JOURNAL OF EPIGENETICS

Journal homepage: http://jep.usb.ac.ir/

Document Type: Original Article

Heterosis and Combining Ability Evaluation in Different Tomato Inbred-lines for Salinity Tolerance During the Seedling Stage

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ARTICLE INFO

Article history

Received 01 October 2019

Accepted 11 October 2020

Available online 11 October 2020

DOI:10.22111/jep.2020.31828.1017

KEYWORDS:

Cytoplasmic effect, Reciprocal cross, Seedling growth, Dominance, Diallel-cross.

ABSTRACT

Vast numbers of studies on tomato breeding have been performed for resistance to environmental stresses and pests. In genetic investigation and breeding of tomato, it is tried to use tomato lines with the most variance on morphological and agronomical traits to obtain better results, like maximum heterosis on F1 hybrids. In this survey combining ability and heterosis of 7 inbred lines (ME, KaLN3, Fla, CT6, LA3770, R2-05, and DB) of tomato and their F1 hybrids were investigated for salinity tolerance during the seedling stage using the diallel-cross. Therefore, seed germination percentage, seedling emergence percent and rate under salt-stress, tested in a complete randomized design with four replications. Results show that the reciprocal effects and SCA were significant for all traits on the probability of 1 %. Analysis of Wr-Vr regression also shown that seed germination percentage and seedling emergence percentage and rate under salt stress controlled by over-dominance effects. The best line for resistance to salt stress in juvenility was R205, which has the most GCA for all three traits connected to salt resistance. It may be beneficial to combine this line as a parental line in breeding programs for increasing salinity tolerance of tomato during the seedling stage.

Introduction

Tomato (*Solanum lycopersicom*) (2n=2x=24) is one of the most important solanaceous vegetable crops of Peru-Ecuador origin. It is a moderate nutritional crop and a good source of vitamin A, vitamin C, and minerals. The use of F1 hybrids is one of the means of ever-increasing demand for tomato the demand (Peralta et al., 2005; Nuez, 2008). F1 hybrids in tomato offer several advantages such as earliness, higher productivity, improved quality, resistance to biotic and abiotic stresses. It also provides a quick and convenient way of combining desirable characters (Omholt, 2000).

It has been realized that hybrids perform better than self-pollinated cultivars in respect of uniformity of product, yield, quality, resistance to pests and diseases, Adoption to different environmental conditions, and long storage life (Titok, 1994). Heterosis breeding is used to improve yield quantity and quality of Tomato (Solanum lycopersicum L.) because traditional methods cannot be used to achieve this goal. Tomato hybrids first made through hand emasculation and hand pollination (Omholt, 2000).

Availability of enough irrigation water with acceptable quality in arid and semi-arid regions of the world, limiting agriculture activities.

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(Munns, 2002; Al-Busaidi, 2009). While crop productivity reduces by high soil salinity level in these areas and salt susceptible vegetable crops either die or have a yield or growth reduction (Kaveh, 2011). High concentrations of salt (150 mM NaCl and 15 mM CaCl2) in the germination media delays germination and reduce its rate, significantly (Foolad, 1997; Foolad, 1999; Foolad, 2004; Kaveh, 2011). In different developmental stages, tomato regulates different responses to salt stress. Usually, shoot growth and vigor of younger seedlings become stunted while salinity increases (Foolad, 2004; Li et al., 2011). Zhang et al. reported that the occurrence of a slight saline condition during the seedling stage increases the final plant height and fresh biomass compare to the same saline condition in the flowering or fruiting stage (Zhang, 2017).

The genetic transformation is a tool in tomato breeding for higher salinity tolerance. Different studies showed that the expression of one gene could increase tomato tolerance to salinity conditions (Arrillaga, 1998; Gisbert, 2000; Moghaieb, 2000; Zhang, 2001).

In this study, we aimed to find the best possible line for salinity tolerance, which can be used in breeding programs.

Materials and Methods

Plant material

In this study, seven Lines ME, KaLN3, Fla, CT6, LA3770, R2-05, and DB selected from 133 tomato lines of the Ferdowsi University of Mashhad collection, based on variation in morphological, biological, and agronomical traits.

Table 1. Crossing method of different tomato lines.

	ME	KALN$_3$	Fla	CT$_6$	LA$_{3770}$	R$_{205}$	DB
ME	ME* ME	ME* KALN$_3$	ME * Fla	ME* CT$_6$	ME* LA$_{3770}$	ME* R$_{205}$	ME*7 DB
KALN$_3$	KALN$_3$* ME	KALN$_3$* KALN$_3$	KALN$_3$* Fla	KALN$_3$* CT$_6$	KALN$_3$* LA$_{3770}$	KALN$_3$* R$_{205}$	KALN$_3$* DB
Fla	Fla * ME	Fla* KALN3	Fla * Fla	Fla * CT$_6$	Fla* LA$_{3770}$	Fla * R$_{205}$	Fla * DB
CT$_6$	CT$_6$* ME	CT$_6$* KALN$_3$	CT$_6$ * Fla	CT$_6$* CT$_6$	CT$_6$* LA$_{3770}$	CT$_6$* R$_{205}$	CT$_6$* DB
LA$_{3770}$	LA$_{3770}$* ME	LA$_{3770}$* KALN$_3$	LA$_{3770}$ * Fla	LA$_{3770}$* CT$_6$	LA$_{3770}$* LA$_{3770}$	LA$_{3770}$ * R$_{205}$	LA$_{3770}$ * DB
R$_{205}$	R$_{205}$* ME	R$_{205}$* KALN$_3$	R$_{205}$ * Fla	R$_{205}$* CT$_6$	R$_{205}$* LA$_{3770}$	R$_{205}$* R$_{205}$	R$_{205}$* DB
DB	DB * ME	DB * KALN$_3$	DB * Fla	DB* CT$_6$	DB* LA$_{3770}$	DB* R$_{205}$	DB * DB

Crossing method

After choosing the parental lines, six plants of each line cultivated in separate rows in the greenhouse condition, there was an 80 cm distance between rows and 50 cm between the plants on each row. Hand emasculation and hand pollination of different tomato lines were done after flowering (Table1). Pollinated flowers are covered with cover sheets to prevent unwanted pollinations.

Seed Extraction

Full ripe tomato fruits were collected, and seed extraction was done using the fermentation method (Nemati, 2010). Seeds and attached fruit pulp were crushed in a non-metallic container and kept at room temperature for fermentation for 48 hours. During fermentation, the seeds get detached from the adhering pulp and settle to the bottom of the container. Then, seeds being washed and dried to 12-14% moisture level.

Germination

Seeds were plated into Petri dishes under aseptic conditions. Each Petri dish contains 20 seeds of one specific cross. Petri dishes were placed in a controlled-environment growth chamber at 25 °C and 80% relative humidity. Irrigation was begun immediately after adding 20 mL of NaCl (0.5, 2.5, 5, and 10 ds·m−1) solutions daily. Avoiding salt aggregation in dishes, irrigation with distilled water was done every day. When the radical was at least 2 mm long, seeds were considered germinated (Al-Harbi, 2008).

Emergence and Seedling Growth

Tests were carried out in seedling trays with 190 cells (4 ×4 × 6 cm), which were filled with

DOI:10.22111/jep.2020.31828.1017

sterilized coco peat and sand 1:1 v/v and placed in a greenhouse at 25 ± 3°C 60% ± 15% relative humidity. Seeds of the tomato Inbred-lines and their cross offspring have sawn one seed per cell. Then, irrigation was begun immediately after adding 200 mL of NaCl (0.5, 2.5, 5, and 10 ds·m–1) solutions daily. Irrigation with distilled water was repeated every two days to avoid salt aggregation in the medium. A seedling considered emerged when the hypocotyls hook was visible above the media surface. The emerged seeds number was recorded daily (emergence rate), and after ten days, the final emerged seedling percentage determined. The experiment was a factorial, with a completely randomized design, with four replications.

Data Analysis

Diallel cross for general and specific combining ability and heterosis of each studied characteristics analyzed with Dial 98 (Nemoto et al., 2004).

Results and Discussion

Germination:

ANOVA for germination under saline conditions based on Griffing (1956) revealed that combining ability and cytoplasmic effects are significant (P≤0.01). Progeny of CT6 × LA3770, CT6 × R205, and Fla × R205 crosses with 64,62 and 62, respectively, had the highest percentage of germination. For this trait, average of F1 hybrids had a 1.92 fold more than the best parent. CT6 × R205 had the highest amount of heterosis (Table 2). The highest value for SCA has happened in CT6 × LA3770 (Table 3).
Due to the lack of significance in the GCA/SCA ratio in the F test, it could be said that additive effects had no significant impacts on germination percentage.

Table 2. Heterosis value for germination percentage of each cross progeny.

	Me	KalN3	Fla	CT6	LA3770	R205	DB
Me	-	20.5	-2.5	33.75	-8.25	32	-2.75
KaLN3	4	-	-3.5	3.75	-16.75	24	15.25
Fla	-20	-7.5	-	-18.25	0.25	38	0.75
CT6	-3.25	10.75	5.25	-	30.5	52.25	-2.5
LA3770	-8.75	-5.25	-20.25	-7.5	-	-8.75	-14.5
R205	21	24	20	4.75	-5.25	-	21.25
DB	9.25	4.25	-18.25	-1	-25	5.25	-

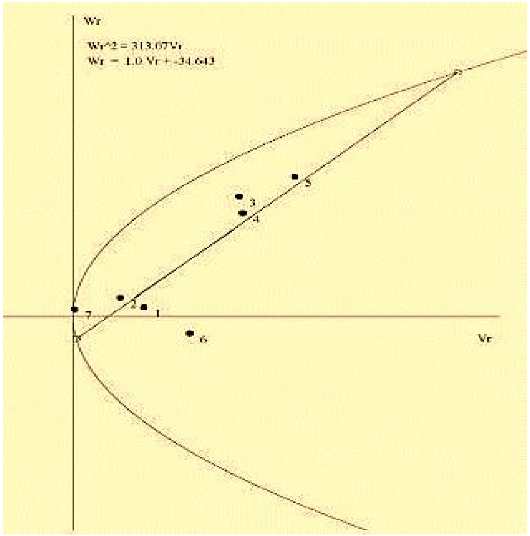

Fig.1- Wr-Vr diagram of Germination Percentage, Wr = aVr + b.

1-ME, 2-KaLN3, 3-Fla, 4-CT6, 5-LA3770, 6- R205, 7-DB.

Table 3. Specific Combining Ability of Germination Percentage

	Me	KalN3	Fla	CT6	LA3770	R205	DB	σSij^2
Me	-2.43	2.3	-9.55	1.75	-1.25	4.65	2.1	22.03522381
KaLN3	-2.7	-3.98	-3.75	-6.2	-3.7	2.7	8.65	27.86208095
Fla	-5.9	-2.55	1.87	-8.3	8.95	10.85	1.8	63.03622381
CT6	-4.25	-0.15	5.9	2.82	15.25	3.9	-6.4	67.65689048
LA3770	2.35	1.2	2.5	2.15	2.57	-17.6	-1.65	107.6768905
R205	4.65	3.75	2.05	-3.55	-5.45	9.17	-4.5	93.98853333
DB	5.85	0.45	-2	-0.1	-2.75	-1.45	-10.03	38.77239048
σSij^2	21.0986	7.18291	15.5954	12.8244	10.1269	26.0619	22.3882	

DOI:10.22111/jep.2020.31828.1017

According to figure 1 and average dominance of this trait (1.201), overdominance effects are more responsible for germination percentage in saline condition. Researchers have reported different inheritance method for the germination percentage. Some researchers have reported that only additive effects play a role in controlling seed germination (Farzaneh,2012).

The results of research by Sekhar et al. (2010) showed that non-additive effects are responsible for the inheritance of this trait. Salinity tolerance was mainly controlled by additive effects with the tolerance allele showing partial dominance (Chen, et al; 2008). When High positive SCA was also found between two tolerant cultivars, it is indicating possible different tolerant genes or some minor genes in these cultivars

Table 4. Analysis of variance for seedling emergence based on Griffing (1956)

	df	SS	F	
Block	3	1.71	0.63 n.s	(Rep/Error)
GCA[1]	6	710.33	1.17 n.s	(GCA/SCA)
SCA[2]	14	608.59	222.22 **	(SCA/Error)
Maternal effects	21	462.29	168.8 **	(Rec/Error)
Error	123	2.74		

n.s: not significant

**: significant at P≤1%.

1,2: General and specific combining ability

Table 6. Specific Combining Ability for Emergence Percentage

	Me	KalN3	Fla	CT6	LA3770	R205	DB	σSij^2
Me	-14.15	12.88	-10.07	7.63	21.54	-10.80	-21.17	254.30
KaLN3	6.65	-2.68	10.61	-13.64	1.54	-8.51	-2.87	92.24
Fla	-11.50	-13.35	0.75	8.54	-3.69	-13.56	8.17	91.92
CT6	-4.60	11.80	2.15	-2.90	-14.51	13.46	-1.49	119.72
LA3770	-4.20	3.70	10.05	-10.55	-12.61	-1.42	-3.46	140.09
R205	-1.65	-19.25	12.35	14.25	4.15	13.35	20.82	190.38
DB	15.30	10.45	0.30	-13.05	-3.15	-9.85	18.25	209.37
σSij^2	103.94	143.55	94.87	109.49	67.86	165.97	151.00	

Seedling Emergence

Analysis of variance for seedling emergence showed that maternal effects and SCA were significant (P≤1%) (Table 4).

The total mean of F1 hybrids was higher than the best parent value of 7.5 units that show heterosis for this trait. Progeny of R205 × CT6 (42.5) and CT6 × KaLN3 (39.5) had the highest value for heterosis for this trait (Table 5). Maximum SCA for this trait in direct crosses went to Me progenies, and for reciprocal crosses, it went to R205 hybrids (Table 6).

The average degree of dominance, which is equal to 1.44 and b value of figure 2 (-65.97), showed that overdominance controlling this trait in most gene loci. For emergence percentage, there was no specific research on Tomato, but Zengin (2016) described the genetic structure of the 30 F1 hybrid tomato combinations and Two Male Testers. It has been suggested that both additive and non-additive effects control this trait.

Seedling Growth

Analysis of variance for this trait (Table 7) showed that general and specific combining ability and maternal effects were significant.

The average of heterosis for this trait was equal to -3.3. Although there were high positive heterosis values in this trait for some of the inbred-lines like R205 and LA3770 negative heterosis was more favorable. As much as the heterosis value became more negative, the time that seedling reached six leaves-stage was reduced.

This means that the progeny of LA3770 and CT6 had the fastest growth rate during the seedling stage (Table 8).

Table 5. Heterosis value for emergence percentage of each cross progeny.

	Me	KalN3	Fla	CT6	LA3770	R205	DB
Me	-	-3.75	-6	7.25	-6.75	19.25	16.25
KaLN3	29.25	-	-2.25	12.5	-21.5	-3	18
Fla	-11	-8.25	-	15.25	0.25	26.75	16.25
CT6	1.75	39.5	11.25	-	-11	22.5	-6
LA3770	-6.75	34	18.75	1	-	32.5	-18.5
R205	-2.25	-5	22.25	42.5	-11	-	3
DB	20.25	19	-5.75	2	11	1.5	-

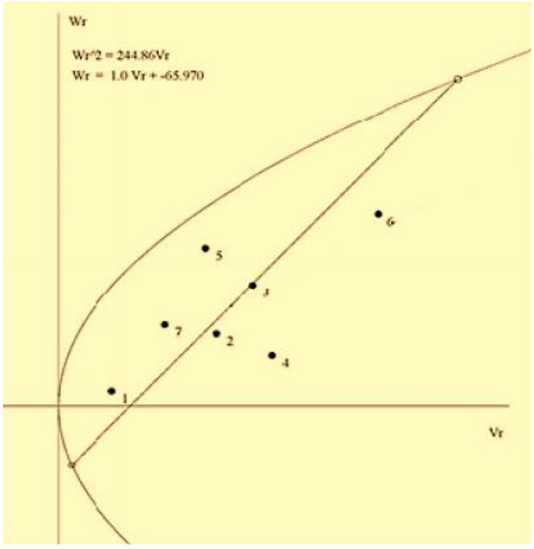

Fig. 2- Wr-Vr Diagram of Emergence Percentage, Wr = aVr + b.

1-ME, 2-KaLN3, 3-Fla, 4-CT6, 5-LA3770, 6- R205, 7-DB.

Table 7. Analysis of variance for seedling emergence rate based on Griffing (1956)

	df	SS	F	
Block	3	105.33	0.63 $^{n.s}$	(Rep/Error)
GCA	6	4220.72	2.04 *	(GCA/SCA)
SCA	14	2068	12.46 **	(SCA/Error)
Maternal effects	21	2413.33	14.54 **	(Rec/Error)
Error	123	165.97		

n.s: not significant

*: significant at P≤5%.

**: significant at P≤1%.

In this trait, lower values of specific combining ability mean that the F1 hybrid was more resistant to saline conditions, so CT6 × LA3770 and R205 × DB hybrids were the best (Table 9). Analysis of figure 3 showed that the b value was equal to -452.866. Considering b value and average dominance degree (2.79), we can conclude that overdominance may have a significant role in controlling this trait in saline conditions.

Conclusion

For germination percentage and seedling emergence and growth in studied tomato lines, we had concluded that R205 ، CT6 ، LA3770 , KaLN3 and their progeny had better performance in saline condition, and the best line for tolerance to salinity during the seedling stage was R205, which had a high amount of GCA in total, and in it's progeny, or one of them performed better than the others. It may be beneficial to combine this line as a parental line for salinity tolerance increasing of tomato seedlings in breeding programs.

Table 8. heterosis value for emergence rate of each cross progeny

	Me	KalN3	Fla	CT6	LA3770	R205	DB
Me	-	-15.85	-30.1	17.25	-24.55	-24.8	-0.5
KaLN3	-28.85	-	21.55	-25.6	18.1	65.85	-6.85
Fla	-43.1	-77.95	-	-37.35	20.35	51.6	3.9
CT6	-58.25	-51.6	43.15	-	-65.3	46.95	-53.25
LA3770	7.95	-20.4	-58.65	-31.3	-	18.65	40.95
R205	21.7	53.35	-29.4	51.95	8.15	-	-3.3
DB	-18	9.65	2.4	32.75	19.45	10.7	-

DOI:10.22111/jep.2020.31828.1017

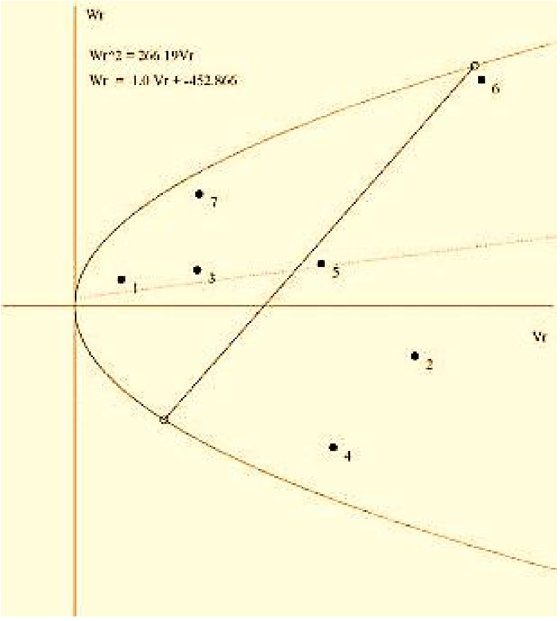

Fig. 3- Wr-Vr Diagram of Emergence Rate, Wr = aVr + b.

1-ME, 2-KaLN3, 3-Fla, 4-CT6, 5-LA3770, 6- R205, 7-DB.

Table 9. Specific Combining Ability for Emergence Rate

	Me	**KalN3**	**Fla**	**CT6**	**LA3770**	**R205**	**DB**	σSij^2
Me	-24.83	-1.40	-8.15	7.65	13.45	-13.60	2.05	170.90
KaLN3	3.80	8.37	-13.60	-24.30	6.75	33.70	-1.15	336.67
Fla	1.45	-16.25	-11.88	24.70	-3.75	-7.30	8.10	185.90
CT6	-21.15	-18.10	34.05	-4.43	-33.20	30.75	-5.60	559.18
LA3770	18.10	3.65	-10.70	-12.05	5.27	-11.70	28.45	385.48
R205	5.85	21.40	-15.20	10.45	-5.30	16.32	-31.85	616.52
DB	-8.05	5.50	6.65	6.80	6.30	-17.20	11.17	338.88
σSij^2	236.49	194.62	322.72	375.30	115.37	230.77	104.66	

DOI:10.22111/jep.2020.31828.1017

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DOI:10.22111/jep.2020.31828.1017