Comparative expression of different characters in 
cultivated tomato and its wild relatives

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Abstract
Tomato domestication experienced a severe genetic bottleneck as the crop was carried from the Andes to Central America and from there to Europe. Selection of a horticultural crop like tomato is usually done on a single plant basis and with small numbers of selected plants. Domestication has triggered a wide range of morphological and physiological traits that distinguished domesticated crops from their wild ancestors. These characteristics are collectively referred to as the domestication syndrome (Frary and Doganlar, 2003). Studies on the domestication syndrome and domestication process have revealed that numerous traits that distinguished crop plants from their wild relatives which include a more compact growth habit, increased earliness, reduction / loss of seed dispersal and dormancy, gigantism and increased morphological diversity. These characters are often controlled genetically by a relatively small number of loci with effects of unequal magnitude (Frary and Doganlar, 2003). In tomato, domestication syndrome traits have been studied for growth habit (self-pruning, plant height and earliness) and fruit traits (set, size, shape, colour and morphology) and the qualitative genes and quantitative trait loci (QTLs) underlying these syndrome characteristics have been identified (Grandillo and Tanksley, 1996; Doganlar et al., 2000; Frary and Doganlar, 2003; Tanksley, 2004). In the present investigation, mean of the 25 plant, fruit, fruit quality, physiological and seed characters of the 4 wild relatives of tomato viz Solanum pimpinellifolium, Solanum chilense, Solanum lycopersicum var. ceraiformae (EC 514013) and Solanum peruvianum (EC251790) were compared with that of the 5 cultivated tomato genotypes viz Berika, BCT-115d, Aft, Alisa Craig Aft, BCT-59 (IC 0585694) and BCT 82 (IC 0585697) to test the significance of difference between the two mean by paired ’t’ test to document the array of characters that distinguished cultivated species from their wild relatives.

Keywords: Wild relatives, cultivated type, characters, mean difference, tomato

Introduction
Tomato (Solanum lycopersicum) originated from the Andean region now encompassed by part of Chile, Bolivia, Ecuador, Colombia and Peru. The time and place of domestication of tomato are not known with certainty. Tomato had reached a fairly advanced stage of domestication before being taken to Europe in the 15th century and further domestication on a much more intense level occurred throughout Europe in the 18th and 19th centuries (Sims, 1980) [131]. Since the 20th century, human beings have created a huge array of morphologically different cultivars and forms from the single cultivated species Solanum lycopersicum through different aspects of plant breeding. The cultivated tomato is genetically poor compared with the rich reservoir in wild species. It is estimated that the genomes of tomato cultivars contain <5 % of the genetic variation of their wild relatives (Miller and Tanksley, 1990) [90]. The lack of diversity in the cultivated tomato can be visualized using DNA technologies. Very few polymorphisms within the cultivated tomato geneepool have been identified, even using sensitive molecular markers (Garcia-Martinez et al., 2005; Tam et al., 2005) [49, 142]. Tomato domestication experienced a severe genetic bottleneck as the crop was carried from the Andes to Central America and from there to Europe. Selection of a horticultural crop like tomato is usually done on a single plant basis and with small numbers of selected plants. In a predominantly inbreeding species, genetic variation tends to decrease, even without selection. Therefore, genetic drift is a major process that reduces genetic variation.

Domestication has triggered a wide range of morphological and physiological traits that distinguish domesticated crops from their wild ancestors. These characteristics are collectively referred to as the domestication syndrome (Frary and Doganlar, 2003) [46]. Studies on the domestication syndrome and domestication process have revealed that numerous traits that distinguish crop plants from their wild relatives which include a more compact growth habit, increased earliness, reduction / loss of seed dispersal and dormancy,
Materials and Methods

Nine widely divergent genetic materials including four wild relatives of tomato viz. Solanum pimpinellifolium, Solanum chilense (EC 513698), Solanum lycopersicum var. cerasiforme (EC 514013) and Solanum peruvianum (EC251790) and five breeding lines and lines-bred varieties of cultivated tomato Berika, BCT-115 dg, Alisa Craig Aft, BCT-59 (IC 0585694) and BCT 82 (IC 0585697) were employed in the present investigation. The genotypes were evaluated at Central Research Farm, Gayeshpur, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal, India situated at 22°57’N latitude and 88°20’ E longitude with average temperature range of 22.5º to 31.9ºC and night temperature range of 8.4º to 22.4ºC, the average day/ night being 27.6º/15.1ºC.

Five random plants from each genotype per replication were selected for recording the data on different characters. Five fruits from each of the 5 plants per genotype per replication were sampled periodically at advanced turning stage and kept in the room temperature condition till the fruits did ripe completely. The plant, fruit and seed characters recorded were plant height (cm), primary branches per plant, days to first flowering (after transplanting), flower clusters per plant, flowers per cluster, fruits per cluster, fruits per plant, fruit weight (g), equatorial diameter of fruit (mm), polar diameter of fruit (mm), locule number per fruit, pericarp thickness (mm), seed number per fruit and 100 seed weight (g). Total number of fruits of marketable size and maturity of the periodical harvests were considered in recording fruit yield per plant. All misshaped, small and cull fruits were discarded at the time of fruit harvest. After taking fruit weight (g), the fruits were cut into two halves and pericarp thickness (mm) and locule number was recorded. The jelly-like materials along with the seeds were kept in small container for fermentation. After two days, all pulpy substances and chaffy seeds which floated were discarded and the seeds which settled at the bottom of the container were collected after thorough washing in running water. Total seed number of five sampled fruits was then averaged to record seed number per fruit in each replication. Weight of 100 dry seeds from the five sampled fruits per replication was recorded as test weight of seed. Pulp of the cut fruits were used to make replication-wise composite sample to estimate different fruit quality characters on fresh weight basis viz., total soluble solids (Brix) by hand refractometer, total sugar content (%) by anthrone method as per Dubois et al., (1951) [37], reducing sugar (%) by anthrone method as per Dubois et al., (1951) [37], titratable acidity (%) as per Sadashivam and Manickam (1996) [120], ascorbic acid content (mg/100g fresh weight) by titration with 2.6-dichlorophenolindophenol sodium salt solution (AOAC, 1990) [5], lycopene and β carotene content (mg/100g fresh weight) as per Davies (1976) [32], total chlorophyll content of leaf and immature fruit (mg/100g fresh weight) as per Sadashivam and manickam (1996) [120], total phenol content of leaf (mg/100g fresh weight) as per Malick and Singh (1980) [82] and total soluble protein content of seed (mg/g) as per Lowry et al., (1951) [100].

Two sample 't' test was done separately for each character to compare the means of wild and cultivated species of tomato by assuming the null hypothesis (H0) as there was no significant difference between the mean values of wild and cultivated species for various characters under study (µ wild = µ cultivated). The alternate hypothesis assumed for this test was significant difference between the mean values of wild and cultivated species was present for various characters under study (µ wild ≠ µ cultivated). The test was done based on following assumptions.

1. The two samples were drawn from a normally distributed population
2. The variance of both the samples was not same

Calculated ‘t’ value was compared with table ‘t’ value at (5-1=4) degree of freedom with 5% level of significance (2.776). For the characters, where calculated ‘t’ value was greater than table ‘t’ value the null hypothesis was rejected i.e., significant difference between the mean values of the wild species and cultivated genotypes was present for those characters. For the characters, where calculated ‘t’ value was not greater than table ‘t’ value the null hypothesis was accepted i.e., there was no significant difference between the mean values of the characters of the wild species and cultivated genotypes for those characters.

Results and Discussion

Character expression and mean difference for different characters between cultivated tomato and its wild relatives are presented and discussed below.

Plant height: The wild relatives were long viny plant with indeterminate growth habit however, domestication triggered the self-pruning or determinate growth habit in cultivated tomato (Bai and Lindhout, 2007) [111]. In the present investigation, genotypes of cultivated tomato showed all the growth habits viz., indeterminate and viny, semi-determinate and dwarf and bushy. Expectedly, mean plant height of the wild relatives (163.21 cm) was significantly higher than that (69.14 cm) of the cultivated tomato (Table 1).

Primary branches per plant: Mean primary branches per plant of the wild relatives (13.72) was significantly and conspicuously higher than that (6.96) of the cultivated tomato. Long and vine plant with high number of primary branches indicated high biological yield in the plant system of the wild species. However, physiological capacity was limited compared to the cultivated species to mobilize the photosynthates and their onward translocation into organs having economic yield.
Days to first flower: Average days to first flower after transplanting in the wild species (32.07 days) were slightly lesser than the average (34.12 days after transplanting) of the cultivated tomato (Table 1) however, the difference was not statistically significant. Restrepo-Salazar et al., (2008) [108] also could not record statistically significant difference with respect to days to first flowering among different accessions of Solanum habrochaites var. glabratum, Solanum habrochaites var. typicum, Solanum peruvianum and cultivated tomato.

Flower clusters / plant: The distant wild relatives had very high number of flower clusters / plant so that huge number of small fruits was produced in them. Fruit size in the cultivated tomato increased many folds through conspicuous decrease in the number of flower cluster as well as fruit number per plant. Average number of flower clusters / plant in the wild species (77.02) was significantly and conspicuously higher than the average (15.62) of the cultivated tomato (Table 1).

Number of flowers / clusters: Solanum lycopersicum var. cerasiformae, Solanum pimpinellifolium and Solanum chilense presented simple inflorescence but Solanum peruvianum showed compound inflorescence. Average number of flowers / clusters in the wild species (12.73) was significantly and conspicuously higher than the average (7.16) of the cultivated tomato (Table 1).

Fruits / cluster: Average fruit number / cluster in the wild species (11.05) was more than double of that (5.37 fruits / cluster) of the cultivated tomato.

Fruits per plant: Huge fruit bearing capacity due to very high number of flower clusters / plant, flowers/cluster and fruits/cluster was the typical characteristics of the wild relatives. Fruit number was reduced with concomitant increase in fruit weight through the process of domestication in cultivated tomato. Average fruit number/ plant in the wild species (718.01) was more than 23 times higher than that (31.78 fruits/ plant) in the cultivated tomato.

Fruit weight: Mean fruit weight of the wild relatives (4.55 g) was significantly and conspicuously lower than that (92.98 g) of the cultivated tomato (Table 1). An obvious feature of tomato domestication is the massive increase in fruit size. Domestication has transformed the once small wild tomato into the big-fruited present-day cultivars. The evolution pathway related to the transition in tomato fruit size is unknown because domestication occurred in prehistoric times and most likely, mutations associated with larger fruit were selected and accumulated during selection by early humans (Bai and Lindhout, 2007) [11]. By crossing a wild and a cultivated tomato, mutations in about six QTLs seem to have been responsible for transforming the small berries of wild tomatoes to the extremely large fruit now associated with modern cultivars and one of these QTLs is fruit weight 2.2 (fw2·2) that changes fruit weight by up to 30% (Tanksley, 2004) [143].

It was believed that mutation (s) in the fw2·2 locus was the first step on the road to domestication and responsible for a key transition during tomato domestication (Alpert et al., 1995; Frary et al., 2000) [47]. Cloning of fw2·2 has shown that this locus codes for a negative repressor of cell division and the changes from small to large fruit are caused by mutations in the promoter sequence. These mutations are associated with a lower total transcript level of fw2·2 during the cell-division phase of fruit development as well as a shift in the timing of expression (Cong et al., 2002) [28].

Equatorial diameter of fruit: Mean equatorial diameter of the fruits of the wild relatives (17.00 mm) was more than 3 times lower than that (53.03 mm) of the cultivated tomato. Polar diameter of fruit: As expected, mean polar diameter of the fruits of the wild relatives (15.25 mm) was more than 3 times lower than that (51.89 mm) of the cultivated tomato.

Number of locules / fruit: In addition to an increase in fruit size, the domestication of fruit-bearing species often has resulted in tremendous shape variation. Increase in size and shape of fruit has resulted in the increase in the number of locules per fruit. Wild tomatoes bear fruits that are almost invariably round with two locules per fruit, while the cultivated tomato genotypes show a wide variety of shapes: round, oblate, pear, torpedo and bell-shaped (Tanksley, 2004) [143]. Average locule number per fruit in the wild relatives (2.00 per fruit) was significantly lower than the average of 3.59 locules per fruit in the genotypes of cultivated tomato.

Genetic studies have revealed several loci that cause phenotypic differences in fruit shape that distinguish the domesticated tomato from its wild relatives (Tanksley, 2004) [143]. The ovate gene determines a change from round to elongated or pear-shaped tomato fruit (Liu et al., 2002) [78]. Allelic variation in sun and fs8·1 loci can cause elongated and square fruit shape, respectively, mainly for processing tomatoes (Ku et al., 2000; Van der Knaap and Tanksley, 2001) [72, 150].

Pericarp thickness: Fruit weight in the cultivated tomato increased with the increase in the size and thickness of pericarp. Average pericarp thickness in the fruits of the wild species (2.44 mm) was significantly and conspicuously lower than that (6.42 mm) of the cultivated tomato (Table 1).

Seed number per fruit: With the dramatic increase in the size of fruit in the cultivated tomato, seed number per fruit also increased by more than two-fold. Average seed number / fruit in the genotypes of wild species was 44.99 which was significantly lower than that (96.17 seeds/ fruit) in the genotypes of cultivated tomato (Table 1).

Seed weight: Mean 100-seed weight of the wild relatives (1.77 g) was significantly and conspicuously lower than that (3.95 g) of the cultivated tomato (Table 1). Compared with their wild relatives, the seeds of cultivated tomato have become more than 2-fold larger. Change in seed weight was is most likely in response to the selection pressure for uniform germination and seedling vigour (Doganlar et al., 2000) [35]. QTLs for seed weight have been identified that are often in close proximity to loci for fruit weight and soluble-solids content (Doganlar et al., 2000; Tanksley, 2004) [35, 143] which indicated that seed weight was positively correlated to fruit weight but negatively correlated to soluble-solids content (Goldman et al., 1995; Grandillo and Tanksley, 1996) [91, 52]. One QTL, sw4·1, contributes significantly to seed weight variation and it is likely that it differentiated large-seeded cultivated tomato from its small-seeded wild relatives (Doganlar et al., 2000) [35].
TSS content of fruit: Negative correlation between seed weight and total soluble-solids content (Goldman et al., 1995; Grandillo and Tanksley, 1996) has been established in this investigation. Mean total soluble-solids content of the small seeded wild species (6.59 °Brix) was about 30% higher than the mean (4.60 °Brix) of the cultivated tomato although the difference was not statistically significant (Table 2). Several earlier studies also revealed that wild tomato species contained higher TSS content in the fruits compared to that of the cultivated tomato (Chen et al., 1999; Yousef and Juvik, 2001; Lecomte et al., 2004) which agreed well to the results of the present investigation.

Total sugar content: Mean total sugar content in the ripe fruits of the wild species (3.20%) was higher than that (2.89 %) of the cultivated tomato (Table 2). The sugars and acids, together with small amounts of dissolved vitamins, fructans, proteins, pigments, phenolics, and minerals, are commonly referred to as soluble solids (Chope et al., 2006; Kader, 2008). Hence, high degree of Brix does not all the time indicate high sugar content as sugars are not the only components contributing to TSS.

Reducing sugar content: Mean reducing sugar content in the ripe fruits of the wild species (2.35 %) was also higher than that (2.15 %) of the cultivated tomato although the difference was not statistically significant (Table 2).

Titratable acidity: Mean titratable acidity in the fruit pulp of the wild species (0.71 %) was conspicuously higher than that (0.55%) of the cultivated tomato although the difference was not statistically significant (Table 2).

Ascorbic acid content: Mean ascorbic acid content in the fruit pulp of the wild species (40.79 m/100 g fresh) was conspicuously higher than that (34.09 mg/100 g fresh) of the cultivated tomato although the difference was not statistically significant. The Solanum peruvianum genotype was the best for improvement of vitamin C content with 38.08% more ascorbic acid content than the average of the cultivated tomato (Table 4.2). This result is corroborated with the earlier report of Top et al., (2014) who recorded twice as much vitamin C as cultivated tomato in Solanum peruvianum LA 2172.

Lycopene content: Mean lycopene content in the fruit pulp of the wild species (3.72 mg/ 100 g fresh) was conspicuously lower than that (4.45 mg/ 100 g fresh) of the cultivated tomato although the difference was not statically significant indicating up-regulation of carotenoid biosynthesis in the cultivated tomato through the process of domestication. Schauer et al., (2004) reported that of the five wild species (S. pimpinellifolium, S. neorickii, S. chimelewskii, S. habrochaites and S. pennellii), only Solanum pimpinellifolium was red-fruited which also suggested down-regulated carotenoid biosynthesis in the wild species.

β carotene content: Mean β carotene content in the fruit pulp of the wild species (0.27 mg/ 100g fresh) was almost half of that (0.48 mg/ 100g fresh) of the cultivated tomato indicating down-regulated carotenoid biosynthesis in the wild species.

Total leaf chlorophyll content: Chlorophyll synthesis was markedly up-regulated in the cultivated tomato through domestication process resulting increased chlorophyll content in both leaf and immature fruit. Mean chlorophyll content of the wild species (107.55 mg/ 100 g fresh) was significantly lower than that (156.23 mg/ 100 g fresh) of the cultivated tomato. Increased chlorophyll content in the cultivated species indicated their better photosynthetic ability resulting higher accumulation of photosynthates in the sink.

Total fruit chlorophyll content: Mean chlorophyll content in the immature fruits of the wild species (8.52 mg/ 100 g fresh) was markedly lower than that (12.68 mg/ 100 g fresh) of the cultivated tomato.

Total leaf phenol content: Mean total phenol content in the leaf of the wild species (17.58 mg/ 100 g fresh) was slightly higher than that (16.41 mg/ 100 g fresh) of the cultivated tomato although the difference was not statistically significant (Table 2). Top et al., (2014) also recorded high phenolic content in the wild species, Solanum pimpinellifolium, Solanum habrochaites and Solanum peruvianum compared to the cultivated tomato which agreed well to the present findings.

Total seed protein content: Mean soluble protein in the seed of the wild species (69.24 mg/g) corresponded closely to that (67.73 mg/g) of the cultivated tomato (Table 2).
Conclusion
As a consequence of inbreeding during tomato domestication, the genetic diversity in cultivated tomato is now very narrow and much of the genetic variation was due to spontaneously occurring mutations that were rapidly introduced into new cultivars if these had added value however, sooner or later, a ceiling of the potential of tomato breeding by only using the cultivated germplasm will be reached (Bai and Lindhout, 2007) [13]. The present investigation amply suggested that large variation for most of the characters was present and exploitable in the wild relatives of tomato. To explore tomato biodiversity, “Introgression lines” (ILs) that carries small introgressed chromosome fragments from related wild species in a cultivated tomato background are most useful. With techniques like eco-tilling, allele mining will greatly facilitate the identification of useful genes in the wild tomato germplasm (Comai et al., 2004) [27].

Table 2: Test of significance of difference between the mean of different characters in the wild relatives and the cultivated genotypes

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Population</th>
<th>TSS (%Brix)</th>
<th>Total sugar (%)</th>
<th>Reducing sugars (%)</th>
<th>Titrable acidity (%)</th>
<th>Ascorbic acid (mg/100g)</th>
<th>Lycopene (mg/100g)</th>
<th>β-Carotene (mg/100g)</th>
<th>Total leaf chlorophyll (mg/100g)</th>
<th>Total fruit chlorophyll (mg/100g)</th>
<th>Total leaf phenol (mg/100g)</th>
<th>Total soluble seed protein (mg/g)</th>
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<tr>
<td>Mean</td>
<td>Wild</td>
<td>6.59</td>
<td>2.95</td>
<td>2.35</td>
<td>0.71</td>
<td>40.79</td>
<td>3.72</td>
<td>0.27</td>
<td>107.55</td>
<td>8.52</td>
<td>156.23</td>
<td>9.46</td>
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<tr>
<td></td>
<td>Cultivated</td>
<td>4.95</td>
<td>2.99</td>
<td>2.15</td>
<td>0.55</td>
<td>34.09</td>
<td>4.48</td>
<td>0.48</td>
<td>165.23</td>
<td>12.68</td>
<td>16.41</td>
<td>67.73</td>
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<tr>
<td>Standard deviation</td>
<td>Wild</td>
<td>0.45</td>
<td>0.66</td>
<td>0.84</td>
<td>0.12</td>
<td>9.59</td>
<td>0.89</td>
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<td>18.77</td>
<td>2.02</td>
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<td>0.71</td>
<td>0.23</td>
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<td>2.94</td>
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<td>0.42</td>
<td>0.06</td>
<td>4.79</td>
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<td>0.08</td>
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</table>

*Significantly different at 5% level of significance; t value for two sample t test at 4 df. = 2.776

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