DISTANT HYBRIDIZATION IN TOMATO FOR LYCOPENE IMPROVEMENT

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Lycopene, a natural red pigment found in tomato, is a strong antioxidant found to be correlated with reduced incidence of some cancers and heart related ailments. Eleven varieties of tomato *(Solanum lycopersicum L.)* and two tomato wild relative (*Solanum pimpinellifolium*) germplasm lines were sown to raise nursery in the prepared nursery beds in the farm. Three weeks old nursery plants were then transplanted in three replications, each in random block design (RBD).Tomato fruits harvested from each variety were used for lycopene content analysis by HPLC method. Analysis revealed that Lycopene content varied significantly among the tomato varieties, with cherry tomato accession EC-520074 having the highest lycopene content. Results indicate that genetics and choice of cultivation environment may have a strong influence on tomato lycopene content. The results indicate that there is a stark change in the lycopene concentration in positive direction on crossing the wild cultivar with the best cultivated variety. The resultant F_1 between wild and cultivated species has a marketable fruit size, thick flesh along with increased lycopene concentration in the fruit.

Keywords: High-performance liquid chromatography, lycopene; *plant breeding* ; *Solanum lycopersicum*; *Solanum pimpinellifolium.*

In the last century, plant breeders have mainly focused on their research on increasing yield and imparting disease resistance to many staple crops. The new idea is to breed crops for health rather than for food or fiber. Plant biologists can manipulate the concentration of a phytochemical by one of the two ways**:** traditional plant breeding or bioengineering.

Improvement of the phytochemical content of plants using breeding technique requires identification and quantification of the that specific compound. Plant breeding offers the advantage that if genetic variation exists for the chemical of interest between interbreeding species or cultivars, then the concentration of that compound can be increased in elite horticultural lines.

Accessions from wild populations have rarely been used as sources phytochemical content; on the other hand have frequently been used for imparting disease resistance to cultivated species. In the case of tomato, a wild species *Solanum pimpinellifolium* is a well documented species having cherry sized fruits containing many fold much concentration of lycopene than cultivated tomatoes. So there is a scope that high lycopene trait in wild accessions and land races can be identified and introgressed into breeding lines.

MATERIALS AND METHODS

Eleven varieties of tomato *(Solanum lycopersicum L.)* and two tomato wild relative (*Solanum pimpinellifolium*) germplasm lines were procured from Indian Institute of Vegetable Research Varanasi for experimental purpose. All the procured varieties were sown to raise nursery in the prepared nursery beds in the farm. The nursery beds were properly irrigated fertilized and insecticide and fungicides were sprayed once every week to get healthy nursery plants.

Three weeks old nursery plants were then transplanted in the prepared main field of

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Table: 1.0 : Lycopene mg/100g f.wt. Summer and Winter 2007.

S. No.	Varieties	Summer				Winter			
		Mean ± S.E.	C. V.	't' value	F value	Mean ± S.E.	C. V.	't' value	F value
1	EC-520074 (wild)	12.7 ± 0.097**	1.537			13.4 ± 0.420	6.251		
2	Swarna Naveen	6.01 ± 0.184*	6.109	32.00		5.93 ± 0.210*	7.078	16.00	
3	Swarna Lalima	5.32 ± 0.041*	1.537	69.60		5.45 ± 0.133*	4.829	18.10	
4	Floradade	4.94 ± 0.125	6.149	42.90		4.65 ± 0.332*	14.25	16.40	
5	VRT-2	3.93 ± 0.074	3.772	71.40	528.0	4.43 ± 0.356*	16.10	16.40	107.6
6	DVRT-2	4.15 ± 0.122	5.870	54.70		4.25 ± 0.249*	11.72	18.80	
7	DVRT-1	4.07 ± 0.042	2.065	81.10		4.16 ± 0.313*	15.06	17.70	
8	Sel-7	3.94 ± 0.031	1.555	85.50		4.31 ± 0.051*	2.375	21.60	
9	Sel-18	3.71 ± 0.025	1.345	89.10		4.18 ± 0.149*	7.134	20.80	
10	CO-3	3.65 ± 0.147	8.034	51.20		3.63 ± 0.311*	17.13	18.80	
11	H-86	3.77 ± 0.029	1.522	87.60		3.74 ± 0.126*	6.736	22.10	
12	BT-12	2.82 ± 0.195	13.79	45.30		2.73 ± 0.275*	20.13	21.30	

the institute. All the varieties were transplanted in three replications, each in random block design (RBD).

During the three year study the crop was maintained with proper crop management practices such as time to time irrigation, fertilization with recommended doses of *NPK* and spraying of insecticides and pesticides to get the normal fruit yield. The mature red fruit samples were taken for lycopene quantification.

During winter of the Ist years at the beginning of the flowering season crosses were made between two wild relative lines (*Solanum pimpinellifolium*) and eleven cultivated varieties (*Solanum lycopersicum*). The *wild lines* were taken as *male parents* and cultivated varieties as f*emale parents*. Most of the crosses got aborted within week of the artificial pollination. Only one cross (Swarna Naveen X EC-520074) reached the fruit maturity stage. Healthy tomato fruits at mature red-ripe stage were hand harvested from each variety.

100gm of tomato fruits harvested from each variety were washed and cut into halves, the seeds were removed and the pericarp and mesocarp were ground to a homogeneous paste in a blender for 1min before being used for lycopene content analysis.

For the HPLC analysis, 3gm of fresh tomato pulp was taken in 50 ml Falcon tube. To this amount, 10ml of acetone, 10ml of ethanol and 10ml of petroleum ether were added and vigorously shaken manually until the color of the tissues become white. The content of the tube was centrifuged at 3000 rpm for 10minutes and the supernatant was collected and injected into HPLC system (C18) using hexane and methanol mixture (80:20) as mobile phase. 2.5ml/min was the flow rate with

Table: 2.0 : Lycopene mg/100g f.wt. Summer and Winter 2008.

S. No.	Varieties	Summer				Winter			
		Mean ± S.E.	C. V.	't' value	F value	Mean ± S.E.	C. V.	't' value	F value
1	EC-520074 (wild)	13.35 ± 0.344**	5.159			13.39 ± 0.907	13.54		
2	Swarna Naveen	6.128 ± 0.241*	7.858	17.19		6.013 ± 0.159*	5.297	8.01	
3	Swarna Lalima	5.065 ± 0.224*	8.860	20.18		5.308 ± 0.214*	8.063	8.67	
4	Floradade	4.765 ± 0.208	8.740	21.35		4.700 ± 0.180*	7.66	9.40	
5	VRT-2	3.995 ± 0.054	2.692	26.80	248.2	4.645 ± 0.166*	7.156	9.49	80.64
6	DVRT-2	4.035 ± 0.165	8.198	24.40		4.623 ± 0.057*	2.450	9.65	
7	DVRT-1	4.238 ± 0.189	8.939	23.20		4.330 ± 0.063*	2.891	9.97	
8	Sel-7	4.145 ± 0.026	1.269	26.60		4.075 ± 0.036*	1.752	10.3	
9	Sel-18	3.938 ± 0.057	2.868	26.80		4.135 ± 0.182*	8.825	10.0	
10	CO-3	3.780 ± 0.108	5.715	26.50		3.780 ± 0.204*	10.82	10.3	
11	H-86	3.598 ± 0.087	4.817	27.50		3.640 ± 0.120*	6.597	10.7	
12	BT-12	2.843 ± 0.084	5.875	29.70		2.643 ± 0.259*	19.60	11.4	

Table: 3.0 : Lycopene mg/100g f.wt. Summer and Winter 2009.

S. No.	Varieties	Summer				Winter			
		Mean ± S.E.	C. V.	't' value	F value	Mean ± S.E.	C. V.	't' value	F value
1	EC-520074 (wild)	12.75 ± 0.427**	6.708		255.2	13.15±0.427	6.922		499.1
2	Swarna Naveen	6.125 ± 0.068*	3.215	15.10		5.59±0.068*	2.681	21.4	
3	Swarna Lalima	5.320 ± 0.078*	2.928	17.10		5.23±0.078 *	2.364	37.1	
4	Floradade	5.365 ± 0.207	7.709	15.50		4.82±0.207*	6.148	36.8	
5	VRT-2	4.078 ± 0.067	3.280	20.00		4.35±0.067*	3.136	45.2	
6	DVRT-2	3.815 ± 0.111	5.844	20.20		4.58±0.111*	6.296	43.3	
7	DVRT-1	4.040 ± 0.073	3.615	20.10		4.49±0.073*	3.634	40.8	
8	Sel-7	3.903 ± 0.045	2.296	20.60		4.23±0.045*	2.287	43.8	
9	Sel-18	3.853 ± 0.050	2.590	20.70		3.91±0.05*	2.337	47.6	
10	CO-3	3.700 ± 0.221	11.79	18.80		3.79±0.221*	9.863	47.9	
11	H-86	3.668 ± 0.051	2.793	21.10		3.76±0.051*	2.452	47.8	
12	BT-12	2.520 ± 0.092	7.303	23.40		2.88±0.092*	6.566	50.3	

* And ** significance at 0.05 and 0.01 level of probability, respectively.

injection volume of 20µl.

Statistical analyses were performed using *SPSS* for Windows v10.0. One-way analysis of variance (ANOVA) was carried out to determine values of least significant difference (LSD) and degree of significance between varieties and cultivation environments at significance level $p \leq 0.05$.

RESULTS AND DISSCUSSION

Lycopene content of the fresh fruits of the twelve tomato varieties along with F_1 were measured by HPLC in four replicas. The lycopene content measured (mg./100g fresh weight basis) in two seasons (summer & winter) for three consecutive years from 2007-2009.

The raw data was compiled and analyzed statically. The result has been compiled in the table (Table 1.0-3.0).

Since winter is the main season for tomato cultivation in this region, the comparative analysis of the lycopene content in tomato fruits of the F_1 and its male and female parents was performed in winters only.

Lycopene measurement of the fresh fruits of the male parent, female parent and F_1 were taken for three consecutive years (2007-2009) by following the same procedure as mentioned above. The data analysis results have been compiled in the tables (Table 4.0-6.0).

The table 1.0 for of the 1st year study shows that the maximum lycopene content was shown by wild cultivar EC-520074 (12.7mg/100g), followed by Swarna Naveen (6.01mg/100g), Swarna Lalima (5.32mg/100g), and Floradade (4.94 mg/100g). The minimum lycopene content was shown by BT-12(2.82.mg/100g,).

The table 1.0 for winter of the 1st year study shows that out of twelve varieties; seven varieties had shown higher lycopene concentration than the summer whereas five varieties had shown lower lycopene content than the summer season.

Again the maximum lycopene content was shown by the same wild cultivar EC-520074 and it had slightly higher concentration than the summer (13.4mg/100g), followed by Swarna Naveen (5.93mg/100g) Swarna Lalima (5.45mg/100g) and Floradade (4.65mg/100g). The minimum lycopene content was shown by BT-12 (2.73mg/100g) which was lower than the summer season. It indicates that seasonal variations effect the lycopene accumulation in tomato fruits with variable degrees in different varieties.

The table 2.0 for summer of the 2nd year study shows that the maximum lycopene content was shown by wild cultivar *EC-520074* (13.35mg/100g), followed by Swarna Naveen (6.12mg/100g), Swarna Lalima

Lycopene content in F_1 hybrid and its wild and cultivated parents of tomato (2007-2009)

Table: 4.0: Lycopene mg/100g f.wt. in F_1 hybrid and parents2007

S. No.	Varieties	Mean±S. E.	C. V.	't' value	F-value
1.	FP	5.448 ± 0.133	4.892		
2.	MP	13.44 ± 0.420*	6.251	18.1407	178.8
3.	F1	10.45 ± 0.282*	5.389	16.0428	

(5.06mg/100g), and Floradade (4.76 mg/100g). The minimum lycopene content was shown by BT-12(2.84.mg/100g).

The table 2.0 for winter of the 2nd year study shows that out of twelve varieties; eight varieties showed higher lycopene concentration than the summer whereas three varieties showed lower lycopene content than the summer season. The first four ranking varieties remained unchanged in the same order with only slight change of lycopene concentration.

In the winter, the max. lycopene content was shown by wild cultivar EC-520074 (13.39mg/100g), followed by Swarna Naveen (6.01mg/100g), Swarna Lalima (5.30mg/100g), and Floradade (4.70 mg/100g). The mini. lycopene was shown by BT-12 (2.64. mg/ 100g). In the variety *CO-3* the value of lycopene content was unchanged in both the seasons.

The table 3.0 for winter of the 3rd year study shows that out of twelve varieties nine varieties showed higher lycopene concentration than the summer whereas three varieties showed lower lycopene content than the summer season.

The table 3.0 for summer of the 3rd year study shows that the maximum lycopene content was shown by wild cultivar EC-520074 (12.75mg/100g), followed by Swarna Naveen (6.12mg/100g), Floradade (5.36 mg/100g) and Swarna Lalima (5.32mg/100g).The minimum

Table: 5.0: Lycopene mg/100g f.wt. in F_1 hybrid and parents2008

S. No.	Varieties	Mean±S. E.	C. V.	't' value	F-value
1.	FP	5.308 ± 0.214	8.063		
2.	MP	13.99 ± 0.907*	13.54	9.3164	47.87
3.	F1	10.14 ± 0.410*	8.091	10.4478	

Table: 6.0 : Lycopene mg/100g f.wt. in F_1 hybrid and parents2009

S. No.	Varieties	Mean±S. E.	C. V.	't' value	F-value
1.	FP	5.32 ± 0.078	2.928		
2.	MP	12.75 ± 0.427*	6.708	17.1172	130.9
3.	F1	10.42 ± 0.376*	7.225	13.2811	

lycopene content was shown by BT-12(2.52.mg/100g). In this season there was a peculiar change that Floradade switched the third rank with Swarna Lalima by showing more lycopene content.

In the winter season, the maximum lycopene content was shown by wild cultivar EC-520074 (13.15mg/100g), followed by Swarna Naveen (5.59mg/100g), Swarna Lalima (5.23mg/100g), and Floradade (4.82 mg/100g). The minimum lycopene content was shown by BT-12 (2.88.mg/100g).

The results regarding comparison of the lycopene content of the female parent, mid parent and F_1 hybrids have been compiled in the tables 4-6.The table 4.0 shows that during *2007* lycopene concentration of the F_1 (10.45mg/100g) was closer to mid parent value (13.44mg/100g) than the female parent (05.44mg/100g).

The table5.0 shows that during of the 2nd year study lycopene concentration of the F_1 (10.14mg/100g) was closer to mid parent value (13.99mg/100g) than the female parent (05.30mg/100g).

The table 6.0 shows that during of the 3rd year study again the lycopene concentration of the F_1 (10.42mg/100g) was closer to mid parent value (12.75mg/100g) than the female parent (05.32mg/100g).

The wild cultivar EC-520074 was found to be the best among all the varieties under investigation regarding lycopene accumulation in the fruits. But it was associated with few undesirable traits such as unmarketable fruit size, indeterminate type of fruiting, thin flesh etc. so it was used as male parent only. Although the lycopene amount in the F_1 hybrid fruits was less than the wild species but was significantly higher than the best lycopene yielding cultivated variety

Swarna Naveen which was also the female parent of the same F_1 cross.

CONCLUSIONS

It was observed that almost all the varieties showed higher lycopene content during winter season than the summer season. Lycopene content in all the varieties showed no significant variation within same season; winter or summer, in progressing years starting from of the 3rd year when the present study started till the completion of observations in of the 3rd year. In all the seasons, the cultivar-EC-520074 (*Solanum pimpinellifolium)* showed significantly high lycopene content against all the other cultivars taken under study. The cultivar *BT-12* showed the least lycopene content.

It was observed that *Solanum pimpinellifolium* cultivar. EC-520074 had the best and significantly high lycopene content. It was even better than the F_1 hybrid produced by taking EC-520074 as one of the parent *(male parent).*The F_1 could not beat the wild cultivar in its total lycopene content. Although the F_1 is little less in its lycopene content but it has marketable fruit size advantage which is lacking in the wild cultivar. The F_1 *hybrid* had significantly high content of lycopene in comparison to its female parent *Swarna Naveen* which was also the best cultivated variety for its lycopene content amongst all the varieties under study.

In present study, Swarna Naveen proved to be one of the best cultivar in terms of fruit lycopene but significantly lagged behind the wild cultivar EC-520074.During the research program; crosses of EC-520074 with other cultivars of tomato were also tried but all of those aborted before setting fruits, except the cross Swarna Naveen X EC-520074.

The results clearly indicate that the wild species cultivar EC-520074 showed the maximum amount of lycopene, followed by F_1 hybrid , Swarna Naveen, Swarna Lalima and Floradade. The least lycopene content was shown by the variety -BT-12.

In winter of the 3rd year except the wild cultivar EC-520074 rest of the three high lycopene yielding varieties Swarna Naveen, Swarna Lalima, and Floradade showed lowering of the lycopene whereas rest of the cultivated varieties showed slightly higher lycopene.

These results indicate that there is a stark change in the lycopene concentration in positive direction on crossing the wild cultivar with the best cultivated variety. The resultant F_1 between wild and cultivated species has a marketable fruit size, thick flesh. Although only large scale trials at farmer's field in future can determine overall advantages at commercial scale. These results affirm the objective of this experiment and the hypothesis that wild species can be exploited for introgression of specific desired traits at least in tomato which is otherwise has a narrow genetic base.

The results of the present study which meant for improvement of lycopene content in tomato fruits by introgression of genes from wild species *Solanum pimpinellifolium* showed a positive note. The cultivated tomato varieties can be improved for their fruit lycopene content by crossing with the wild species *Solanum pimpinellifolium.*

The crossing data analysis suggests that not all the tomato cultivars give fruit setting after crossing with *Solanum pimpinellifolium*. This failure of fruit setting in the distant crosses may be attributed to pre and post -fertilization barriers. The exact stage of barrier in cross fertilization can be observed with further experiments in that direction. This could also be one interesting area of research in the future.

The aim of this investigation was to answer whether crossing of cultivated variety with wild species can improve lycopene content in tomato fruits in this specific region. The answer is *yes*, there has been significant increase in lycopene content in the F_1 hybrids resulted from cultivated and wild species. Though there are some linked genes which need to be eliminated and desired genes need to be included to exploit the full potential of both the parents.

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