

Population Parameters and Path-Coefficient Analysis of Tomato Grown under Heat Stress

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ABSTRACT

Development of genetically improved heat-tolerant varieties is one of the most important approaches for minimizing the negative effects of heat stress. Although there are many factors causing similar negative effects of high temperature, screening of heat tolerance using fruit set percentage as a direct selection for promising genotypes is the common approach. This study aimed to enhancement of selection capacity for heat stress through dual trends; analysis the genetic and phenotypic information derived from generation analysis in tomato and study the causes and effects of indirect selection for heat stress by path coefficient analysis. Homogenous and heterogeneous generations (P₁, P₂, F₁ and F₂) were used for estimation the following traits; fruit weight (g), fruit set %, total yield per plant (kg), fruit firmness (inch/cm²), chlorophyll a/b ratio, total chlorophyll/carotenoids ratio and electrolytes leakage at vegetative (ELV) and fruiting (ELF) stages. High genetic variability (GCV, 37.24) with moderate value of heritability (72.02%) was recorded by total chlorophyll/carotenoids followed by electrolytes leakage at vegetative stage (ELV) with genotypic coefficient of variance of 25.89% along with high heritability that estimated by 82.20%. Results of the path analysis along with heritability and selection gain showed that fruit set % and electrolytes leakage are the two most critical component traits for fruit yield under heat stress condition. On the other hand, the average fruit weight had low indirect effect but highly significant with fruit yield per plant. The electrolytes leakage showed high ability to distinguish the sensitive and tolerant tomato plants at early stage of plant development which can be relied upon in the selection of heat tolerant genotypes. Among the evaluated materials, ten genotypes; 130, 129, 127, 08, 11, 10, 131, 155, 161 and 124 that possessing better heat tolerance performance with high yield that could be incorporated in further tomato improvement programs.

Keywords: path analysis, tomato, electrolyte leakage, heat stress.

INTRODUCTION

Temperature is the main environmental factor affecting the plant growth and development as well as physiological and biochemical characters (Nahar and Ullah, 2012; Bitu and Gerats, 2013). Among abiotic stress, the high temperature is one of the most critical factors that determine fruit set in tomato during summer season (Rivero *et al.* 2004). The optimal temperature for photosynthesis and fruit set in tomato is between 25-

30°C and 22–25°C, respectively (Khavari-Nejad 1980, Peet and Bartholomew 1996). Since the reproductive phase is the most critical stage and sensitive to heat stress in tomato (Abdul-Baki 1991, Sato *et al.* 2000; Soyly and Comlekcioglu 2009) the final yield depends on the tolerance of plants to heat stress at anthesis stage.

Development of genetically improved varieties is vital approach for minimizing the negative effects of heat stress (Heywood *et al.* 2007; Tester and Langridge, 2010). Under heat stress, the breeder usually follows direct methods for screening of heat tolerance and the promising genotypes with high performance are then selected and developed. This approach is commonly used by breeder in environments where heat is the only major stress. But in fact, there are other stresses as high intensity of insects, irrigation irregularity, lack of calcium and boron, high levels of salinity in soil or irrigation water; spraying with copper pesticides during hot times, misuse of growth regulators and spraying with high pressure causing similar negative effect under heat stress. Hence, the evaluation efficacy of heat impacts like fruit set percentage may become far from the fact. An alternative approach is applying indirect selection for specific traits that have strong correlation with heat tolerance during reproductive development. Leaf electrolytes leakage (Alsadon *et al.*, 2006; Camejo *et al.* 2005; Naveed *et al.* 2016a; Azhar *et al.* 2009), leaf photosynthesis content (Camejo *et al.* 2006, Sharma *et al.* 2014) are some examples for indirect selection that have been used to confer heat tolerance.

In this context, correlation and path coefficient analyses are prerequisites for improvement of any crop including tomato for selection of heat tolerant superior genotypes. The utilization of information obtained from the correlation coefficients can be enhanced by partitioning into direct and indirect effects for a set of a pair-wise cause-effect inter relationships (Kang *et al.* 1983). It is basically a standardized partial regression analysis and deals with a closed system of variables that are linearly related. In tomato, correlation and path coefficient analyses have been used by many authors to estimate the associations between yield and other traits and to identify interrelationships between fruit yield and other traits. Among those studies, Khapte and Jansirani, (2014) and Maurya *et al.* (2011) reported high positive direct effect of average fruit weight on fruit yield

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followed by number of fruits per plant. In addition, Salehur *et al.* (2015) demonstrated highest positive direct effects of number of fruit/cluster on fruit yield/plant while imposing selection for correlation of yield in tomato. In the same context, fruit weight had the most positive direct effect on yield per plant followed by number of fruit per plant, fruit diameter, and number of fruit per cluster. Other studies of Adams *et al.* (2001) and Islam (2011) showed significant negative correlations between high temperature and both yield, number of fruit and fruit weight in tomato. In addition, Linda and Scott (1992) reported that yields/plant may increase with selection for fruit set under high temperature, but with decreasing of fruit weight.

On the other hand, understanding of the genetic as well as the phenotypic parameters is another factor affecting genetic advance. Among these parameters, genotypic and residual variances, heritability, heterosis, degree of dominance, and number of genetic factors controlling the character of interest. In certain cases, breeder face inferior genetic gain in F₂ generation may be attributed to magnitude of environmental factors causing mask of genes expression. In this case, we cannot discriminate neither high nor low yielding genotypes. Hence, analysis of heritability and estimate genetic advance together with selection response and selection differential are essential especially when we deal with abiotic stress as high temperature. Mehboob *et al.* (2017) recorded genetic advance % in F₂:F₃ tomato populations estimated by 20.69% for fruit weight, 8.05 % for yield/plant. While, low response to selection, 0.18 % was noted for flowers/cluster. Heritability coefficient was found to be high for all studied traits including yield/ Plant and its components under heat stress condition (Rashwan, 2016). Similar values of heritability were also noticed by several authors among them (Ghosh 2010; Jiregna *et al.* 2011; Kamel *et al.* 2010).

This study aimed to enhancement of selection capacity for heat stress through dual trends; analysis the genetic and phenotypic information derived from generation analysis (P₁, P₂, F₁ and F₂ scale) in tomato and study the causes and effects of indirect selection for heat stress through adopting path coefficient analysis.

MATERIALS AND METHODS

Genetic materials

Fifty genotypes of tomato (*Lycopersicon esculentum*, Mill.) were compared for their performances under heat stress field conditions which revealed the genotype BGH-7466 as heat tolerant and BGH-2000 as heat susceptible (Elsayed *et al.* 2015). The first genotype characterized by its ellipsoid fruits,

average fruit weight of 80 g, TSS (5.5%), high fruit set (\approx 60%) with inferior fruit firmness. The second genotype, have 100-120 g fruit weight, regular round, TSS (6.2%), low fruit set (\approx 15%), the fruits relatively more firm than BGH-7466. The parental genotypes were kindly provided by Prof Derly Henriques da Silva, Horticultural Germplasm Bank (HGB), UFV-Brasil. BGH-7466 was used as the male parent and crossed with BGH-2000 as the female to generate F₁ generation. The F₁ seed along with its parents was grown in the field during summer season of 2015. F₂ generation was developed by selfing some of F₁ plants during the same season.

Field experiment

Seeds of the parents and their F₁ and F₂ generations were sown on 20 April 2016 in 209-seedling trays. By the end of May at 40-old days, the seedlings were transplanted to the field at private farm in Elgmiza, district of Mansoura, Dakahlia governorate. The field experiment included two main parts, the first one consisting of 30 plants of each parent and F₁ through three replications, while the second part included a total of 400 plants of F₂ population were distributed through a randomized complete-block design. Plants were spaced about 0.40 m apart with 10 plants per ridge (4.5 m long). A check heat-tolerant, strain B variety was used to compare the performance of selected genotypes. The average maximum temperature during the flowering and fruit set for July and August was 38 and 35 °C, respectively while the nighttime averages were 29 and 24°C, for July and August, respectively.

After identified the plants of both homogenous and heterogeneous generations, observations were recorded on the following traits; fruit weight (g), fruit set %, total yield per plant (kg), fruit firmness (inch/cm²), chlorophyll a/b ratio, total chlorophyll/carotenoids ratio and electrolytes leakage at vegetative (ELV) and fruiting (ELF) stages. Chlorophyll a, chlorophyll b and carotenoids content were estimated according to Camejo *et al.* (2005). For chlorophyll a and chlorophyll b, the absorbance was adjusted at 663 nm and 647 nm, respectively, and at 470 nm for carotenoid content. The concentrations for Chlorophyll a, Chlorophyll b, and the total leaf carotenoids (xanthophylls and carotenes) were calculated in mg/ml extract solution according to Lichtenthaler and Buschmann (2001). Electrolytes leakage was used as indicator for cell injury which determined by conductivity method according to Lafuente *et al.* (1991) with some modification Camejo *et al.* (2005) and Alsadon *et al.* (2006) as follow: uniform leaf segments from the new leaves were taken from the marked plants at vegetative and fruiting stages, 30 and 75 days from transplanting, respectively. Four

leaf disks of one cm in diameter from each plant were washed with distilled water three times and transferred in 20 ml tubes completed with distilled water and maintained for 20 hours at room temperature. Then, the tubes were placed in water bath at 40°C for two hours, and then kept for 20 hours at temperature room. The electrical conductivity (EC) was measured using Hanna Combo EC meter model HI98129 as an indicator of cell injury caused by electrolytes leakage.

Statistical analysis

Data were collected on P₁, P₂, F₁ and F₂ generations for yield components and other physiological and biochemical traits under heat stress field conditions. Twenty plants selected randomly in each homogenous generation and 200 plants for segregation population were identified and used for different measurements. Means, variances, genetic and environmental parameters were estimated for each trait and analyzed using the scale (P₁, P₂, F₁ and F₂) according to Mather and Jinks (1982). Phenotypic (PCV) and genotypic (GCV) coefficients of variation were calculated according to Singh and Chaudhary (1985). Estimation of correlation coefficient was accomplished as described by Weber and Moorthy (1952). Path coefficient analysis was carried out according to Wright (1921 and 1923) and modified by Li (1975). Before applying path coefficient analysis, a diagnosis of multicollinearity was performed as described by Chatterjee and Price (1991).

Genetic gain (ΔG) was estimated as follows: $h \cdot SD$ where; h : the absolute value of heritability; SD : selection differential. Selection differential: $(SD) = \bar{x}_s - \bar{x}_0$ where: \bar{x}_0 the original mean of F₂; \bar{x}_s the general mean of the selected individuals. Number of genes (n) was calculated according to the formula:

$$n = \frac{R^2}{8Vg(F_2)}$$

where; $R^2 = F_2 \text{ max} - F_2 \text{ min}$, vg :

genetic variance in F₂ population. The degree of dominance (D) for genetic factors was calculated by formulas described by Petr and Frey (1966) as follows D

$= (F_1 - mp) / (hp - mp)$ where F_1 is the mean of F₁, mp is the mid-parent, and hp is the high parent mean. All statistical procedures were accomplished using the software GENES program version 2009.7.0 for analysis and data processing based on genetic and experimental statistical models (Cruz, 2006).

RERSULTS AND DISCUSSION

Means and variances

For yield components and fruit firmness, the highest mean performance of average fruit weight and fruit firmness were recorded by the parent BGH-2000, with 100.3 g and 3.255 inch/cm², respectively. On the other hand, the same parent showed a relative high variation of 40.47 than BGH-7466 parent regarding average fruit weight (Table 1). While the mean performance of fruit set % and fruit yield per plant were higher in BGH-7466 than BGH-2000 with relatively high variation within BGH-2000 plants for fruit set %.

Regarding the performance of F₁ generation, the fruit weight was similar to BGH-2000 susceptible parent, but with high yielding estimated by 2.93 kg/plant with inferior firmness of fruits. Overall view unfavorable firmness that was similar in both parents and in their F₁ generation (Table 1). High variability was observed in F₂ generation especially in average fruit weight followed by fruit set %, estimated by 193.1 and 128.2, respectively.

For physiological and biochemical traits, chlorophyll a/b ratio showed values more than one indicating that content of chlorophyll a was high than chlorophyll b, however, their relative amount deferred upon parents and F₁, F₂ generations. In general, chlorophyll a/b ratio was higher in the leaves of the P₁ but with relative moderate range of variability among its plants more than the other generations (Table 2). In contrast, the P₂ plants recorded the highest ratio of total chlorophyll /carotenoids with lowest variance. The electrolytes leakage (EL) has a great range of variability in F₂ generation in both vegetative and reproductive stages estimated by 222.4 and 211.7, respectively.

Table 1. Means and variances of parents and their F₁ and F₂ generations for yield components and fruit firmness in tomato evaluated under heat stress field condition

G.	No	Average fruit weight (g)			Fruit set (%)			Fruit yield (Kg)/plant			Firmness (inch/cm ²)		
		Mean	Variance	V(μ)	Mean	Variance	V(μ)	Mean	Variance	V(μ)	Mean	Variance	V(μ)
P ₁	20	89.10	19.88	0.994	55.95	19.40	0.970	2.779	0.047	0.002	2.508	0.028	0.001
P ₂	20	100.3	40.47	2.024	36.92	4.445	0.222	1.876	0.048	0.002	3.255	0.024	0.001
F ₁	20	103.7	41.64	2.082	56.47	13.13	0.656	2.935	0.023	0.001	3.420	0.017	0.001
F ₂	200	101.9	193.1	0.966	43.01	128.2	0.641	2.288	0.362	0.002	2.796	0.303	0.001

Table 2. Means and variances of parents and their F₁ and F₂ generations for physiological and biochemical parameters in tomato evaluated under heat stress field condition

G. No	Chl a/b ratio			Total chl/ carotenoids ratio			Electrolyte leakage at vegetative st.			Electrolyte leakage at fruiting st.			
	Mean	Variance	V(μ)	Mean	Variance	V(μ)	Mean	Variance	V(μ)	Mean	Variance	V(μ)	
P ₁	20	2.146	0.531	0.027	1.365	0.076	0.004	39.83	46.58	2.329	46.88	59.74	2.987
P ₂	20	1.958	0.108	0.005	1.497	0.066	0.003	60.78	57.68	2.884	59.05	54.23	2.711
F ₁	20	1.574	0.221	0.011	0.963	0.118	0.006	35.65	14.24	0.712	44.95	37.65	1.882
F ₂	200	1.908	0.443	0.002	1.271	0.311	0.002	52.24	222.4	1.112	62.62	211.7	1.058

Regarding EL, the mean of P₁ was relatively low than P₂ in both stages of evaluation. Since variability is pre-requisite, here high variability detected in certain traits under study revealed that there is scope for achievement effective selection of these traits.

Population parameters

The majority of studied traits showed high genotypic variances conferred by the superior values of genotypic coefficient of variance (Table 3). Except the chlorophyll a/b ratio that was the only trait that affected by the environmental conditions than genotypic factors (GCV/ECV, 0.741). Moderate to high values of heritability 72.02% to 92.3% were obtained for the studied traits except chlorophyll a/b ratio that showed inferior value of heritability estimated by 35.37 % (Table 3). Also, high genetic variability (GCV, 37.24) with moderate value of heritability (72.02%) was recorded by total chlorophyll/carotenoids followed by electrolytes leakage at vegetative stage (ELV) with genotypic coefficient of variance of 25.89% along with high heritability that estimated by 82.20%.

On the other hand, low variability, GCV was recorded by average fruit weight followed by fruit firmness, (12.38% 18.93%, respectively) however with high coefficients of heritability, 82.40% and 92.3%, respectively. In contrast, high genetic variability was observed for both fruit set % and total fruit yield per plant with GCV of 25.03% and 24.80%, respectively. These findings are in agreement with (Sivaprasad *et al.* 2009; Ahmad *et al.*, 2016). While heterosis relative to mid parent ranged from 9.50% to -32.74%. The estimation average degree of dominance revealed that all the traits under study exhibited complete dominance under the current conditions of field experiment. The number of genetic factors controlling the studied traits ranged from 3 to 9 factors while chlorophyll a/b ratio showed the highest number of genetic factors that controlling this trait among others (Table 3).

Response to selection under heat stress

Actual and expected genetic gain for yield components, physiological traits and electrolytes leakage in F₂ population grown under heat stress

conditions were estimated and shown in (Table 4). High positive genetic gain (ΔG %) was recorded by fruit set %, (37.25%) followed by total yield per plant (34.25%), fruit firmness (30.23%) and 19.41% for average fruit weight. High genetic advance was obtained by Haydar *et al.* (2007) for fruit weight/plant. High heritability with moderate genetic gain observed for average fruit weight is in accordance with findings previously reported by Mohanty *et al.* (2003). On the other hand, negative genetic gain -44.27%, -33.65% and 27.53%, was observed for total chl/car, ELV and ELF, respectively. Selection towards the negative or the lowest values shall be desirable for electrolytes leakage and total chl/car ratio. Based on heritability coefficient and genetic variability among the individuals of F₂ population, it's expected to achieve an increment in fruit weight estimated by 121.6 g by the next cycle of selection regardless if this increase would be achieved under heat stress or not. Also, an increasing in Chla/b ratio with decreasing of total chl/car ratio and electrolytes leakage could be used in selection enhancement for heat tolerance if there was enough evidence of the association between these characters and fruit yield under heat stress field conditions.

Mean performance of selected individuals and check variety

Superior individuals with the extreme values are identified, selected and selfed generation after generation to develop inbred lines. Twenty individuals for each studied trait, 10 % of selection intensity, were selected among F₂ population that showed best values regarding each evaluated trait under high temperature. Plant number and its mean performance for each trait are presented in (Table 5). Regarding average fruit weight, mean performance of all selected individuals showed average fruit weight more than the check variety. For fruit set percentage, high relative values of fruit set simultaneously with fruit yield per plant were obtained in this season compared with last summer season of 2015 including check variety (Elsayed *et al.* 2015).

Table 3. F₂ population parameters for yield components and physiological and biochemical traits in tomato evaluated under heat field stress

Parameter	Average fruit weight (g)		Fruit set (%)		Fruit yield/plant		Firmness (inch/cm ²)		Chl a/b ratio		Total chl/ carotenoids		Electrolyte leakage (Vegetative st.)		Electrolyte leakage (Fruiting st.)	
	Value	SD	Value	SD	Value	SD	Value	SD	Value	SD	Value	SD	Value	SD	Value	SD
\bar{C}_1	193.1	19.26	128.2	12.79	0.362	0.036	0.303	0.030	0.443	0.044	0.311	0.031	222.4	22.19	211.7	21.12
\bar{C}_2	159.1	24.54	115.9	16.07	0.322	0.053	0.280	0.038	0.157	0.290	0.224	0.092	182.9	26.70	161.1	26.77
\bar{C}_3	34.00	6.314	12.32	2.452	0.039	0.007	0.023	0.004	0.286	0.060	0.087	0.016	39.50	7.766	50.54	9.159
\bar{C}_4	82.40	6.342	90.39	8.766	89.11	3326.5	92.3	4056.3	35.37	2395.8	72.02	3895.4	82.20	5.253	76.12	6.055
GCV %	12.38		25.03		24.80		18.93		20.77		37.24		25.89		20.27	
ECV %	5.722		8.161		8.631		5.424		28.03		23.21		12.03		11.35	
GCV/ECV	2.163		3.067		2.873		3.489		0.741		1.605		2.152		1.785	
Heterosis %	9.504		21.61		26.10		18.69		-23.29		-32.74		-29.14		-15.13	
H-P ₁	16.39		0.929		5.614		36.36		-26.65		-29.49		-10.51		-4.117	
H-P ₂	3.390		52.95		56.45		5.069		-19.61		-35.70		-41.35		-23.88	
H-Strain B	68.43		8.89		23.68		-0.14		-5.07		-55.71		73.32		54.96	
D	1.594		-1.05		-1.346		1.44		5.07		-7.10		-1.40		-1.32	
Range	65-133		11-65		0.9-3.4		1.2-4.2		1.0-4.2		0.2-3		25-88		33-110	
No of genes	≈4.0		≈3.0		≈3.0		≈4.0		≈9.0		≈4.0		≈3.0		≈5.0	

(*)VM = (VP1+VP1+VP2)/3

Table 4. Actual and expected response to selection considering selection intensity of 10% in F₂ population for yield components, fruit firmness, physiological and electrolytes leakage in tomato under heat stress field conditions

Items*	Traits							
	Average fruit weight (g)	Fruit set (%)	Fruit yield/plant (kg)	Fruit firmness (inch/cm ²)	Chl a/b ratio	Tchl/car. ratio	Electrolyte leakage at vegetative st.	Electrolyte leakage at fruiting st.
\bar{x}_0	101.9	43.01	2.288	2.796	1.908	1.270	52.23	62.62
\bar{x}_s	125.9	60.73	3.167	3.712	3.327	0.490	30.87	39.97
SD	23.99	17.72	0.879	0.915	1.419	-0.780	-21.37	-22.65
ΔG	19.77	16.02	0.783	0.845	0.502	-0.562	-17.58	-17.24
ΔG (%)	19.41	37.25	34.23	30.23	26.30	-44.27	-33.65	-27.53
\bar{x}_{c_1}	121.6	59.03	3.071	3.641	2.410	0.708	34.66	45.38

\bar{x}_0 : Original mean of F₂; \bar{x}_s : general mean of selected individuals; SD: Selection Differential; ΔG : Selection gain; \bar{x}_{c_1} : Expected mean after first cycle of selection. *For the last three traits, selection realized towards the lowest values.

Table 5. Mean performance of eighty seven genotypes with selection intensity of 10 % among F₂ population for yield components, fruit firmness, physiological and electrolytes leakage in tomato under heat stress field conditions

G.	Average fruit weight (g)		Fruit set (%)		Fruit yield/plant (kg)		Fruit firmness (inch/cm ²)		Chl a/b ratio		Tchl/car. Ratio		Electrolyte leakage at vegetative st.		Electrolyte leakage at fruiting st.	
	Value	G.	Value	G.	Value	G.	Value	G.	Value	G.	Value	G.	Value	G.	Value	G.
54	133	130	65.8	130	3.45	155	4.25	45	4.18	27	0.234	128	25.32	125	33.55	
51	132	129	63.5	127	3.24	161	4.10	84	3.94	26	0.324	129	26.54	04	34.54	
55	132	09	62.4	124	3.23	68	4.00	147	3.94	25	0.344	127	27.43	06	35.65	
130	132	127	62.3	129	3.22	67	3.90	36	3.68	124	0.367	130	29.34	01	39.54	
36	130	08	61.4	55	3.21	20	3.80	134	3.58	132	0.408	08	29.43	14	39.54	
50	130	11	61.4	123	3.21	21	3.70	129	3.55	127	0.445	09	30.12	123	39.54	
52	130	131	61.4	132	3.21	27	3.70	35	3.54	128	0.453	10	30.32	05	39.65	
122	128	07	60.4	199	3.17	29	3.70	77	3.42	133	0.464	131	30.43	143	40.32	
157	125	10	60.4	192	3.14	46	3.70	24	3.33	65	0.468	133	30.43	119	40.34	
23	124	128	60.4	49	3.13	52	3.70	40	3.23	156	0.468	125	31.32	07	40.54	
99	124	132	60.4	12	3.12	18	3.60	196	3.21	117	0.479	11	31.43	12	40.54	
103	123	126	60.3	51	3.12	19	3.60	23	3.21	129	0.517	132	31.43	187	40.54	
53	122	124	60.3	53	3.12	45	3.60	78	3.18	126	0.533	126	32.32	02	40.65	
56	122	12	60.2	125	3.12	70	3.60	41	3.13	102	0.589	01	32.40	131	40.76	
57	122	125	60.2	126	3.12	114	3.60	42	3.10	64	0.594	134	32.43	199	41.3	
59	122	123	59.8	131	3.12	16	3.56	124	3.04	155	0.594	12	33.23	09	41.32	
129	122	133	58.8	54	3.11	49	3.55	01	2.87	85	0.623	53	33.23	128	42.43	
35	121	19	58.7	08	3.1	180	3.54	97	2.81	176	0.623	56	33.23	120	42.54	
22	121	134	58.7	19	3.1	44	3.53	109	2.81	131	0.636	55	33.43	148	42.56	
107	121	56	57.7	128	3.1	126	3.50	160	2.81	07	0.641	04	33.54	129	43.55	
Strain B	60.5	----	39.5	----	1.85	----	2.8	----	2.01	----	2.87	----	30.14	----	40.41	

The percentage of fruit set range from 57.7% to 65.8% in the selected individuals which 75% of the selected individuals have more than 60% of fruit set %. High fruit firmness was obtained by the individuals 155, 161, 68 and 67 estimated by 4.25, 4.10, 4.00, and 3.90 inch/cm², respectively.

Regarding physiological traits and electrolytes leakage in vegetative and reproductive stages, the selected individuals showed high chl a/b ratio since the highest value was twofold comparing with the check variety. Camejo *et al.* (2005) reported that an increase in the Chl a/b ratio was correlated with alteration in pigment composition of the photosynthesis towards a more sun-type as chloroplast become less content of light harvesting chlorophyll proteins.

For Tchl/car ratio and electrolytes leakage, the selection practiced towards the lowest values. All selected individuals were less in their Tchl/car ratio than the check variety (Table 5). The electrolytes leakage ELV and ELF expressed as the absolute values of the electrical conductivity EC in leaves were used as an indicator for heat stress tolerance. Hence, five individuals 128, 129, 127, 130 and 08 exhibited low electrolytes leakage at vegetative stage ELV than the tolerant check variety while seven individuals 125, 04, 6, 01, 14, 123 and 05 had EL less than the tolerant check variety at reproductive stage.

Correlation and coincidence coefficients

For quantification the magnitude and direction of interrelationship among studied characters under the effect of heat stress, simple correlation coefficients were estimated among examined yield components, firmness, physiological and electrolytes leakage (Table 6). Positive and significant correlations were obtained for the average fruit weight, AFW with both fruit set %, FS% and fruit yield per plant, FYP (0.218, and 0.317, respectively, $p < 0.01$) with coefficient coincidence (C %) of 57.69% and 58.46, for FS% and FYP, respectively. Similar findings reported by Islam *et al.* (2010) and Rashwan, (2016) who found highly significant and positively correlation between yield and both flowers per plant and average fruit weight. In contrast, Haydar *et al.* (2007), found negative correlation between FS % and AFW under high temperature stress. A significant negative association was observed between AFW with ELV at vegetative stage (-0.238, $p < 0.01$, C % 44.62). In addition, positive and highly significant correlation coefficients were observed for FS % with FYP (0.901, C % 90). Contrary, this relationship of FS % with ELV and ELF was negative (-0.896 and -0.709, respectively). Regarding fruit firmness, it had a negative significant correlation

with fruit yield (-0.132, $p < 0.05$) indicating the difficulty a simultaneous improvement of yield and fruit firmness. No significant association could be observed between fruit firmness with any of the studied physiological and biochemical traits. A significant positive association 0.799 with high coefficient coincidence 81.54% was estimated between ELV and ELF indicating the possibility of practicing of early selection for heat stress tolerance at vegetative stage using electrolytes leakage as a form of indirect selection. Although leaf photosynthesis is one of the most physiological process affected by heat stress (Wahid *et al.* 2007, Allakhverdiev *et al.* 2008), our results showed that photosynthesis parameters; chlorophyll a/b and chlorophyll/carotenoids ratios, could not reflect a straight association of this effect with any of the studied traits.

Path coefficient analysis

As a previous step for path analysis conducting, a multicollinearity test was applied on studied traits. The variance inflation factors, VIF was inferior than 10 indicating absence of multicollinearity (Table 7). Moreover, the condition number, ratio between maximum and minimum eigenvalues was 50.16 revealed the presence of weak collinearity. In conclusion, multicollinearity test could not detect collinearity effects within the explanatory variable matrix. Under these circumstances, the outcomes of the direct and indirect effects of the physiological and electrolytes leakage parameters on total fruit yield can reliably be drawn by adopting path coefficient analysis.

As it explained, the correlation of fruit yield with most of the studied traits except photosynthesis traits indicated that indirect selection of yield through electrolytes leakage is possible because of the high and significant indirect effects. The non- significant negative correlation between yield and fruit firmness confirmed that fact a simultaneous improvement of yield and quality traits is a complicated task. As outlined previously, nonetheless the highest direct effect of fruit set on fruit yield, electrolytes leakage at vegetative stage was found as the most important selection index of tomato under heat stress field conditions. In contrast, Shushay *et al.* (2014) revealed that average fruit weight had the highest direct effect on total fruit yield (0.644). Direct selection on the basis of electrolytes leakage at vegetative stage can reliably be drawn for yield improvement under high temperature in tomato. The residual effects estimated by 0.3894 revealed the casual factors explain about 61.06 % of the variability in the dependent factor, total fruit yield per plant. The coefficient of determination was 0.8483 between fruit

set percentage and total fruit yield per plant meaning that 84.83% of the variance in total yield can be explained by fruit set %.

Results of the path analysis along with heritability and selection gain showed that fruit set percentage and electrolytes leakage are the two most critical component traits for fruit yield under heat stress condition. On the other hand, the average fruit weight had low indirect effect but highly significant with fruit yield per plant. Among the evaluated materials, ten plants; 130, 129, 127, 08, 11, 10, 131, 155, 161 and 124 that possessing better heat tolerance performance with high yield under the current conditions of our investigation.

However of the common usage of fruit set percentage as a direct indicator for heat tolerant breeding programs in tomato, in many cases selection could be unreliable as a result of the effect of annual climatic conditions besides certain biotic causes and the instability of these factors from year to another (Ismail and Hall, 1999; Naveed *et al.* 2016a). Furthermore, this approach requires more time until reaching flowering and fruiting, more efforts and labor cost especially when screening for huge number of genotypes.

Table 6. Correlation and coincidence coefficients between yield components and physiological and biochemical parameters in tomato under heat stress field condition

Traits*		Cov(X,Y)	<i>r</i>	Probability (%)	Coefficients of Coincidence (C %)		
(x)	(Y)				Data size	(No)	(%)
AFW	FS%	32.04	0.218	0.05 **	260	75	57.69
AFW	FYP	2.422	0.317	0.00 **	260	76	58.46
AFW	Firm	-0.315	-0.045	52.7	260	56	43.08
AFW	a/b	0.793	0.095	12.3	260	65	50.00
AFW	Tch/Car	0.628	0.093	12.9	260	69	53.08
AFW	ELV	-45.21	-0.238	0.02 **	260	58	44.62
AFW	ELF	-8.086	-0.043	50.4	260	66	50.77
FS%	FYP	6.025	0.901	0.00 **	260	117	90.00
FS%	Firm	-0.433	-0.071	25.1	260	51	39.23
FS%	a/b	0.093	0.013	83.4	260	59	45.38
FS%	Tch/Car	-0.607	-0.103	9.35	260	57	43.85
FS%	ELV	-149.3	-0.896	0.00 **	260	23	17.69
FS%	ELF	-116.5	-0.709	0.00 **	260	32	24.62
FYP	Firm	-0.042	-0.132	3.10 *	260	48	36.92
FYP	a/b	0.019	0.051	58.0	260	57	43.85
FYP	Tch/Car	-0.019	-0.063	31.0	260	60	46.15
FYP	ELV	-7.272	-0.840	0.00 **	260	22	16.92
FYP	ELF	-5.718	-0.670	0.00 **	260	36	27.69
Firm	a/b	0.008	0.023	71.2	260	64	49.23
Firm	Tch/Car	0.007	0.025	68.7	260	70	53.85
Firm	ELV	0.664	0.084	17.1	260	76	58.46
Firm	ELF	0.357	0.046	53.4	260	65	50.00
a/b	Tch/Car	0.035	0.103	9.38	260	75	57.69
a/b	ELV	-0.388	-0.041	51.9	260	73	56.15
a/b	ELF	0.569	0.061	67.0	260	74	56.92
Tch/Car	ELV	0.733	0.096	11.8	260	73	56.15
Tch/Car	ELF	0.562	0.075	22.8	260	67	51.54
ELV	ELF	170.0	0.799	0.00 **	260	106	81.54

AFW: average fruit weight; FS%: fruit set percentage; FYP: fruit yield/plant; Firm: fruit firmness; a/b: chlorophyll a/b ratio; Tch/Car: Total chlorophyll/ carotenoids ratio; ELV: Electrolyte leakage at vegetative stage and ELF: Electrolyte leakage at fruiting stage.

Table 7. Multicollinearity diagnosis of correlation matrix including yield components and physiological parameters in tomato under heat stress field condition

Order	Eigenvalues	Singular value	Condition index	VIF _k
1	3.4741	1.8639	1.0000	1.5066
2	1.2252	1.1069	1.6839	8.6675
3	1.0125	1.0062	1.8524	6.5939
4	0.9031	0.9503	1.9614	1.0353
5	0.8885	0.9426	1.9774	1.0634
6	0.3274	0.5722	3.2574	1.0368
7	0.0999	0.3161	5.8957	9.0593
8	0.0692	0.2631	7.0830	2.8622

*Small single value indicates collinearity while high condition index indicates collinearity. Condition number (Max/Min): 50.16

Table 8. The direct and indirect effects of seven secondary components on the total fruit yield per plant as the primarily component in tomato evaluated under heat stress field condition

Effects	Secondary variables*							Correlations with FYP
	Average fruit weight	Fruit set %	Firmness	Chl a/b	Tchl/car.	Electrolyte leakage at vegetative st.	Electrolyte leakage at fruiting st.	
Direct effect on FYP	0.178	0.578	-0.057	0.014	0.010	-0.310	-0.003	
Indirect effect via:								
Average fruit weight	-----	0.039	-0.008	0.017	0.017	-0.004	-0.008	0.317**
Fruit set %	0.126	-----	-0.041	0.008	-0.060	-0.518	-0.410	0.901**
Firmness	0.003	0.004	-----	-0.001	-0.001	-0.005	-0.003	-0.132*
Chl a/b	0.001	0.000	0.000	-----	0.001	-0.001	0.001	0.051
Total chl/car.	0.001	-0.001	0.000	0.001	-----	0.001	0.001	-0.063
ELV	0.007	0.278	-0.026	0.013	-0.030	-----	-0.248	-0.840**
ELF	0.000	0.002	0.000	0.000	0.000	-0.002	-----	-0.670**
Total:	0.317	0.901	-0.132	0.051	-0.063	-0.84	-0.670	
Coefficient of determination			0.8483					
Residual Effects			0.3894					

*ELV and ELF are electrolyte leakage at vegetative and fruiting stages, respectively.

Although the well-known role of carotenoids in the protection of chlorophylls from photo-oxidation and as essential light harvesting pigments, it was difficult to demonstrate their relationship regarding tolerant to heat stress along the evaluated genotypes in current investigation. However slight differences in these photosynthesis parameters were observed between both tolerant and susceptible parents in our results. In contrast, Camejo *et al.* (2006) and Sharma *et al.* (2014) demonstrated that different responses to leaf photosynthesis under heat stress differed from one genotype to another, which revealed its potential role in detection of heat tolerant and susceptible genotypes.

An increase in electrolyte leakage was observed by Camejo *et al.* (2005) in stressed plants caused by changes in membranes permeability as a result of reduction in their capacity to maintain solutes and water. The same authors reported that in the tolerant genotypes,

the permeability of the membrane was not modified under heat stress which reflected the maintenance of its functioning. Furthermore, the leakage of electrolytes through the plasmalemma was found to be associated with photosynthetic and mitochondrial activity reductions (Shanahan *et al.* 1990; Ristic *et al.* 1996).

From our point of view, the results implied that assessment of cell membrane thermo-stability as presented by electrolyte leakage at vegetative stage proved to be effective in differentiating 200 plants of F₂ population for high-temperature tolerance. The estimated mean, variances, heritability and genotypic and phenotypic coefficients data besides correlations and path coefficient analysis regarding fruit set and electrolyte leakage suggested the different reactions of the F₂ individuals to high temperatures indicating divergent responses of genetic materials to heat-stressed conditions. Many authors among them Blum and

Ebercon, (1981), Agari *et al.* (1995), Ibrahim and Quick, (2001) and Muhammad *et al.* (2016) have been used cell membrane thermo-stability as an efficient parameter for screening against abiotic stresses as drought and heat stress tolerance.

The impact of additive variance in the inheritance of fruit set percentage, firmness, total soluble solids and lycopene content was confirmed by analysis of combining ability and higher broad sense estimates under heat stress (Elsayed *et al.* 2015). Hence, the results obtained here about electrolyte leakage may suggest the role of additive genetic variance in determining response to selection in succeeding plant progenies confirmed by its high genetic gain values.

Path analysis consists of study of direct and indirect effects of certain characters under basic variable using regression. Sewall Wright (1921) is considered the first one that applied path analysis technique in genetics to quantify interrelationships, while the first application of this technique in breeding reported by Dewey and Lu (1959). In spite of much previous studies on path analysis in plant breeding and the appraisal of the available literature on tomato (Maurya *et al.* 2011; Manoj 2011; Roa *et al.* 2013; Pemba *et al.* 2014; Meena and Bahadur 2015), this investigation is the first report applying path analysis to describe the cause and effect of electrolytes leakage on yield as a form of indirect selection for heat tolerance in tomato. According to our results, it could be recommended that the utilization of electrolytes leakage in tomato as indirect selection in early stage of plant development for discover the promising genotypes and discard the susceptible ones.

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الملخص العربي

المؤشرات العشائرية ومعامل المرور للطماطم النامية تحت ظروف الإجهاد الحراري

أحمد يوسف عبدالنبي محمد السيد

توريث بلغ ٨٢ % . نتج عن تطبيق الإنتخاب بشدة ١٠ % بالنسبة لحجم عشيرة الجيل الثاني عشرون تركيب وراثي لكل صفة تحت الدراسة على حدى بواقع سبعة وثمانون تركيب وراثي غير مكرر لتلك الصفات المدروسة.

كما أوضحت نتائج تحليل معامل المرور بالتوازي مع قيم معامل التوريث و المكسب الإنتخابي أهمية الإنتخاب على أساس نسبة العقد كمكون أساسى للمحصول و درجة الإرتشاح الإلكتروليتي في المرحلة الخضرية التي أظهرت اعلى تأثير غير مباشر لمعامل المرور (٠,٨٤-) بعد نسبة العقد مباشرة (٠,٩٠١) مما يوضح أهمية الإنتخاب الغير مباشر للمحصول تحت ظروف الإجهاد الحراري في الطماطم. بينما أظهرت صفة متوسط وزن الثمرة أقل تأثير غير مباشر على الرغم من إرتباطها المعنوي بالمحصول الكلى للنبات. من خلال التقييم الحقلى للجيل الاول الإنعزالي، أظهر عشرة تراكيب وراثية أداء متفوق تحت ظروف الإجهاد الحراري والتي يمكن إدخالها في برامج التربية لاستكمال التحسين الوراثي. من خلال النتائج المتحصل عليها من تحليل التباين، الارتباط و معامل المرور اثبتت درجة الإرتشاح الإلكتروليتي قدرتها العالية على تمييز النباتات المحتملة والحساسة في مرحلة مبكرة من تطور النبات والتي يمكن الاعتماد عليها في الانتخاب للحرارة العالية نظراً لسهولة وسرعة تقديرها عملياً بالإضافة الى قلة العوامل الأخرى التي قد تضلل من تقديرها كما هو الحال عند الإعتماد على نسبة العقد فقط.

يعتبر تطوير أصناف متحملة للحرارة المرتفعة عن طريق التحسين الوراثي من احد العوامل الرئيسية للحد من التأثيرات السلبية الناتجة عن إرتفاع درجات الحرارة. على الرغم من وجود العديد من العوامل التي تؤثر على عقد الثمار في الطماطم بخلاف درجة الحرارة، إلا أن الإنتخاب المباشر عن طريق تقدير نسبة العقد مازال الأسلوب الأكثر إستخداماً عند التقييم الحقلى والإنتخاب للتراكيب الوراثية المتفوقة. لذا كان الهدف من هذه الدراسة هو محاولة رفع كفاءة الإنتخاب تحت ظروف الإجهاد الحراري من خلال دراسة البيانات الوراثية والبيئية الناتجة من تحليل المتوسطات والتباين في الطماطم بالإضافة الى تقييم جدوى إستخدام بعض الصفات التي تتميز بثبات تعبيرها إتجاه التغيرات الحرارية تحت هذه الظروف عن طريق تحليل معامل المرور.

تم تنفيذ هذه الدراسة بإستخدام سلالتين من الطماطم بالإضافة الى الجيل الأول والثاني الناتج عن التهجين بين الأبوين المتحمل والحساس للحرارة المرتفعة. تم تقدير كل من متوسط وزن الثمرة، نسبة العقد، المحصول الكلى للنبات، صلابة الثمار، نسبة كلوروفيل أ إلى ب، نسبة الكلوروفيل إلى الكاروتينات ودرجة الإرتشاح الإلكتروليتي في المرحلة الخضرية والثرمية. أوضحت النتائج وجود مدى واسع من التنوع الوراثي بالتزامن مع قيم متوسطة من معامل التوريث لصفة نسبة الكلوروفيل الكلى إلى الكاروتينات متبوعاً بدرجة الإرتشاح الإلكتروليتي التي أظهرت معامل تباين وراثي بنسبة ٢٥,٨٩ % مع معامل