

Production of Tomato Hybrids for Dry Zone Conditions of Sri Lanka Using Combining Ability Analysis, Heterosis and DNA Testing Procedures

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ABSTRACT. *The field performance of 45 tomato hybrids produced according to a half diallel genetic design was evaluated together with their parents at the Field Crops Research and Development Institute, MahaIlluppallama, Sri Lanka, during January to May 2004 in order to select suitable hybrids for cultivation under dry zone conditions. Molecular investigations were carried out to test for true hybridization. Yield, net photosynthesis rate, instantaneous transpiration efficiency, stomatal conductance and root length were evaluated in the parents and hybrids. Analysis of variance revealed existence of highly significant genetic variability between genotypes for all the traits studied. The general combining ability (GCA) was significant for all characters while the specific combining ability (SCA) was significant for all traits except root length, indicating the importance of both additive as well as dominant gene effects. The dominance variance was much higher than the additive variance indicating the possibility of producing superior hybrids. The variety LC5 showed the highest value for GCA for yield, stomatal conductance and root length while UP8 was the best combiner for photosynthetic rate and transpiration efficiency. Based on heterosis in all traits considered, hybrids (UP5 x UP1), (UP5 X UP3), (UP2 X UP3), and (UP8 X LC5) were recommended as new tomato hybrids with potential for cultivation under dry zone conditions. The micro-satellite molecular technique was effective in testing true hybrids.*

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) is an important vegetable crop which provides an array of fresh as well as processed products. Although it is not extensively grown in the dry zone of Sri Lanka due to lack of varieties suitable for cultivation under dry zone conditions, there is a great potential to grow tomatoes in these areas as they fetch very high returns. Therefore, one of the most important requirements at present is to breed and select high yielding tomato varieties especially for dry zone conditions in Sri Lanka. An understanding of the genetic basis of variation in the characters under the dry zone stress conditions such as high temperature and limited water availability will enable breeders to decide on the end-product of their improvement programmes, be they pure lines or hybrids, although hybrids showing heterosis are becoming increasingly

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popular world-wide. The choice of the breeding methodology is a function of the genetic architecture of the economic traits in the crop to be improved upon (Thirunani *et al.*, 2000). Combining ability is an effective tool, which gives useful genetic information for the choice of parents in terms of the performance of their hybrids (Dhillon, 1975; Chezian *et al.*, 2000; Sprague and Tatum 1942; Virmani, 1994, Singh, *et al.*, 1976).

Heterosis (hybrid vigour) refers to an improved performance of F1 hybrids over their parents. Heterosis is of great commercial importance, since it enables the breeder to generate a product (F1 hybrid seed) with preserved values, which in turn, allows the farmer to grow uniform plants expressing these heterotic features. Hybrid vigor has been exploited in the production of many crops such as rice, corn, sugar beet, canola, sunflower, cauliflower, onion and tomato. Hybrid vigour in tomato was first observed by Hedric and Booth (1907). Since then a number of workers have reported heterosis in tomato (Singh *et al.*, 1978; Bhatt *et al.*, 1998; Bhatt *et al.*, 2001). Kumar *et al.* (2003) reported 60% hybrid vigor in tomato. Champoux *et al.* (1995) observed correlations of root parameters in rice measured in greenhouse experiments with field drought avoidance/tolerance and Price *et al.* (2000) identified QTLs for root-penetration ability in rice. However, the studies of physiological parameters in relation to hybrid vigor have not been exploited to a great extent and information available on the genetic basis of quantitative traits under dry zone conditions in Sri Lanka is scanty.

The present research was, therefore, initiated to produce new tomato hybrids suitable for cultivation under dry zone conditions, by combining six local and four foreign varieties in a diallel genetic design and testing them under field conditions in the dry zone. Analysis of important quantitative traits such as yield, photosynthetic rate, transpiration efficiency and stomatal conductivity would provide useful information on the genetic basis of variation of these characters as well as on hybrid vigour.

Based on this information, hybrids suitable for cultivation under dry zone conditions can be selected. Microsatellite molecular markers were used to test for true hybridization in producing hybrids. Microsatellites or SSR or short tandem repeats (STRs) is a class of repetitive sequences which are widely distributed in all eukaryotic genomes. Being a codominant marker, the SSR technique is used to evaluate information on the expected heterozygosity. Microsatellite markers consisting of (GATA) repeats and dinucleotide motifs extracted from sequence databases have been studied (Arens *et al.*, 1995).

MATERIALS AND METHODS

A total of 45 F1 hybrids were produced using 10 varieties, including foreign varieties UP1, UP2, UP3, UP6, UP8, UP10 and local varieties LC4, LC5, LC7, and LC9 in a half diallel genetic design at the Agricultural Biotechnology Center, University of Peradeniya, from May to December 2003. They were evaluated using two replicates in a randomized complete block design at the Field Crops Research and Development Institute, Mahalluppallama, Sri Lanka from January to May 2004. Each treatment consisted of 10 plants at a row spacing of 80 cm and plant spacing of 50 cm. Applications of fertilizer and other cultural practices were carried out according to the recommendations of the Department of Agriculture.

DNA was extracted using modified CTAB method (Sanghai-Marooof *et al.*, 1984; Doyle and Doyle, 1987). Three grams of tender leaves were ground in liquid

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nitrogen until a powder was formed and transferred into 15 ml of pre-warmed (60°C) 4% CTAB buffer with 0.1% β -mercaptoethanol. After incubating for 30 min. at 60°C, 15 ml of Chloroform : Isoamyl alcohol (24:1) was added and gently shaken for 10 min. Thereafter, the solution was centrifuged for 10 min. at 5000 x g and the supernatant was filtered into a 50 ml glass tube. Finally, 0.6 volume of ice-cold isopropanol was added and gently mixed by inverting the tube. DNA was hooked out and washed twice with washing solution (1M Ammonium acetate and 70% ethanol). After drying the samples DNA was dissolved in 100-500 μ l of TE buffer and stored at 4°C. DNA was quantified using 260 and 280 nm wave lengths by diluting the stock 200 times. Diluted DNA of 50 ng/ μ l was used in the PCR reaction.

PCR was performed using 94°C for 3 min for initial denaturation and then 45 cycles (1 min. at 94°C for denaturation, 1 min. at 55°C for primer annealing, 2 min. 72°C extension) and a final extension at 72°C for 10 min. The reaction volume was 25 μ l. The final concentration in the reaction solution contained 0.5 μ M for each forward and reverse primer, 200 μ M dNTPs each, 1X PCR buffer, 2.5 mM of MgCl₂ and 0.025 U/ μ l of Taq DNA polymerase (Abgene) and 4 ng / μ l of DNA template.

SSR (microsatellite) primers selected at random from a sequence database (Table 1) were used in the PCR for amplification, and the resulting reaction products were electrophoresed on 6% denaturing polyacrylamide gels stained with silver nitrate (Samarasinghe *et al.*, 2002). DNA profiles were analyzed to test for true hybridization.

Table 1. SSR primers used to test true hybridization.

SSR Marker	Repeat Sequence	Fragment Size (bp)	N	Primer Sequence * 5'.....3'
TMS 45	(GA) ₁₇	249	4	CCGTCCAGAAGACGATGTAA
	(GT) ₆			CAAAGTCTTGCCAACAATCC
TMS 6	(GATA) ₄₅	335	3	CTCTCTCAATGTTTGTCTTTC
				GCAAGGTAGGTAGCTAGGGA
TMS 23	(GT) ₃₂	412	4	GGATTGTAGAGGTGTTGTIGG
	(AT) ₆₇			TTTGTAATTGACTTTGTCTGAIG

N = Number of alleles; * First and second sequences represent forward and reverse primers, respectively.

The Licor-6400 portable Photosynthetic Measurement System (LICOR, Nebraska, Lincoln USA) was used to measure the net photosynthesis rate (P_n), and the transpiration rate of fully expanded young leaves. Instantaneous transpiration efficiency (ITE) was calculated as the ratio between instantaneous photosynthesis and transpiration rates. Stomatal conductance (g_s) was recorded at the flowering stage using the LI-1600 Steady State Porometer. The root length was measured by collecting soil cores from 10cm depth and using a root grid (Newman, 1966). Yield data was collected from 5 competitive plants at the time of harvest.

The diallel crosses were analyzed following Griffing (1956). The additive and dominance variance, s^2_A and s^2_D were calculated (Table 2), and heterosis over the better parent was considered for all the traits.

Table 2. Estimation of expected mean squares

Source	Expected Mean Square
GCA	$S^2_e + s^2_s + (n+2)/2 s^2_g$
SCA	$S^2_e + s^2_s$
Error	S^2_e

The estimates of variance components, s^2_e , s^2_s and s^2_g represents error, general combining ability and specific combining ability, respectively. 'n' is the number of parents used in the diallel cross. The additive genetic variance and dominance variance were interpreted as follow:

$s^2_g = 1/2 s^2_A$ and therefore, $s^2_A = 2 s^2_g$, and
 $s^2_D = s^2_s$
 where, s^2_A and s^2_D refer to variances due to additive gene action and dominant gene action, respectively.

RESULTS AND DISCUSSION

The analysis of variance revealed highly significant ($P < 0.01$) genetic variability between genotypes for all the traits under study. GCA and SCA were highly significant ($P < 0.01$) for all the traits except SCA for root length ($P < 0.05$). This indicated the importance of both additive and non-additive genetic components in the genetic determination, expression and inheritance of these traits. Gunasekera and Perera (1999), Dod *et al.* (1992), Srivastava *et al.* (1998) have also observed significant GCA and SCA effects together with significant additive genetic variance as well as dominance for tomato yield and fruit quality characters. The parents and hybrids with the highest GCA and SCA values are shown in Table 3.

It can be observed that LC5 was the best combiner for yield, stomatal conductance and root length, whilst UP8 was the best for net photosynthesis rate and transpiration efficiency. Different parents were the best combiners for different traits respectively indicating differences in genetic superiority for different characters among the parents. Similar observations were reported by Bhatt *et al.* (2001) for tomato yield and quality characters. It is significant to note that LC5 did not appear as parents in the best hybrids for 5 characters, although UP8 was one parent in the best hybrid for yield.

Table 4 shows that the variance due to dominance gene effect was much greater than the additive gene effects for all characters. This indicated the possibility of exploiting dominance variance by producing hybrid varieties instead of pure lines.

Table 3. Parents and hybrids having the highest GCA and SCA values respectively.

Character	Best parent (GCA)	Mean	Best hybrid (SCA)	Mean
Yield (g/plant)	LC5	830.50	UP8 x UP1	1196.00
Pn ($\mu\text{mol}[\text{CO}_2]\text{m}^{-2}\text{s}^{-1}$)	UP8	28.88	LC4 x UP3	22.20
ITE ($\mu\text{mol}[\text{CO}_2] \mu\text{mol}[\text{H}_2\text{O}]^{-1}$)	UP8	2.67	UP1 x LC4	2.12
g_s (cm s^{-1})	LC5	0.24	UP10 x UP1	0.16
Root length (cm)	LC5	541.80	LC9 x UP3	214.50

Table 4. Estimates of additive genetic and dominance variances for the traits.

Character	Additive genetic variance	Dominance variance
Yield (g/plant)	115345.7	187203.1
Pn ($\mu\text{mol}[\text{CO}_2]\text{m}^{-2}\text{s}^{-1}$)	-1.93	7.9
ITE ($\mu\text{mol}[\text{CO}_2] \mu\text{mol}[\text{H}_2\text{O}]^{-1}$)	0.015	0.102
g_s (cm s^{-1})	-0.0002	0.0007
Root length (cm)	3013.2	5840.6

Accordingly, the 10 best hybrids were selected and ranked based on per cent heterosis over their respective better parent (BP). LC5 appeared as one parent in six of the best hybrids for yield (Table 5).

In the present study, very high heterosis was detected for yield with UP2 x UP10 showing >200% heterosis (Table 5). Ahmed *et al.* (1988), Patil and Bojappa (1988) and Valicek and Obeidat (1987) have also reported similar results. Heterosis for yield is the end product of many intricate physiological and biochemical processes. Sinha and Khanna (1975) observed that, although some hybrids showed heterosis for photosynthesis at the seedling stage, clear evidence for the superiority of hybrids for photosynthesis was lacking. Akita (1988) reported both positive heterosis and little heterosis for photosynthesis efficiency in rice. But the genetic basis of heterosis for yield in tomato remains elusive. In some crosses of tomato, Lu (1983) reported heterosis for photosynthesis rate while in other crosses there was no heterotic effect. However, Griffing (1990) reported that heterosis is a consequence of faster hybrid growth rate by exploiting available nutrients. In the present study most hybrids showed high heterosis for net photosynthesis rate with UP10 x UP3 showing >50% heterosis (Table 6).

Table 5. Yield performance of the best hybrids based on per cent heterosis.

Hybrid Combination	Mean (g/plant)	% Heterosis (>BP)
LC5 X UP1	1930.50	132.45
LC5 X UP6	1769.50	113.06
LC5 X UP3	1591.50	91.63
UP2 X UP10	1589.00	202.38
LC5 X UP8	1519.50	82.96
LC5 X UP10	1517.50	82.72
UP2 X UP3	1442.50	174.50
UP2 X UP1	1373.00	161.27
UP2 X LC 9	1287.50	145.00
LC5 X UP2	1270.00	52.92

Table 6. Performance of the best hybrids in net photosynthesis rate.

Hybrid combination	Mean ($\mu\text{mol} [\text{CO}_2] \text{ m}^{-2}\text{s}^{-1}$)	% Heterosis (>BP)
LC7 X LC4	31.39	41.14
LC7 X UP3	30.91	39.09
UP8 X LC4	30.85	- 8.94
UP10 X UP3	30.55	54.89
UP10 X LC9	30.11	33.23
UP2 X UP3	29.75	27.50
LC5 X UP3	29.42	8.50
LC9 X UP1	29.42	21.29
LC7 X UP2	29.16	24.48
UP10 X UP2	29.04	24.47

UP10 x UP3 and LC7 x UP3 showed >50% heterosis for ITE (Table 7). Many studies have reported gene cetic variability in the number of moles of CO_2 fixed per unit of water lost from leaf ITE. High heterosis was observed for stomatal conductance with UP2 x UP3, UP10 x UP3, UP6 x UP3 and UP6 x UP2 showing >40% heterosis (Table 8). Reduced stomatal conductance will reduce water loss and dehydration. However because, stomata influence the influx of CO_2 into leaves, it could lower the photosynthetic rate. Jones (1987) reported the genetic variability and the heritability of various stomatal characteristics.

Table 7. Performance of the best hybrids in instantaneous transpiration efficiency.

Hybrid combination	Mean ($\mu\text{mol} [\text{CO}_2] \mu\text{mol} [\text{H}_2\text{O}]^{-1}$)	% Heterosis (>BP)
UP2 X UP3	3.11	39.28
LC9 X UP1	2.94	34.85
LC7 X UP3	2.89	55.53
UP8 X UP6	2.83	-10.49
UP6 X UP3	2.74	9.47
UP8 X LC4	2.67	-15.52
LC7 X UP6	2.63	4.77
UP10 X UP3	2.58	54.47
LC5 X UP1	2.57	15.15
LC7 X LC4	2.57	36.54

Table 8. Performance of the hybrids in stomatal conductance.

Hybrid combination	Mean (cm s^{-1})	% Heterosis (>BP)
LC5X UP3	0.30	22.92
UP2X UP3	0.28	75.00
UP1X UP3	0.27	58.82
LC5X UP1	0.27	12.50
UP1X LC5	0.26	8.33
UP6X UP3	0.26	47.22
UP6X UP2	0.26	44.44
UP6X UP1	0.26	36.84
LC5X UP8	0.25	-6.00
LC5X UP6	0.25	4.17

UP10 x UP6 showed >300% heterosis for root length (Table 9). Heterosis for root growth has been observed in many studies (Sinha and Khanna, 1975). Root growth depends upon shoot growth and it serves as a sink to excess photosynthates until an alternative “sink” is developed in the shape of storage organs like fruits, grains or tubers. It is therefore, likely that the enhanced photosynthetic potential of a hybrid could lead to enhanced root growth. In contrast, the cost of root growth maintenance represents clear diversion of assimilate which might have been used for shoot growth and thus may decrease yield potential. Moreover greater depth and extent of soil water extraction could increase the amount of water transpired, and avoid water deficits at critical growth stages. Therefore could increase photosynthesis, transpiration (Jones and Zur, 1984) and yield (Machow and Sinclair, 1986).

Table 9. Performance of the best hybrids in root length.

Hybrid combination	Mean (cm)	% Heterosis (>BP)
UP8X LC4	451.50	72.00
UP8X UP2	387.00	17.45
UP10 X UP6	359.00	348.75
LC5X LC9	326.00	-50.15
UP6X UP1	300.50	61.13
UP1X UP2	258.50	-21.55
LC9X UP3	258.00	-33.25
UP8X UP1	223.00	19.57
LC5X UP8	221.50	-66.13
LC5X LC4	220.50	-66.28

A significant feature in the analysis is that the hybrids with the best SCA were not necessarily those that showed high heterosis for the respective characters. The hybrids were therefore ranked according to the 7 characters (Table 10). Accordingly, as expected, it is evident that no single hybrid showed superiority for all characters. Nevertheless, the hybrids LC5 X UP1, LC5 X UP3, UP2 x UP3 and LC5 X UP8 can be selected as superior hybrids over the others, although a selection method such as the index method can be used to make the final decision amongst them.

The hybrids were identified as true hybrids by using molecular techniques. SSR markers were used to identify the hybrids as shown in Fig. 1. Since tomato is a highly self-pollinated crop, the microsatellite primers very clearly identified the hybrids as true hybrids as shown in Fig. 1. Apart from the DNA band present in female parent, the band belonging to the male parent appeared in all the hybrids as expected in true hybridization.

Table 10. Overall ranking of ten best hybrids.

Hybrid	Yield	Net Photosynthesis rate	Instantaneous transpiration efficiency	Stomatal conductance	Root length
CL5 x UP1	1	*	9	4	*
CL5 x UP6	2	*	*	10	*
CL5 x UP3	3	7	*	1	*
UP2 x UP10	4	10	*	*	6
CL5 x UP8	5	*	*	9	9
CL5 x UP10	6	*	*	5	*
UP2 x UP3	7	6	1	2	*
UP2 x UP1	8	*	*	*	*
UP2 x CL9	9	*	*	*	*
UP2 x CL5	10	*	*	*	*

* represents ranking beyond 10.

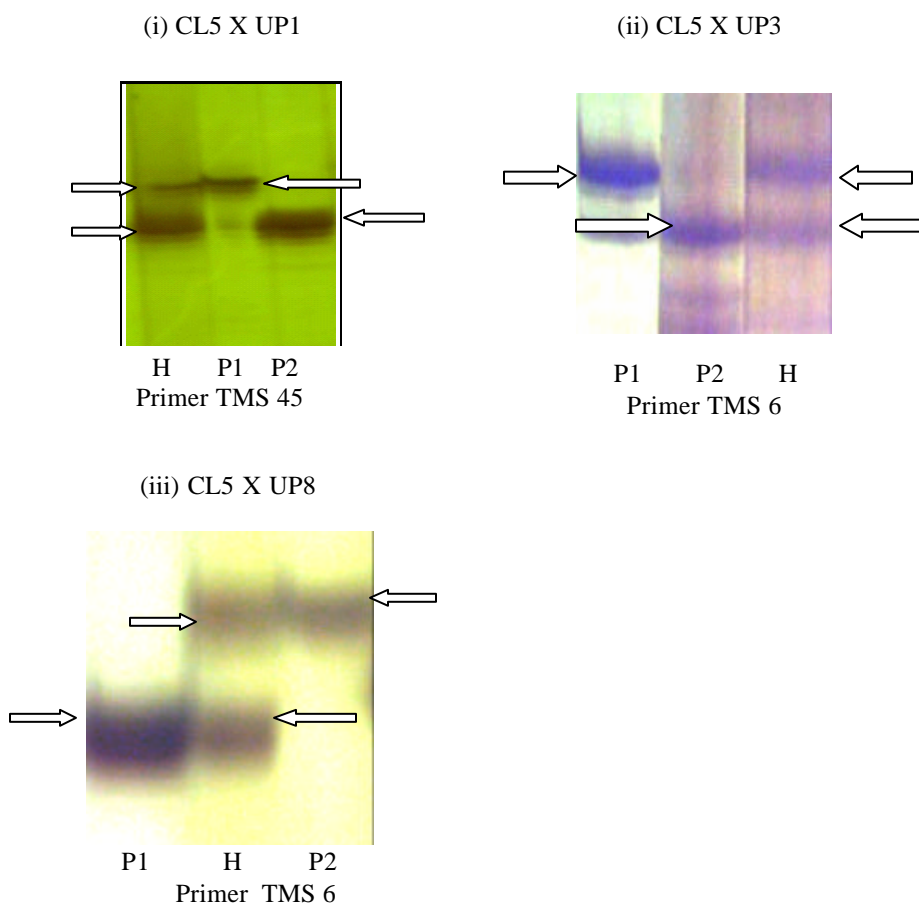


Fig 1. Testing of tomato hybrid DNA using microsatellite primers. (H: Hybrid and P1, P2: parents).

CONCLUSIONS

Since the variance due to dominant gene effects for the 5 characters under study was much higher than that due to additive genes, promising hybrids could be produced from the germplasm used in this study. LC5 is a superior parent showing very high general combining ability with respect to yield, stomatal conductance and root length. UP8 is the superior variety with respect to photosynthesis rate and instantaneous transpiration efficiency. The very high heterosis values shown in this study indicate that producing superior hybrids with high yield potential, specially for the dry zone conditions in Sri Lanka, could be achieved successfully by hybridization programmes. LC5 X UP1, LC5 X UP3, UP2 X UP3 and LC5 X UP8 could be recommended as superior hybrids for the dry zone conditions in Sri Lanka. Further selection of the best hybrid should be based on the Index selection approach. Microsatellite markers can be used to test for true hybrids.

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