



Phenotypic characterization and selection of F₂ tomato population for fruit uniformity and nutrient improvement quality. (*Lycopersicon esculentum* L.)

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Abstract

Fruit uniformity and physiochemical nutrients improvement define the quality of tomato (*Lycopersicon esculentum* L.) The objective of this study was to improve the fruit uniformity and nutrients in F₂ population, while selected two parents for crossing. The donor parent (*Lycopersicon esculentum* var *cerosiforme*, cherry tomato, LA 1421, TGRC) used as a male and (*L. esculentum*) accession of LA 2711, TGRC) used as a female. These selected parents crossed to generate F₁ hybrids in 2013. these F₁ crosses along with parents were planted in second season and evaluated. From the F₁ hybrids plants are selected and back crossed and also the F₁ selfed to produce F₂ seeds in 2014. The (P₁ and P₂) parent cultivars the F₁ and F₂ first and second filial generations and the BC₁ is first back crosses all these generation produced in two cropping seasons and grown in the same cropping season. Analysis data showed significantly improvement in F₂ population compare to P₁ LA 2711 as recorded number of branches per plant in F₂ (41.66%), number of cluster per plant (1.13%) and plant yield in kg (13.81 %) improvement, Nutrients analysis recorded better results and improved in F₂ population as pH (6.01%), ascorbic acid (8.46%), TSS (17.95), fruit firmness (23.17%), total phenols (35%) and fruit uniformity of F₂ population recorded 62.4% in total. So it can be concluded that the F₂ population can be used as a better source for next plant breeding and selection process.

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Introduction

Tomato (*Lycopersicon esculentum* L) is diploid all with same chromosome number $2n=2X= 24$ is self-pollinated crop that cross pollination occur around 5%.fruit uniformity and nutrients improvement is the objectives of breeders, so a small diploid genome and autogamous reproductive system makes tomato ideal for genetic studies and clarify how Genetics, Genomics and Breeding of Tomato is important.

The present research endeavors was to improve fruit quality by inter-specific crossing of two parents. So the "F1" come from interspecific crossing between, (*L. esculentum*, LA2711 and (*L. esculentum* var. *cerusiform*, LA1421), and the "F2" come from the selfing of F1, while the "BC1" generation come from the crossing of F1 with the (*L. esculentum* LA2711). After evaluation of data observed that "F2" population significantly improved quality in case of fruit uniformity and nutrients improvement.

So in commercial cultivated tomato quality of fruits such as fruit uniformity and nutritional quality are much more important to meet the consumers needs and reduce the losses with high income and tomato are consuming widely as a fresh and processed Causse *et al.* (2002); Powell *et al.* (2012); Hasan N *et al.* (2014).

Golani *et al.* (2007) reported that for improvement on quality and production of tomato first breeder should know nature and genetic variation of tomato that is prerequisite for breeding programme, and many researches are focusing on estimation of genetic components like co-efficient of variation, heritability and expected genetic advance on qualitative and quantitative traits selection.

Majid. (2007) reported that better fruit quality of fruit and long shelf life of tomato fruits need to have pH less than 4.1 and concentration of acid should be higher than 0.35 g/100 g in fresh fruit weight in other hand fruit firmness is one of the important factors which dealing with shelf life and choose the fruit quality.

Hounsome *et al.* (2008) reported tomato fruit gives important parts of antioxidants in human diet with the form of ascorbic acid, carotene and total phenolic compound, while Valverde *et al.* (2002) reported total of organic acid, phenolic compound, fruit firmness, fruit color as well as fruit shape and texture is an index of fruit quality which can be improved by breeding program with carrying desire genes from donor parent to the offspring.

Ahmad M. (2015) reported for increasing tomato production, needs successful breeding techniques to produce high yielding with disease resistance variety. Nechifor, B *et al.* (2011) reported that for any breeding programme it is indispensable to have information about the genetic variability and corresponding heritability, as the selection of success superior genotypes depends on the degree of genetic variability and extent to which the characters are inherited.

The objective of this research was

1. Develop appropriate segregating population(s) F2 for fruit quality trait(s).
2. Genetic analysis of fruit quality trait(s) using quantitative data.
3. Develop new breed line(s) of tomato carrying the desired fruit quality trait.

Material and methods

Plants

The parental line seeds of tomato (*Lycopersicon esculentum* L.), P1 LA 2711, and LA 1421 (*L. esculentum* var. *cerasiforme*) collected from tomato genetic research centre (TGRC), the experimental plot laid out in randomized complete block design (RCBD) with three replication, the research conducted in open field and greenhouse of the agriculture research station, King Abdul-Aziz University, Had Al-Sham during two year each of two seasons in (2013/2014). Which parent line LA2711 considered to have high growth vigour, medium yield, and big fruits and is considered as a salinity tolerance? But the fruits of LA2711 are soft, high TSS and misshaped (not uniform).

The donor parent (*L. esculentum* var. *Cerasiforme* LA 1421, TGRC) reported to have a uniform fruit, compact fruit, high lycopene contents, long shelf life fruits with small size fruit, but donor "LA1421", have a medium growth and lower fruit yield.

The "F1" generation obtained by traditional hand crossing techniques between LA1421 as (male) and 2711 as (female). Self-pollination is applied for the "F1" plants to produce the "F2 seeds". Quantitative and qualitative measure used for all segregating and non-segregating population of (F2, P1, P2, and BC1). The F2 populations derived from the self-pollination of F1 plants. Agronomic data recorded on (days to first flowering, No. of branches/plant , sing fruit weight(g), plant height(cm) and plant yield kg/plant), physicochemical traits (total soluble solids content (TSS%), citric acid concentration in (g), pH, vitamin C mg /100 g of fruit weight , total phenolic compound in (g) and fruit firmness (N).

Traits evaluation

Sampling

Agronomic data were collected from different traits in parents BC1 and F2 population. Plant height (cm) No of branches per plant, total No. of fruits per plant, No. of uniform fruits/plant and No. of deform fruits/plant, total soluble solids (TSS%), measured by hand refractometer and firmness of fruits [lbs/cm²; measured using a fruit penetrometer, pH were identified by complete analytical titrate systems instrument so identification of acidity 40 ml of well homogenized juice putted for titration of 8.0 using 0.1 mol/L of KOH the titrated volumes (ml) correspond directly the total acidity and expressed, expressed as g/L citric acid, for determination of ascorbic acid , 60 g of oxalic acid was dissolved in 80 ml of water gently homogenized, then 40 g of fruit weight added to this solution homogenized the filtered and tooked 5 ml of supernatant filtrated juice sample and putted in 50 ml beaker glasses , added 2-4-dichlorophenol by dropping funnel until the collar of tomato juice changes from dark to light yellow for reading of vitamin C total phenol analysis which 0.2 g of fresh fruit was added on 2ml of methanol with 50% methanol and 50% distilled water then shake for one hour from shake sample toked 10 micro litre (µl)

and added 190 micro litre of distilled water, added 1.5 ml of Folin reagent keep it for two minutes, added 1.5 ml of sodium carbonate for coloration keep them for one hour, blank sample contain all except the fruit sample and finally put in spectrophotometer for absorption and reading of total phenol. Statistical analysis done by general mean analysis formula suggested by Fathy S. El Nakhawy, and the correlation coefficient analysed by method of, Phundan Singh. (2008). And fruit uniformity analysed by chi- square, formula, $\text{Chi-square} = \frac{\sum(O-E)^2}{E}$, where, o= observed value and E= expected value.

Development of the f2 population

The P1 LA2711 x P2 LA1421 crossed, produced the F1 hybrid. A population of 100 F2 individuals was developed by selfing the F1 hybrids. The parental lines and F2 population was evaluated for growth, yield, and fruit quality traits during 2014.

Results and discussion

This research studies has developed by possibility of breeding not only for high yielding and nutrient improvement, but also better quality of crops in F2 population for the fruit uniformity and shape. The values observed higher physical traits high percentage of fruits uniformity with improved nutrients in F2 population.

The analyzed data showed significantly improvement percentage in F2 population recorded number of branches per plant (41.66 %), number of cluster per plant (1.13 %) yield improvement (13.81 %). Nutrients analysis shows improvement in F2 population recorded pH(6.08%), ascorbic acid (8.46 %), TSS (17.95 %)fruit firmness (23.17 %), total phenol (35%) and fruit uniformity improvement recorded (62.4%) in total. So in F2 population shows large physical variation in most of the traits with high heritability's. while this research shows an agreement with finding of recent studies of Kader *et al.*, (1978), sugars/acids ratio as important parameter in differentiating tomato flavour among varieties. Stevens. (1972). pH values equal or below 4.49, which is considered to be ideal for correct fruit sourness. Higher vitamin C value recorded in F2 population, whereas lower vitamin C concentration recorded in P1 LA2711.

Table 1. Mean value of agronomic parameters evaluated in four populations (F2, BC1, P1 LA 2711 and P2 LA 1421).

Population	Traits	Mean \pm SE	σ^2	σ	Range
F2	Days to first flowering	46.1 \pm 0.994	20.53	4.53	35-52
BC1		44.8 \pm 1.287	23.20	4.83	36-51
P1 (LA 2711)		45.2 \pm 1.069	16.02	4.00	38-50
P2 (LA1421)		42.1 \pm 1.340	25.14	5.01	35-49
F2	No. branches/plant	6.8 \pm 0.224	1.45	1.20	0.0-6.79
BC1		5.0 \pm 0.33	1.11	1.05	0.0-5.00
P1 (LA2711)		4.8 \pm 0.2	0.4	0.63	0.0-4.8
P2 (LA1421)		5.7 \pm 0.3	0.9	0.94	0.0-5.7
F2	Single fruit weight(g)	157 \pm 20.59	4242.45	65.13	91-280
BC1		91.2 \pm 6.65	443.06	21.04	57-125
P1 (LA2711)		172.6 \pm 843.1	29.03	9.18	119-203
P2 (LA1421)		59 \pm 6.81	463.77	21.53	28-88
F2	No. cluster/plant	10.72 \pm 0.268	7.14	2.67	6-16
BC1		12.3 \pm 0.843	7.12	2.67	8-17
P1 (LA2711)		10.6 \pm 0.566	2.71	1.65	8-13
P2 (LA1421)		13.1 \pm 0.566	3.21	1.29	12-16
F2	Plant height(cm)	58.55 \pm 1.119	72.70	8.50	38-75
BC1		52.3 \pm 2.59	67.12	8.19	42-64
P1 (LA2711)		60.5 \pm 1.38	19.16	4.37	52-67
P2 (LA1421)		67.4 \pm 3.03	92.26	9.60	55-81
F2	Yield Kg /plant	3.79 \pm 0.083	0.71	0.84	2.47-5.97
BC1		3.29 \pm 0.074	0.06	0.24	2.9-3.61
P1 (LA 2711)		3.33 \pm 0.11	0.13	0.37	2.65-3.82
P2 (LA1421)		2.79 \pm 0.074	0.06	0.24	2.25-2.99

SE= standard error, σ^2 = variance, σ = standard deviation, at ($P \leq 0.05$) level of significant.

Correlation coefficient of F2 population summarized in table 4 which is agreement with finding of Gomez *et al.* (2001). While many researchers confirmed the idea of organic acids are produced inside fruits from stored carbohydrate and a portion might be translocate from leaves and roots to fruits Sakiyama *et al.* (1976); Davies and Maw. (1972); Getinet *et al.* (2008).

This research identified that the F2 population has several specific parameters with better nutrient improvement and good uniform fruits vs. parent P1LA2711.

Genetic variability analysis

The availability of genetic variation is essential for initiation of breeding program to facilitate selection in a crop.

Analysis of variance estimated and recorded in all traits, which indicate sufficient diversity among all F2 genotypes that controlled by large number of genes. Phenotypic co-efficient of variance is higher compare to genotypic co-efficient of variance in all traits, same results were findings with Mohamed *et al.*, 2012, Gosh *et al.*, 2010. The results of genetic variability parameters that measured using 100 F2 tomato individuals derived from Inter-specific crosses between LA1421 and LA2711 were presented in Table (3). Plant height of F2 individuals revealed highest genotypic variance (GV), phenotypic variance (PV) and environmental variance (EV). Single fruit weight and yield of fruits were a quantitative traits, that influenced by a large number of genes which strongly controlled by environmental factors that same result with Saleem *et al.*, 2015.

Table 2. Mean value of nutrients parameter evaluated in four populations (F2, BC1, P1 LA2711 and P2 LA1421).

Population	Traits	Mean \pm SE	σ^2	σ	Range
F2	pH	4.32 \pm 0.043	0.13	0.37	3.59-5.68
BC1		4.37 \pm 0.070	0.03	0.17	4.57-4.98
P1(LA 2711)		4.58 \pm 0.074	0.03	0.17	4.32-4.89
P2(LA1421)		4.27 \pm 0.060	0.02	0.15	4.67-4.89
F2	Acidity g/100 g	0.57 \pm 0.0011	0.0095	0.097	0.35-0.72
BC1		0.56 \pm 0.0014	0.0035	0.059	0.48-0.63
P1(LA2711)		0.56 \pm 0.0013	0.0033	0.057	0.48-0.65
P2(LA1421)		0.59 \pm 0.0041	0.0010	0.031	0.54-0.62
F2	TSS (%)	4.79 \pm 0.11	0.93	0.96	3.3-6.9
BC1		4.4 \pm 0.21	0.26	0.52	4.5-6
P1(LA2711)		5.65 \pm 0.24	0.59	0.59	3.6-4.5
P2(LA1421)		5.35 \pm 0.19	0.48	0.48	4.6-6
F2	Ascorbic acid mg/100g	12.04 \pm 1.04	76.22	8.73	14.4-50.4
BC1		12.54 \pm 1.26	9.61	3.10	28.8-37.8
P1(LA2711)		11.10 \pm 1.58	15.12	3.89	25.2-32.4
P2(LA1421)		13.43 \pm 1.17	8.31	2.88	32.4-39.6
F2	Fruit width (cm)	2.11 \pm 0.072	0.109	0.33	1.7-2.7
BC1		1.66 \pm 0.079	0.053	0.23	1.4-1.9
P1(L12711)		2.86 \pm 0.076	0.083	0.28	2.4-3.1
P2(LA1421)		1.34 \pm 0.044	0.013	0.11	1.2-1.5
F2	Fruit firmness (lbs/cm ²)	12.81 \pm 0.40	2.96	1.72	9.9-16
BC1		15.65 \pm 0.45	3.72	1.92	10-18
P1(LA2711)		10.40 \pm 0.0.32	1.84	1.36	8-13
P2(LA1421)		16.25 \pm 0.42	3.20	1.78	12.6-18.5
F2	Total Phenol (mg/g of fruit weight)	0.69 \pm 0.090	0.035	0.597	2.46-0.0291
BC1		0.68 \pm 0.092	0.086	0.293	1.12-0.306
P1(LA2711)		0.34 \pm 0.076	0.058	0.241	0.729-0.029
P2(LA1421)		0.83 \pm 0.11	0.12	0.358	1.27-0.131

TSS = total soluble solids SE= standard error, σ^2 = variance, σ = standard deviation, at (P \leq 0.05) level of significant.

The variability recorded is sum total of hereditary effects of combined genes as well as environmental effect. While the variability is divided in to heritable and non-heritable constituent with acceptable genetic characters such as GCV, PCV, heritability and genetic advances. The estimation of variability characters helps breeders to a gain suitable crop improvement by selection. The results recorded are same as Khanom *et al.*, 2008, Hayder *et al* 2007, Sharanappa and Mogali, 2014. The present study indicates the potential of identifying segregating traits and non-segregating traits for multiple QTL(s) throughout the entire genome. The independent segregating loci can and are likely to make effect on others through epistasis interaction. The PCV and GCV evaluated by considering the respective shows high value for characters selection which indicates wide range of diversity.

Correlation co-efficient

The correlations between measured parameters were presented in Table (4). The results revealed negative and insignificant correlations between growth and yield and yield components parameters except significant correlations between fruit cluster per plant with plant height (0.30) and total fruit weight with both total uniform fruits per plant (0.880) and total deform fruits per plant (0.470) single fruit weight (g) has negative significant correlation with both weight of fruits per plant (-0.220) and plant height (-0.280). The results were comparable with the findings of Agong *et al.*, (2008) and Hadar *et al.*, (2007).

Table 3. Genotypic variance (GV), phenotypic variance (PV), environmental variance (EV), phenotypic coefficient of variance (PCV), genotypic co-efficient of variance (GCV), broad sense heritability (H^2), genetic advances (GA) and mean of genetic advances (MGA%) calculated using 100 F₂ individuals derived from inter-specific crosses between LA1421 (P₁ as a male) and LA2711 (P₂ as female).

Parameters	Range	Mean	$\sigma^2 g$	$\sigma^2 p$	$\sigma^2 e$	GCV	PCV	H^2	GA	(MGA %)
Days to flowering	48-42	44	0.04	2.04	2.00	0.34	3.24	1.86	0.06	0.14
Plant height (cm)	73-32	58.8	2.33	82.5	80.2	2.59	15.4	2.82	0.53	0.90
No. of branches/plant	13-20	7.70	0.31	5.51	5.20	7.23	30.5	5.62	0.27	3.50
no. of flower cluster/plant	16-60	10.90	0.99	5.42	4.43	9.12	21.3	18.2	0.86	7.88
no. of fruit/plant	30-15	22.3	1.20	18.7	17.5	4.91	19.3	6.41	0.57	2.55
No. of uniform fruit/plant	21-90	13.90	0.51	9.07	8.56	5.13	21.6	5.62	0.35	2.52
No. of deform fruits/plant	12-40	8.40	0.51	2.37	1.86	8.50	18.32	21.52	0.68	8.09
Weight of Single fruit (g)	260-85	143.1	1.07	3.15	2.08	0.72	1.24	33.9	1.23	0.86
weight of fruits/plant (kg)	5.97-2.5	3.92	0.05	0.63	0.58	5.70	20.2	7.93	0.12	3.06

Table 4. Correlation between yield and yield component parameters for 100 F₂ tomato individuals derived from inter-specific crosses between LA1421 and LA2711.

Characters	WFP	PH	UFP	DFP	TFP	DF	BP	FCP	SFW
WFP									
PH	-0.060								
UFP	-0.060	-0.050							
DFP	0.060	0.140	-0.020						
TFP	-0.030	0.020	0.880**	0.470**					
DF	0.050	-0.040	-0.090	0.080	-0.040				
BP	-0.080	-0.080	-0.070	-0.020	-0.070	-0.040			
FCP	0.120	0.300**	0.030	0.010	0.030	0.180	0.160		
SFW	-0.220*	-0.080	-0.080	-0.150	-0.130	-0.020	-0.280**	0.040	

WFP= weight of fruits/plant (kg), pH=plant height (cm), UFP= no. of uniform fruit/plant, DFP= no. of deform fruit/plant, TFP=total no. of fruits/plant, DF= days to flowering, BP= no. of branches/plant, FCP= no. of cluster/plant and SFW= single fruit weight (g).

**Significant at 0.01% and * significant at 0.05% level probability, respectively.

Fruit shape and uniformity

Genetic studies in fruits shape and size largely quantitative inherited so the combination of inheritance initially efforts of process that control fruits size and shape.

For fruit uniformity chi square test of statistical significant used between observed value and expected value by the ratio of 3:1 segregation. In plant breeding and genetics usually chi square test applied to test the validity of segregation ratio the advent of marker assisted on quantitative traits loci mapping and cloning methods has made tractable problem.

A number of QTL (s) involved in cross between cultivated local tomato and wild species of varicose size and shape which can improve the fruit uniformity due to fixing of responsible QTL from donor plants to the offspring.

The conclusion of research is that QTL(s) for many variations in both fruit size and shape are not same on their effect that is not complete separate between loci controlling fruit size and those controlling fruit uniformity anyhow the result of this study is acceptable due to collecting more uniform fruits compare to deform fruits in F₂ population as

Fulton *et al.*, 2000. Bia *et al.*, 2003 and Gur and Zamir., 2004, reported that introgression of wild desirable allele into commercial tomato,

for selection process play an important role and provide a basis for breeders for designing optimal strategies program.

Table 5. Data comparing of uniform fruits vs. deform fruits in F2 population.

Plant No	Uniform Fruits	Deform Fruits	Total Fruits/Plant	Uniform Fruits (%)
1	12	10	22	54.5
2	15	7	22	68.2
3	10	7	17	58.8
4	14	10	24	58.3
5	20	9	29	69.0
6	12	11	23	52.2
7	17	9	26	65.4
8	13	8	21	61.9
9	12	6	18	66.7
10	15	9	24	62.5
11	11	8	19	57.9
12	10	10	20	50.0
13	18	6	24	75.0
14	16	8	24	66.7
15	20	7	27	74.1
16	15	8	23	65.2
17	15	6	21	71.4
18	10	7	17	58.8
19	12	8	20	60.0
20	18	9	27	66.7
21	20	6	26	76.9
22	18	10	28	64.3
23	21	9	30	70.0
24	15	9	24	62.5
25	10	8	18	55.6
26	14	10	24	58.3
27	12	7	19	63.2
28	14	8	22	63.6
29	12	9	21	57.1
30	11	7	18	61.1
31	12	11	23	52.2
32	15	7	22	68.2
33	9	6	15	60.0
34	13	9	22	59.1
35	9	7	16	56.3
36	10	5	15	66.7
37	14	8	22	63.6
38	12	7	19	63.2
39	16	10	26	61.5
40	14	8	22	63.6
41	10	7	17	58.8
42	14	9	23	60.9
43	12	7	19	63.2
44	15	9	24	62.5
45	11	12	23	47.8
46	18	9	27	66.7
47	9	11	20	45.0
48	12	8	20	60.0
49	15	7	22	68.2
50	10	10	20	50.0
51	12	10	22	54.5
52	19	10	29	65.5
53	18	9	27	66.7
54	12	8	20	60.0
55	10	7	17	58.8

56	19	9	28	67.9
57	13	7	20	65.0
58	14	10	24	58.3
59	13	8	21	61.9
60	18	9	27	66.7
61	17	7	24	70.8
62	15	7	22	68.2
63	14	11	25	56.0
64	10	8	18	55.6
65	13	6	19	68.4
66	10	8	18	55.6
67	20	6	26	76.9
68	15	7	22	68.2
69	18	9	27	66.7
70	13	4	17	76.5
71	17	9	26	65.4
72	12	10	22	54.5
73	13	11	24	54.2
74	15	6	21	71.4
75	13	8	21	61.9
76	14	10	24	58.3
77	17	5	22	77.3
78	10	9	19	52.6
79	13	9	22	59.1
80	12	10	22	54.5
81	16	9	25	64.0
82	12	11	23	52.2
83	10	10	20	50.0
84	13	11	24	54.2
85	15	9	24	62.5
86	14	9	23	60.9
87	11	6	17	64.7
88	15	9	24	62.5
89	18	8	26	69.2
90	16	11	27	59.3
91	14	8	22	63.6
92	8	4	12	66.7
93	9	6	15	60.0

Summary of chi square test of fruit uniformity by the ratio 3:1.

Segregated Classes	Observed Value (O)	Expected value (E)	O-E	(O-E) ²	X ² Value(O-E) ² /E
Uniform Fruit	1282	1539.85	-257.75	66435.06	43.14
Deform fruit	771	513.25	257.75	66435.06	129.43
Total	2053	2053			172.5

Result. the value calculated chi square ($X^2 = 172.5$) is bigger than table value of X^2 at 93 degree of freedom = 124, hence null hypothesis is rejected and the expected value is not significantly difference with the observed value so the segregation ratio holds good means that is acceptable.

Conclusion

Finally it can be concluded that the present research after analyzing data shows in F₂ population high percentage of fruit uniformity as tested by chi-square test and nutrients improvement compare to parents P₁ LA2711 that used as a female. By the way the F₂ population can be used as improved source material for benefit quality characters that most of the traits have improved in F₂ population.

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