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Assessment of genetic variability among antioxidant constituents in Husk tomato (*Physalis ixocarpa* Brot.) selections grown in temperate region

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Abstract
The current study was carried out at experimental farm of ICAR-Central Institute of Temperate Horticulture, Srinagar during year 2011 and 2012. In this investigation a total ten selections of Husk tomato were screened for chlorophyll, ascorbic acid, phenolic, flavonoids, anthocyanins attributes and antioxidant activities (DPPH). Significant differences among antioxidant attributes were detected in selected husk tomato selections. The range ofchlorophyll a of the tested samples was (1.00-3.28 mg/100g), chlorophyll b (1.03-2.46 mg/100g) and total chlorophyll (2.05-5.17 mg/100g). DPPH % inhibition varied between 42.54 and 84.65 %; however, total anthocyanins ranged between (1.23 and 5.65) mgcyanidin-3-glucoside Eq·100 g–1 fresh weight. Total phenols varied from (15.65-20.84) mg gallic acid Eq·100 g–1 and total flavonoids from (20.18-25.93) mg catechin Eq·100 g^{-1}. Total phenols and DPPH showed close association; however, PCA revealed that the first PC explained 35% of total variation and was positively associated to Chlorophyll a, chlorophyll b, total chlorophyll, total antocyanin, total flavonols and ascorbic acid. All of the diverse cultivars were clustered into two clusters which could be exploited for future qualitative breeding programs in husk tomato. The importance of our findings would be significant for farmers, breeders, consumers and industries concerning food quality, disease prevention and healthcare.

Keywords: Husk tomato, genetic variability, antioxidant, temperate

1. Introduction
The tomatillo or *Physalis ixocarpa* Brot. (2n = 2x = 24), known as, Mexican husk tomato belongs to night shade family. It is also popular as green tomato, berry compote, miltomate or jamberry. The unripe fruit is a bit tart, slightly sweet, earthy, with a hint of citrus and is the key ingredient for Mexican table chili sauces known as salsa verde (green sauce). Fully ripe fruits are eaten raw, like tomatoes or it can be dried like raisins. It is native to Mexico where different types and varieties are cultivated, with significant variability in berry size, colour and flavour (Singh *et al*., 2013) [20]. The fruits have tremendous nutritional and health benefits and can be eaten raw, as a dessert, and appetizer or used as dish decorator. It is rich in vitamin A, B, B_2, C and polyphenols (Gonzalez-Mendoza *et al*., 2010 [8]; Brazanti and Monaresi 1980 [3]; Sarangi *et al*., 1989) [18]. In recent times, awareness about health consciousness among the consumers related to intake of health promoting substances have risen very fast and also the demand of antioxidant rich fruits increased significantly worldwide. Furthermore, recent scientific evidences pointed out the importance of health promoting compounds in husk tomato in relation to their high level of antioxidants including vitamin C and phenolic compounds. Many researchers also reported that ripe fruit have significant antioxidant properties and can be used as functional foods (Medina-Medrano *et al*., 2015 [14], González-Mendoza *et al*., 2010 [8]). However, the consumption of husk tomato mainly limited to the Western and Central regions of Mexico (Santiaguillo *et al*., 1994) [17]. In general this crop is newly introduced to India because of its wide range of adaptation and versatile use as table purpose and processing form and increasing demand in exotic fruit market gives good prospects for the expansion of husk tomato particular in temperate region. Besides this, the information on the antioxidant constituents of fruit is very limited. Considering this the research investigation was initiated to estimate the various antioxidant constitutens in different selections of husk tomato. The information would be useful in increasing the awareness of the consumers regarding the level of beneficial antioxidant constituents present in this nutritious crop.

Materials and Methods

The current investigation was conducted at the research farm of ICAR-Central Institute of Temperate Horticulture (CITH), Srinagar, Jammu and Kashmir, India. The experimental farm is situated at 34° 05 N latitude and 74° 50 E longitudes at an altitude of 1640 m above the mean sea level. A complete randomized block design (CRBD) replicated three times and average data of two years were analyzed as per the method suggested by (Gomez and Gomez, 1994) [7]. Plants from nursery were transplanted during first week of May at a spacing of 30 x 30 cm and no training and pruning was done. Recommended production practices were followed for raising healthy crop. Fruits were harvested at horticultural maturity during (August) 2011 & 2012 from randomly selected plants to represent the population of the plantation. Randomly collected fruits of husk tomato were brought to labs and squeezed manually under ice, filtered with a sieve and centrifuged for 5 min at 500 g. The method of Anderson and Boardman (1964) [2] was followed for the estimation of chlorophylls. The phenol content was measured by the Folin-Ciocalteau reagent (Aaby *et al*., 2005) [1] using gallic acid as standard. DPPH scavenging activity% measures in terms of DPPH (2, 2-diphenyl-1-picrylhydrazyl) was determined according to the method used by Yen and Chen (1995) [23] with some modifications. A 1mL aliquot of husk tomato juice was diluted 200 times and then 3 mL of ethanol (96%) and 1 mL of DPPH (0.012 g DPPH·100 mL) were added. The mixture was shaken and left at room temperature for 10 min; the absorbance was measured spectrophotometrically at 517 nm and reported as mg ascorbic acid equivalent (AAE)·100 g fresh weight. The total flavonoids content was determined using a colorimetric method (Kim *et al*., 2003) [12]. Ascorbic acid contents of fresh fruit were determined spectrophotometrically by metaphosphoric acid extraction of 2,6-dichlorophenol indophenol dye (Robinson *et al*., 1945) [16]. Anthocyanins were determined according to (Shin *et al*., 2008) [19] with some modifications. Total anthocyanins content was calculated using the extinction coefficient (ε) equal to 3.6 x 106 mol-1 m-1 and expressed as mg cyanidin-3-hydrochloride equivalent kg-1FW. Data were subjected to analysis of variance (ANOVA) as per Gomez and Gomez (1994) [7]. Correlations were calculated on a genotype mean basis, according to Pearson's test. Differences at $p < 0.05$ were considered to be statistically significant. The PCA produced eigenvectors andprincipal component scores that were used, respectively, to measure the relative discriminative power of the axes and their associated characters; a dendrogram was constructed using the Ward method. The distance is expressed as average cluster distance. All the statistical analysis was carried out using SAS 9.2 software.

Results and Discussion

The results of 10 husk tomato selections with respect to different antioxidant constituents are presented in the table 1. The perusal of the data revealed significant differences in all the parameters among different selections undertaken for the study. The chlorophyll a was observed to have a maximum content of 3.28mg/100g in CITH-SEL-2 and minimum (1.00mg/100g) in CITH-SEL-3 which was *at par* with CITH-SEL-1, CITH-SEL-5, CITH-SEL-6, CITH-SEL-7, CITH-SEL-8, CITH-SEL-9. Among selections highest (2.45mg/100g) chlorophyll b content was recorded in CITH-SEL-5 whereas, lowest (1.03mg/100g) in CITH-SEL-1 which was statistically similar with all the remaining selections. The maximum DPPH % inhibition of 84.65% was observed in CITH-SEL-7 and minimum (42.54%) in CITH-SEL-5. The genotype CITH-SEL-6 recorded highest total anthocyanin content of 5.65mg cyanidin-3-glucoside equivalents/100g on fresh weight basis whereas, CITH-SEL-10 had the lowest content of 1.23mg cyanidin-3-glucoside equivalents/100g. The maximum content of 5.17mg/100g of total chlorophyll was found in CITH-SEL-2 and minimum (2.05mg/100g) in CITH-SEL-1 which was *at par* with CITH-SEL-3 and CITH-SEL-9. The genotype CITH-SEL-7 recorded highest total phenol content of 20.84mg GA equivalents/100g whereas, CITH-SEL-5 had the lowest (15.65mg GA equivalents/100g). The total phenolic concentrations observed in current study was comparable to those reported by Wang and Lin (2000) [22] and Medina-Medrano *et al*. 2015 [13]. The total flavonols were found to be maximum (25.93mg catechin equivalents/100g) in CITH-SEL-4 and minimum (20.18mg catechin equivalents/100g) in CITH-SEL-6. The ascorbic acid is one of the main nutrients in fruits whose contribution to human diet depends exclusively on these sources, besides vitamin C has an important role in human nutrition because it is an antioxidant that contributes to human health and is credited with strengthening the body in defense of cardiovascular diseases (Carr and Frei, 1999) [4]. Among the series of selections highest ascorbic acid content of 23.27mg/100g on fresh weight basis was found in CITH-SEL-10 and lowest (16.27mg/100g) in CITH-SEL-1 which was at par with CITH-SEL-3. Similar values have been observed in different plants such as roselle of *Hibiscus sabdariffa* (Galicia- Flores *et al*., 2008) [5], *Camellia sinensis* Linn (Khalaf *et al*., 2008) [11] and Tomatillo (González-Mendoza *et al*, 2010) [7]. Taking into account the descriptive statistical analysis for various traits given in table-2 revealed highest standard deviation in DPPH % inhibition followed by ascorbic acid, total flavonols, total phenols, total anthocyanin and total chlorophyll and lowest in chlorophyll b followed by chlorophyll a. Coefficient of variation was found to be maximum in chlorophyl a followed by total anthocyanin and total chlorophyll and minimum in total flavonols followed by total phenols and ascorbic acid. Antioxidant and polyphenol content analysis based on different traits showed the high genetic divergence of the 10 husk tomato cultivars, which could be exploited for future qualitative breeding programs and as a source for making health foods or as raw.

The results of correlation among different antioxidant traits have been presented in the table 3. Highly significant positive correlation has been found between total phenols and DPPH (% inhibition) followed by total chlorophyll and chlorophyll b whereas, significant negative correlation was observed between total phenols and chlorophyll b. Significant positive correlation was found between total chlorophyll and chlorophyll a.

Table 1: Mean performance of 10 husk tomato selections evaluated under temperate condition in relation to different antioxidant attributes.

Genotypes	Chlorophyll a (mg/100g)	Chlorophyll b (mg/100g)	DPPH (% inhibition)	Total anthocyanin (mg cyanidin-3-glucoside equivalents 100 g_1 fw	Total Chlorophyll (mg/100g)	Total Phenols (mg GA equivalents/100 g)	Total Flavonols mg catechin equivalents /100 g)	Ascorbic Acid (mg/100 g of fresh weight)
CITH-SEL-1	1.01	1.03	78.63	2.13	2.05	20.66	22.21	16.27
CITH-SEL-2	3.28	1.55	64.07	3.23	5.17	20.55	23.02	18.19
CITH-SEL-3	1.00	1.27	52.95	2.95	2.27	20.15	22.03	16.64
CITH-SEL-4	2.16	1.57	59.85	3.63	4.07	20.51	25.93	17.45
CITH-SEL-5	1.60	2.45	42.54	4.55	4.39	15.65	21.77	18.19
CITH-SEL-6	1.32	1.28	45.66	5.65	2.61	17.45	20.18	19.08
CITH-SEL-7	1.31	1.51	84.65	4.35	3.16	20.84	23.45	18.51
CITH-SEL-8	1.56	1.66	66.23	3.55	3.56	20.62	22.63	18.07
CITH-SEL-9	1.07	1.27	52.61	2.63	2.27	20.07	20.43	21.20
CITH-SEL-10	2.16	1.57	48.96	1.23	4.07	19.35	24.72	23.27
CD at 5%	0.75	0.68	3.10	0.60	0.48	0.07	0.08	0.50

Table 2: Genetic variability among antioxidant attributes in 10 husk tomato selections.

Characters	Range	Mean	Standard Deviation	Coefficient of Variation (%)
Chlorophyll a (mg/100g)	1.00-3.28	1.65±0.23	0.71	43.03
Chlorophyll b (mg/100g)	1.03-2.45	1.52±0.12	0.38	25.00
DPPH (% inhibition)	42.54-84.65	59.61±4.40	13.93	23.37
Total anthocyanin (mg cyanidin-3-glucoside equivalents 100 g_1 fw)	1.23-5.65	3.39±0.40	1.27	37.46
Total Chlorophyll (mg/100g)	2.05-5.17	3.36±0.33	1.06	31.55
Total Phenols (mg GA equivalents/100 g)	15.65-20.84	19.59±0.54	1.71	8.37
Total Flavonols mg catechin equivalents /100 g)	20.18-25.93	22.64±0.56	1.77	7.82
Ascorbic Acid (mg/100 g of fresh weight)	16.27-23.27	18.69±0.67	2.11	11.29

Table 3: Pearson correlation coefficients among various traits studied for 10 husk tomato selections.

Characters	Chlorophyll a (mg/100g)	Chlorophyll b (mg/100g)	DPPH (% inhibition)	Total anthocyanin (mg cyanidin-3-glucoside equivalents 100 g_1 fw)	Total Chlorophyll (mg/100g)	Total Phenols (mg GA equivalents/100 g)	Total Flavonols mg catechin equivalents /100 g)	Ascorbic Acid (mg/100 g of fresh weight)
Chlorophyll a (mg/100g)	1	.304	-.061	-.104	.898**	.088	.518	.172
Chlorophyll b (mg/100g)		1	-.379	.313	.682*	-.637*	.159	.080
DPPH (% inhibition)			1	-.123	-.152	.727*	.275	-.404
Total anthocyanin (mg cyanidin-3-glucoside equivalents 100 g_1 fw)				1	.053	-.483	-.349	-.334
Total Chlorophyll (mg/100g)					1	-.173	.529	.161
Total Phenols (mg GA equivalents/100 g)						1	.394	-.154
Total Flavonols mg catechin equivalents /100 g)							1	.040
Ascorbic Acid (mg/100 g of fresh weight)								1

**. Correlation is significant at the 0.01 level.

*. Correlation is significant at the 0.05 level.

Highly positive correlations between total phenols and total antioxidantactivity (DPPH, FRAP) were also reported in blueberries (Giovanelli *et al*., 2009 [6]; Kalt *et al*., 1999 [10]; Prior *et al*., 1998 [15]; Heinonen *et al*., 1998) [9] and in strawberry (Lal *et al*., 2013) [13] which confirmed our findings. The high positive correlation among differentpairs can be helpful in breeding for furtherimprovement of cultivars lacking inantioxidant compounds.

The dendrogram generated from the linkage cluster analysis based on average distance, classified 10 husk tomatoselections into two major groups (Fig. 1) at normalized root mean square (NRMS) distance 1.43. The first group consisted of only two selections that contributed 20% of the total selections in this population, and further this group categorized in to two clusters and in each cluster only one genotype existed.The first cluster consisted of genotype CITH-SEL-1 which was characterized by minimum chlorophyll b, total chlorophyll and ascorbic acid however, second cluster consisting of CITH-SEL-7 was characterized by maximum DPPH % inhibition and total phenol.The second group consisted of 8 selections and contributed 80% of the total selections in this population which was further broadly categorized in two broad clusters at 0.81 NRMS. The first cluster includes threeselections (CITH-SEL-2, CITH-SEL-8 and CITH-SEL-4) and it had the highest chlorophyll a and total flavonols. Similarly the second cluster consisted of five selections namely CITH-SEL-3, CITH-SEL-9, CITH-SEL-10, CITH-SEL-5 and CITH-SEL-6. It was characterized by maximum ascorbic acid, chlorophyll b and total anthocyanin and minimum chlorophyll a, DPPH % inhibition, total phenols, total flavonols and total anthocyanin. The diverseselections from different clusters could be utilized in husk tomato improvement programs for introducing desired antioxidant traits. Cultivars with a wide inter-cluster distancecan be used for improving desired traits through hybridization to obtain antioxidant rich varieties.

Principal components analysis is a way of identifying patterns in data, which expresses data in such a way as to highlight their similarities and differences (Verma *et al*., 2013 [21]; Lal *et al*., 2013) [11]. Therefore, it was performed to determine the characters which more strongly contributed to the principal components. Principal components analysis resulted in 8 principal components (Table 4).The first three principal components with eigen values >1 explained 83% of variation among 10 selections (Table 4). Other PCs which had eigen values ≤1 were excluded in interpretation.The first PC, which is the most important component, explained 35% of total variation and was positively related to chlorophyll a, chlorophyll b, total anthocyanin, total chlorophyll, total flavonols and ascorbic acid. The PC2 accounted for 30% of the total variation and the characters with the greatest weight on this component were total phenols, total flavonols, DPPH % inhibition and Chlorophyll a. The PC3 accounted for 18% and positively related to DPPH % inhibition, Chlorophyll b, total anthocyanin, total chlorophyll, total phenols and total flavonols. This suggests that these principal component scores might be used to summarize the 8 variables in any further analysis of data.This situation confirms the suitability of using these traits as abasis for selecting parental sources; however, studies for several years must be conducted before parental selection for a possible plant breeding.

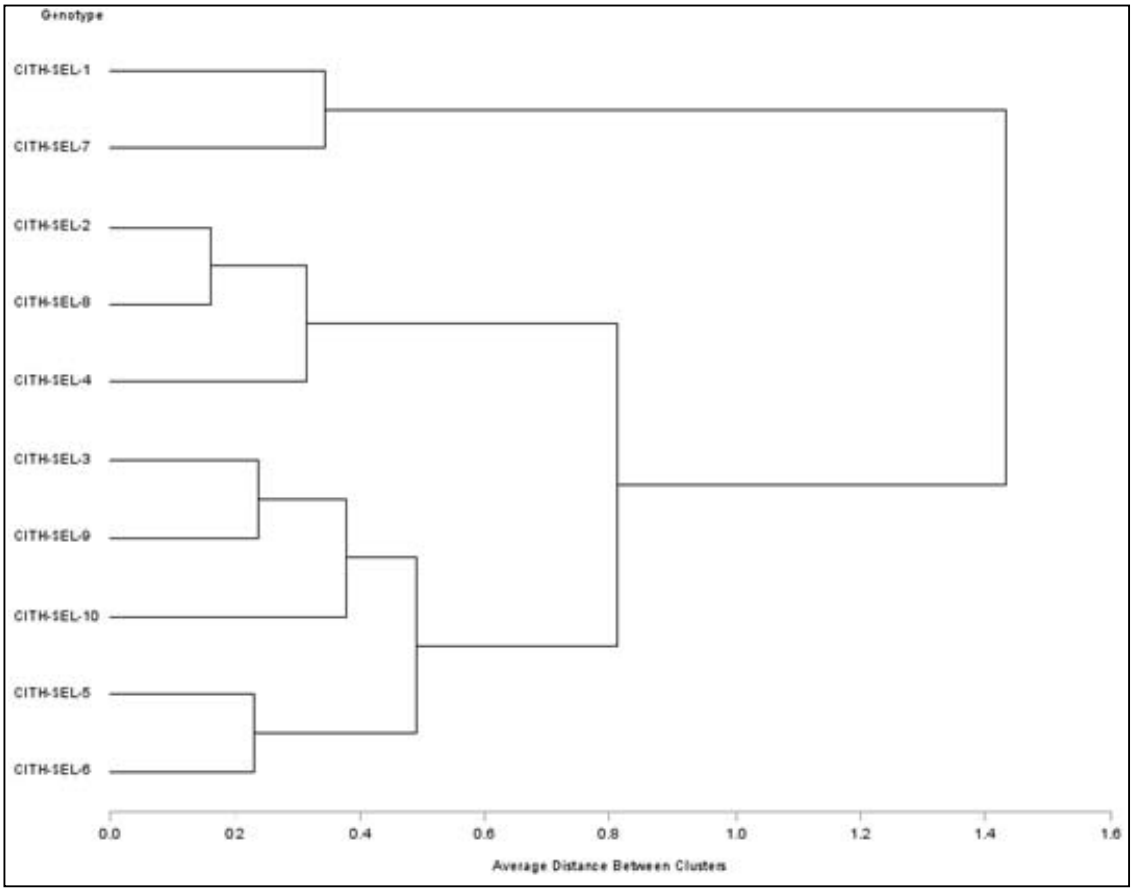

Fig 1: Dendrogram depicting genetic relationships among 10 husk tomato selections based on antioxidant traits produced by average linkage analysis (scale: average distance).

Table 4: Principal component analysis of 10 husk tomato selections showing the principal component scores, eigenvalues and percentage of total variance accounted for the three principal component (PC) axes.

Characteristics	PRIN 1	PRIN 2	PRIN 3
Chlorophyll a (mg/100g)	0.39	0.39	0.05
Chlorophyll b (mg/100g)	0.50	-0.10	0.19
DPPH (% inhibition)	-0.34	0.35	0.38
Total anthocyanin (mg cyanidin-3-glucoside equivalents 100 g_1 fw)	0.13	-0.36	0.54
Total Chlorophyll (mg/100g)	0.52	0.28	0.14
Total Phenols (mg GA equivalents/100 g)	-0.36	0.48	0.02
Total Flavonols mg catechin equivalents /100 g)	0.16	0.52	0.06
Ascorbic Acid (mg/100 g of fresh weight)	0.19	0.02	-0.71
Eigen Value	2.80	2.43	1.41
Difference	0.37	1.02	0.87
Proportion	0.35	0.30	0.18
Cumulative	0.35	0.65	0.83

Conclusion

In the present study, husk tomato selections appear to be good source of various antioxidant constituents. The fruits of selections CITH-SEL-7, CITH-SEL-6, CITH-SEL-10 could be used fordirect consumption as salads or as extracts to increasethe nutritional value of different foods and diets. Further the information generated inthis study can beused for formulatingbreeding and evaluation strategiesand confirming the importance of thegenetic background of cultivars for the availabilityof specific compounds in husk tomato.The choice of cultivar turns out tobe the most important factor to increasehealth- and taste-promoting compounds inhusk tomato fruits.Finally, these results provide useful and important information for researchers in order to increase the antioxidant capacity and functional value of husk tomato.

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