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Ananya Sehanabish
Department of Vegetable Science, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Sourav Roy
Department of Vegetable Science, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Pramit Pandit
Department of Agricultural Statistics, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Upasana Mohapatra
Department of Plant Biotechnology, University of Agricultural Sciences, Bengaluru, Karnataka, India

Pranab Hazra
Department of Vegetable Science, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Correspondence
Pramit Pandit
Department of Agricultural Statistics, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Study on evaluation, characterization and inter-relationships of the novel breeding lines-"purple tomato" for different bio-chemical traits and reaction to tomato leaf curl virus

Ananya Sehanabish, Sourav Roy, Pramit Pandit, Upasana Mohapatra and Pranab Hazra

Abstract
The experiments were carried out at Central Research Farm, Gayeshpur, Bidhan Chandra Krishi Viswavidyalaya, during autumn-winter season of 2017-18 under open field condition to characterize and evaluate the 9 lines of purple tomato along with its two parents and two check genotypes for fruit quality characters with a view to explore the feasibility of commercialization of this nutritionally rich genotypes of tomato and recording TLCV incidence. From the study, wide variation in the traits suggests segregation in different characters in the lines which needs to be stabilized through advancement of generations. Analysis of Variance clearly showed the presence of wide genetic variability for the character concerned among the genotypes. Characters with high Coefficient of variation for instance chlorophyll content suggests comparatively higher influence of the environment for the expression of these characters and vice versa. Lycopene content of purple tomato BCPT 7-4/2-1 was higher than the parental line, BCT 115 *dg*. Anthocyanin content of purple tomato BCPT 7-4/2-2 was higher than the parental line, Alisa Craig *Aft* possessing anthocyanin fruit gene "*Aft*", suggested full expression of this gene in other genetic back ground. On the basis of correlation studies TSS, ascorbic acid and lycopene contents of fruit would be effective for developing plants with higher fruit yield and enhanced quality in tomato.

Keywords: Purple tomato, check genotypes, parental line, anthocyanin

Introduction
Among the many crop improvement works related to yield and yield enhancing traits, recently improvement of food quality has gained prime importance. However, malnutrition still remains a global health problem, including developed countries (e.g. obesity). With this context, tomato is of utmost importance as this versatile vegetable is a rich source of potassium, folate, vitamin E, vitamin A, vitamin B, soluble and insoluble dietary fibers, lycopene and Ascorbic acid (George *et al*., 2004) [5] and grown in almost every corner of the world. There is considerable interest in the dietary role of lycopene in inhibition of heart disease and reducing the risk of certain cancers including prostate (Ansari and Gupta, 2003; Wu *et al*., 2004; Stacewicz-Sapuntzakis and Bowen, 2005) [1, 10, 9], breast (Sesso *et al*., 2005) [8] and other cancers (Bowen-Forbes *et al*., 2010). Other carotenoids present in ripe tomato fruits are β-carotene, phytofluene, phytoene, lutein, neorosporene, d-carotene and z-carotene (Biswas *et al*., 2016) [2]. However, tomato fruits do not usually produce anthocyanins unlike the fruits of the other members of Solanaceae family due to lack of expression of the *chalcone isomerase* (*CH1*) gene in the flavonoid biosynthetic pathway in the peel of the tomato fruit. Having this context, nine novel breeding lines "Purple Tomato" rich in both red carotenoid pigment "lycopene" and purple-violet "anthocyanin" pigment have been developed by introgressing two specific genes: Lycopene enhancing "*dg*" presenent in chromosome 1 of the genotype BCT-115 *dg* and Anthocyanin fruit gene "*Aft*" in chromosome 10 of the genotype Alisa Craig in one genotype (*Aft Aft*/ *dg dg*) following conventional breeding method. In this breeding programme, only 9 segregates bred true with respect to dark green fruit coupled with purple anthocyanin pigmentation which established the presence of two specific genes in one genotype, "Purple tomato" (Li *et al*., 2018) [6]. Hence, the "Purple tomato" line is comprised of 9 segregating lines emanated from the same breeding scheme. With the introgression of two specific genes, lycopene and anthocyanin contents have somewhat been stabilized in those lines however, for different quantitative characters including growth characters and fruit yield components and other proximate compositions, the lines are still segregating. Such breeding

Materials and Methods

The field experiments were carried out at Central Research Farm, Gayeshpur, Bidhan Chandra Krishi Viswavidyalaya (B.C.K.V.), Nadia, West Bengal during autumn-winter season. Topographic situation of the experimental site comes under Gangetic new alluvial plains of West Bengal with sandy loam soil. Nine F_5 lines of purple tomato viz. BCPT 7-4/2-2, BCPT 7-4/2-1, BCPT 7-4-4, BCPT 7-4-3, BCPT 7-4-2, BCPT 7-4-1, BCPT 7-1-3, BCPT 7-1-2, BCPT 7-1-1, two parental lines of purple tomato viz. Alisa Craig *Aft* & BCT-115 *dg* and two check genotypes Arka Rakshak (multiple disease resistant hybrid) and Patharkuchi (popular land race) maintained at the Department of Vegetable Science, B.C.K.V. constituted the genotypes for the investigation.

The 13 genotypes were evaluated considering different fruit quality traits and reaction to diseases. The genotypes were grown in randomized block design with 3 replications under autumn - winter season (planting in mid of October) keeping 30 plants in each replication in 60 x 60 spacing in both ways to study the expression of different characters in them. Five random plants per replication in each genotype were selected for recording the data on different characters. The seeds of all the genotypes, were sown on 25th September, 2017 in raised bed nursery. The seedlings were transplanted in the main field on 30 October, 2017.

Three randomly selected immature fruit at green stage before turning to particular colour were harvested to make a composite sample for estimation of total chlorophyll content of the fruit. The other proximate compositions of the fruits were estimated from the replication-wise composite samples in both matured green and respective coloured stage following standard methods. The quality traits under study were- Total chlorophyll contents of leaves (mg/100g fresh pulp), Total chlorophyll contents of immature fruits (mg/100g fresh pulp), TSS content, Total sugar content (%), Reducing sugar content (%), Lycopene content (mg/100g fresh pulp), titrable acidity (%), β-carotene content (mg/100g fresh pulp), Ascorbic acid content (mg/100g fresh pulp).

The 13 genotypes were grown in autumn-winter season in randomized block design with 3 replications keeping 20 plants each for the genotype. Standard agronomic practices were followed however the plants were not sprayed with any pesticide. The genotypes were evaluated for incidence of tomato leaf curl virus disease. The disease incidence was recorded from all the plants in the block by visual evaluation of disease symptom for single plant after 60 days transplanting which was then expressed as percentage.

Mean values of each entry in each replication for all the traits along with other experimental data were statistically analyzed. For analysis of variance, data were analyzed by the methods out lined by Panse and Sukhatme (1967) using the mean values of random plants in each replication from all treatments to find out the significance of treatment effect. The significance was tested by referring to the values of F table (Fisher and Yates, 1967). Correlation is a measure of linear association between two variables, worked as per the following formula.

$$r(X_1, X_2) = \frac{Cov(X_1, X_2)}{\sqrt{V(X_1).V(X_2)}}$$

Where, r (X_1, X_2) = correlation between X_1, X_2

V (X_1) = variance of X_1

V (X_2) = variance of X_2

Results and Discussions

Results presented in Table 1 clearly suggested significant difference among the 13 genotypes of tomato (9 purple tomato lines, 2 parental lines and 2 check variety /hybrid) for all the quality characters excepting total chlorophyll content of immature fruit even at 1 percent level of significance which clearly endorsed the presence of wide genetic variability for the character concerned among the genotypes. This analysis also justified the utilization of these genotypes for such evaluation study.

Table 1: Analysis of variance for different fruit quality traits and reaction to disease of the lines of purple tomato and other genotypes

Character	Mean sum of squares		
	Genotypes	Replication	Error
Reaction to TLCV	134.335**	0.243	11.914
Total chlorophyll content of leaf	403.611**	7.038	21.384
Total chlorophyll content of fruit	480.922	487.923	519.579
TSS content	1.092**	0.085	0.061
Total sugar content	0.524**	0.147	0.021
Reducing sugar content	0.176**	0.026	0.016
Titratable acidity	0.034**	0.004	0.003
Ascorbic acid content	62.608**	0.002	2.275
Lycopene content	3.729**	0.304	0.158
β carotene content	0.036**	0.006	0.005
Anthocyanin content	196.503**	1.239	1.259

** Significant at 1 percent level, *Significant at 5 percent level

Coefficient of variation expressed as percentage ratio of the standard deviation to the corresponding mean of the concerned characters has been presented in the Table 2 and Table 3. Coefficient of variation was low, below 10 percent for 21 characters indicating minimum influence of environment for the expression of these characters. Coefficient of variation was moderate for 2 characters viz., titratable acidity (11.94 percent) and β carotene content of fruit (12.11 percent) and high (21.73 percent) for total chlorophyll content of fruit suggesting comparatively higher influence of environment for the expression of these characters. Range for the different fruit quality traits of purple tomato lines are discussed character wise.

Table 2: Mean fruit quality traits of the lines of purple tomato and other genotypes

Genotypes	Traits					
Purple tomato	Leaf chlorophyll	Fruit chlorophyll	TSS	Total sugar	Reducing sugar	Titratable acidity
BCPT 7-4/2-2	88.897	20.187	5.577	2.917	1.877	0.410
BCPT 7-4/2-1	88.887	18.310	4.250	2.017	1.727	0.450
BCPT 7-4-4	86.647	17.927	4.623	2.067	1.723	0.423
BCPT 7-4-3	70.300	9.863	4.637	1.363	1.317	0.593
BCPT 7-4-2	85.627	15.350	3.777	1.533	1.277	0.287
BCPT 7-4-1	71.390	11.567	3.723	1.547	1.573	0.310
BCPT 7-1-3	65.187	9.443	5.130	2.083	2.013	0.437
BCPT 7-1-2	66.683	18.037	4.450	1.730	1.863	0.354

BCPT 7-1-1	87.570	16.767	5.090	2.260	1.770	0.457
Alisa Craig *Aft* (Parent)	68.813	11.760	4.137	1.930	1.543	0.470
BCT 115*dg* (Parent)	104.257	25.537	5.187	2.403	2.020	0.480
Arka Rakshak (Check: Hybrid)	73.400	12.017	5.147	1.907	1.960	0.297
Patharkuchi (Check: Cultivar)	81.110	16.657	5.353	2.313	1.837	0.643
Cv (%)	5.787	21.734	5.259	7.263	7.331	11.948
S.E. M	2.670	7.160	0.143	0.084	0.073	0.030
S.E. d	3.776	10.09	0.202	0.119	0.104	0.042
C.D. at 5%	7.839	20.94	0.419	0.247	0.215	0.087

Table 3: Mean fruit quality traits & incidence of TLCV of the lines of purple tomato and other genotypes

Genotypes	**Traits**				
Purple tomato	**Ascorbic acid**	**Lycopene**	**βcarotene**	**Anthocyanin**	**Incidence of TLCV (%)**
BCPT 7-4/2-2	24.650	4.857	0.583	20.920	43.158
BCPT 7-4/2-1	24.037	5.513	0.707	16.713	43.108
BCPT 7-4-4	26.337	4.937	0.663	15.357	39.600
BCPT 7-4-3	19.727	2.827	0.517	23.637	36.432
BCPT 7-4-2	24.673	5.137	0.677	17.813	39.579
BCPT 7-4-1	22.280	2.773	0.633	9.040	41.040
BCPT 7-1-3	16.973	2.717	0.587	9.793	33.542
BCPT 7-1-2	17.023	4.300	0.657	9.783	39.640
BCPT 7-1-1	24.487	4.340	0.470	17.400	34.171
Alisa Craig *Aft* (Parent)	22.703	3.293	0.620	18.783	45.769
BCT 115*dg* (Parent)	33.703	5.490	0.637	0.00	46.989
Arka Rakshak (Check: Hybrid)	17.760	2.563	0.293	0.00	21.612
Patharkuchi (Check: Cultivar)	25.267	3.540	0.580	0.00	33.294
Cv (%)	6.544	9.898	12.108	9.109	9.012
S.E.M	0.871	0.230	0.041	0.648	1.993
S. E. d	1.232	0.325	0.058	0.916	2.818
C.D. at 5%	2.557	0.675	0.120	1.902	5.851

Total chlorophyll content of leaf (mg/ 100 g fresh)

Total chlorophyll content of leaf ranged between 70.30 mg/100g fresh in BCPT 7-4-3 and 88.89 mg/100g fresh in BCPT 7-4/2-2 among the purple tomato lines. Average total chlorophyll content in the leaves of the purple tomato lines was lower than the dark green fruited parent, BCT-115 *dg* and much higher than the other parent, Alisa Craig *Aft* and the check variety/ hybrid.

Total chlorophyll content of immature fruit (mg/ 100 g fresh)

Total chlorophyll content of immature fruit ranged between 9.44 mg/100g fresh pulp in BCPT 7-1-3 and 18.03 mg/100g fresh pulp in BCPT 7-1-2 among the purple tomato lines. Average total chlorophyll content in the leaves of the purple tomato lines was lower than the dark green fruited parent, BCT-115 *dg* and much higher than the other parent, Alisa Craig *Aft* and the check variety/ hybrid.

Total soluble solids content (^{O} Brix)

Total soluble solids content in the ripe fruits ranged between 3.72 ^{O} Brix in BCPT 7-4-1 and 5.57 ^{O} Brix in BCPT 7-4/2-2 among the purple tomato lines. Average total soluble solids content of the purple tomato lines was lesser than the parental average as well as the check variety/ hybrid.

Total sugar content (%)

Total sugar content in the ripe fruits ranged between 1.36 % in BCPT 7-4-3 and 2.92% in BCPT 7-4/2-2 among the purple tomato lines. Average total sugar content of the purple tomato lines was slightly lesser than the parental average but it was higher than the check variety/ hybrid.

Reducing sugar content (%)

Reducing sugar content in the ripe fruits ranged between 1.27% in BCPT 7-4-2 and 2.01% in BCPT 7-1-3 among the purple tomato lines. Average reducing sugar content of the purple tomato lines was slightly lesser than the parental average but it was higher than the check variety/ hybrid.

Titratable acidity (%)

Titratable acidity in the ripe fruits ranged widely between 0.287 % in BCPT 7-4-2 and 0.593 % in BCPT 7-4-3 among the purple tomato lines. Average titratable acidity of the purple tomato lines was slightly lesser than the parental average and the check variety, Patharkutchi but it was higher than the check hybrid, Arka Rakshak.

Ascorbic acid content (mg/100g fresh pulp)

Ascorbic acid content in the fruits ranged between 16.97 mg/100g fresh pulp in BCPT 7-1-3 and 24.67 mg/100g fresh pulp in BCPT 7-4-2 among the purple tomato lines. Average ascorbic acid content of the purple tomato lines was slightly lesser than the parental average and the check variety, Patharkutchi but it was much higher than the check hybrid, Arka Rakshak.

Lycopene content (mg/100g fresh pulp)

Lycopene content in the ripe fruits ranged very widely between 2.72 mg/100g fresh pulp in BCPT 7-1-3 and 5.15 mg/100g fresh pulp in BCPT 7-4/2-1 among the purple tomato lines. Lycopene content of purple tomato line BCPT 7-4/2-1 was higher than the parental line, BCT-115 *dg* possessing lycopene enhancing mutant gene "*dg*" which clearly suggested full expression of this gene in other genetic back ground. However, average lycopene content of the purple tomato lines was slightly lesser than the BCT-115 *dg* parent but much higher than the other parent Alisa Craig *Aft* and the check variety, Patharkutchi and check hybrid, Arka Rakshak.

β carotene content (mg/100g fresh pulp)

β carotene content in the ripe fruits ranged widely between 0.47 mg/100g fresh pulp in BCPT 7-1-1 and 0.707 mg/100g fresh pulp in BCPT 7-4/2-1 among the purple tomato lines. Average β carotene content of the purple tomato lines was almost at par with that of the parental average as well as the check variety, Patharkutchi and the check hybrid, Arka Rakshak.

Anthocyanin content (mg/100g fresh pulp)

Anthocyanin content in the ripe fruits ranged very widely between 9.04 mg/100g fresh pulp in BCPT 7-4-1 and 20.92 mg/100g fresh pulp in BCPT 7-4/2-2 among the purple tomato lines which clearly indicated the presence of *Anthocyanin fruit gene* (*Aft*) in all the purple tomato lines although with widely varied expression with the genotype. Anthocyanin content of purple tomato line BCPT 7-4/2-2 was higher than the parental line, Alisa Craig *Aft* possessing anthocyanin fruit gene "*Aft*" which clearly suggested full expression of this gene in other genetic back ground. The check variety and hybrid did not produce any anthocyanin in the fruit because of the absence of the specific gene *Aft* in them.

Incidences of tomato leaf curl virus disease (%)

Incidence of tomato leaf curl virus disease after 60 days after transplanting ranged between 33.54 % in BCPT 7-1-3 and 43.16 % in BCPT 7-4/2-2 among the purple tomato lines. Average incidence of tomato leaf curl virus disease in the purple tomato lines was almost at par with that of the parental genotype but conspicuously higher than the triple resistant tomato hybrid, Arka Rakshak.

Information generated from the studies of character association serve as the most important indicator (plant character) that ought to be considered in the selection programme. Such studies would also help us to know the suitability of multiple characters for indirect selection, because selection for one or more traits results in correlated response in several other traits (Searle, 1965). Simple correlation coefficients presented in Table 4 clearly suggested that total soluble solids content registered significantly positive correlations with total sugar content (r = 0.763) and reducing sugar content (r = 0.713) and its correlation with tritrable acidity was also high and positive (r = 424). Total sugar content of fruit was also positively correlated with ascorbic acid content (r = 0.454) and lycopene content (r = 0.391).

On the basis of correlation studies total soluble solids content, ascorbic acid and lycopene contents of fruit would be effective for developing tomato plants with higher fruit yield and enhanced fruit quality.

Table 4: Correlations among different physiological and fruit quality characters of tomato

	LCHRPHYL	FCHRPHYL	TSS	TOT SUG	RED SUG	TITAC	AA	LYCPN	β-CARTN	ANTHCYN
LCHRPHYL	1	0.010	0.273	0.551	0.181	0.108	0.902**	0.814**	0.215	-0.088
FCHRPHYL		1	0.005	0.048	0.287	-0.169	-0.070	0.377	0.297	-0.157
TSS			1	0.763**	0.713**	0.424	0.124	-0.011	-0.480	-0.315
TOT SUS				1	0.675*	0.231	0.454	0.391	-0.038	-0.154
RED SUG					1	0.018	0.051	0.047	-0.238	-0.637*
TITAC						1	0.245	-0.059	0.037	0.032
AA							1	0.684**	0.362	-0.118
LYCPN								1	0.570*	0.173
β-CARTN									1	0.258
ANTHCYN										1

LCHRPHYL= Total Leaf Chlorophyll, FCHRPHYL= Total Fruit Chlorophyll, TSS= Total Soluble Solids, TOT SUG = Total Sugar, RED SUG = Reducing Sugar, TITAC= Titatable Acidity, AA= Ascorbic Acid, LYCPN= Lycopene, β-CARTN= β-Carotene, ANTHCYN= Anthocyan

Wide variation could be recorded among the "Purple tomato" genotypes with respect to the parental genotype and check variety / hybrid. It could be concluded that Purple tomato lines can very well be placed as a line bred variety for commercial cultivation in competition with other line bred variety and even hybrids. Purple tomato line significantly outclassed the popular hybrid and cultivar with respect to all the fruit quality parameters. Incidence of tomato leaf curl virus disease in purple tomato lines was comparatively higher than the check variety as well as resistant hybrid. It was not entirely unexpected because this breeding line has been developed employing two parental genotypes of European origin which were also showed comparatively high incidence of tomato leaf curl virus disease. The purple tomato line possessing enhanced nutritional quality need to be cultivated under full protection from white fly infestation which transmit the virus. In line with the findings of Biswas *et al.* (2016) [2], it can be concluded that selection for total soluble solids content, ascorbic acid and lycopene contents of fruit would be effective for developing plants with higher fruit yield and enhanced quality in tomato.

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