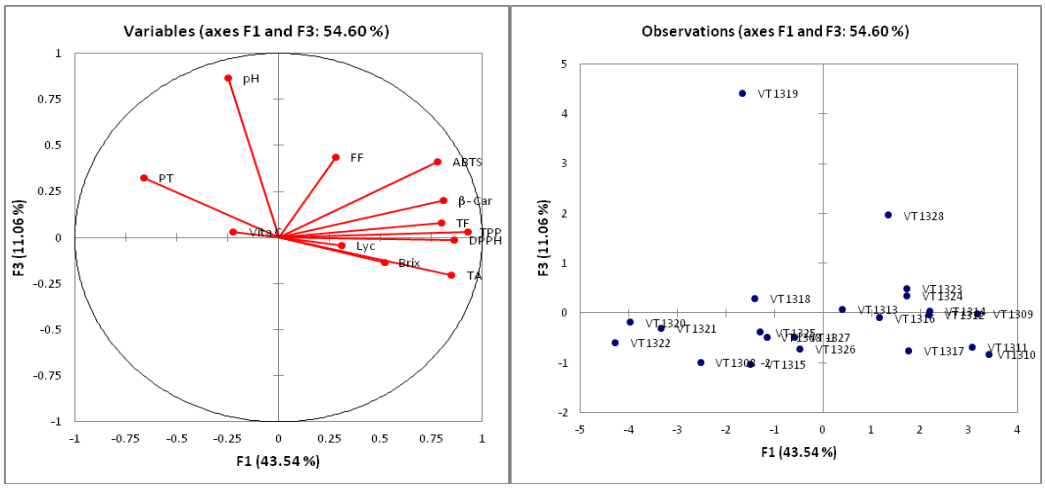

Fig. 1 b. F1:F3 biplot showing relationship of pH and PT with other traits and lines

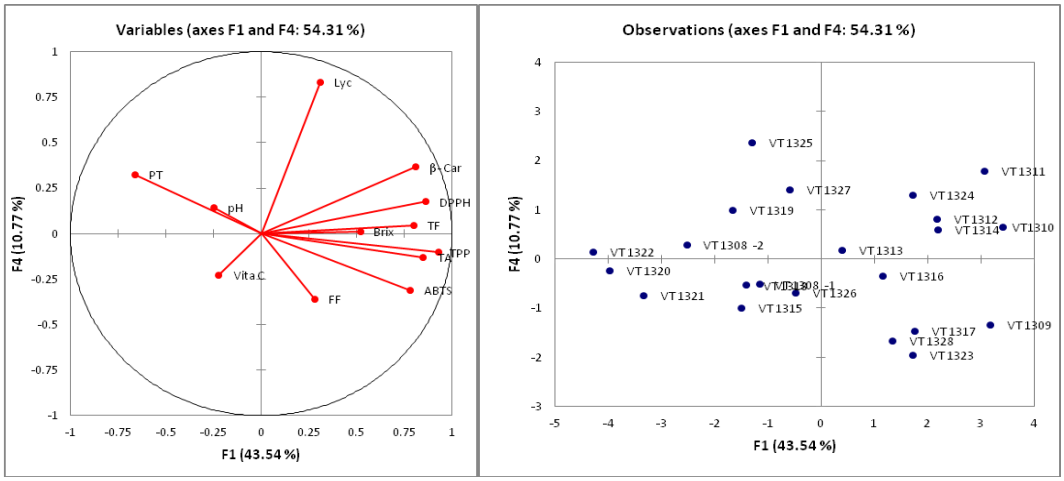

Fig. 1 c. F1:F4 biplot showing relationship of lycopene with other traits and lines

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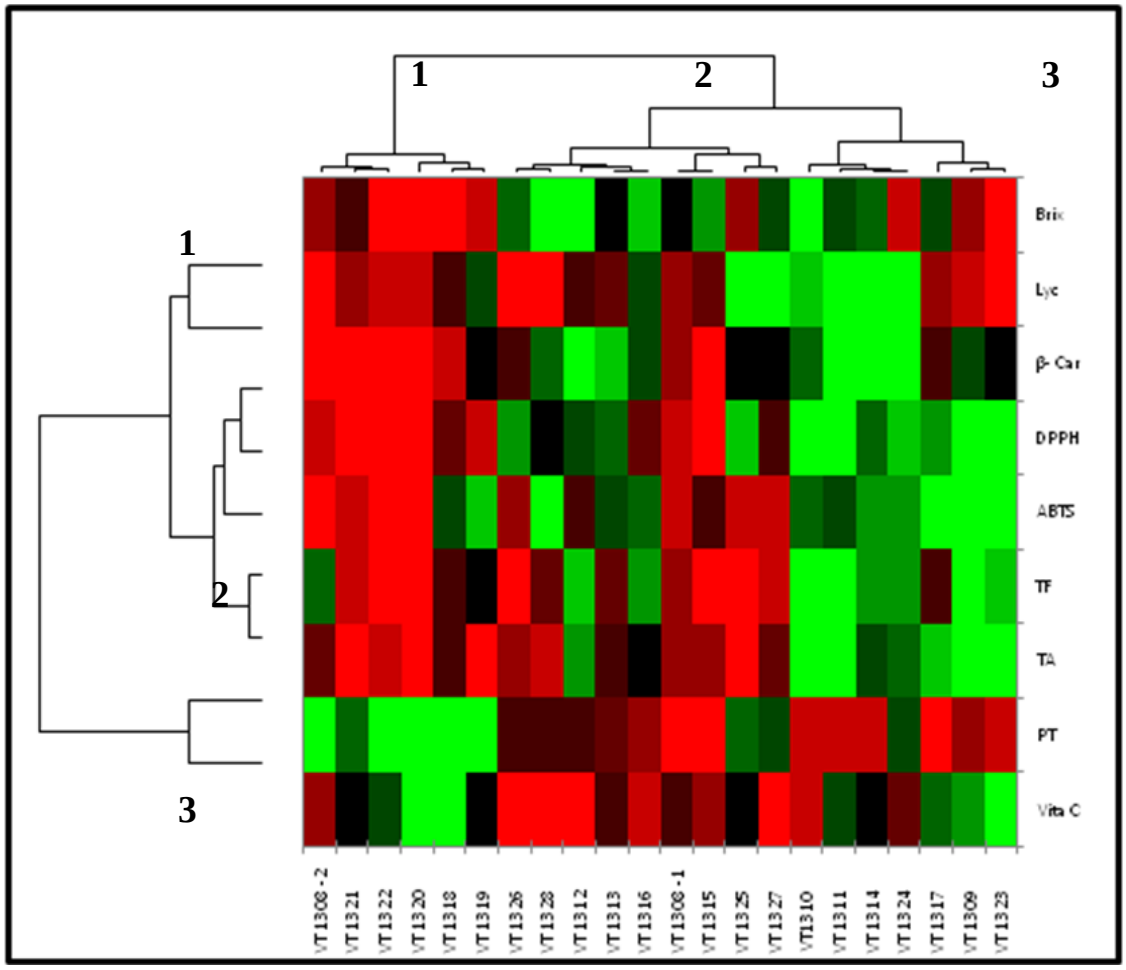

Fig. 2. Heat map showing trait (left dendrogram) and line (top dendrogram) clustering and expression pattern of lines