Zengin S *et al.* (2020)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca 48(1):388-397
DOI:10.15835/nbha48111732
Research Article

Relationship between some morphological traits of the tomato lines and resistance to tomato yellow leaf curl virus disease

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Abstract

Tomato (*Solanum lycopersicum* L.) is the most produced and exported vegetable in Turkey. There are many pathogens to limit tomato production by reducing yield and fruit quality. Among them, Tomato Yellow Leaf Curl Virus (TYLCV) causes important economic losses. The most efficient and environmentally friendly method against TYLCV is the use of resistant varieties. In this trial, it was aimed to determine some morphological traits which were linked to *Ty-1* and *Ty-3a* genes which confer to TYLCV. A commercial hybrid carrying *Ty-1*, *Ty-3a* genes as heterozygous was crossed to a susceptible inbred line from Bati Akdeniz Agricultural and Research Institute (BATEM). Marker assisted selection (MAS) was carried out in F1 and F2 generations and biological tests were done for TYLCV resistance in F3 generation. MAS for *Ty-3a* and *Ty-1* genes were compatible with biological tests. In total of 95 genotypes in F3 were developed with molecular marker selection. It was determined that 30 genotypes having the *Ty-3a* and *Ty-1* genes as homozygous resistant. The nine genotypes carried these genes in heterozygous form. 56 genotypes were identified as susceptible. The 43 morphological traits were observed in identified individuals to correlate with resistant allele, *Ty-3a*. It was found that there was statistically important correlation between *Ty-3a* and length of internode, length of stem at first inflorescence, status of calix, leaf attitude, length of inflorescence and plant habitus. Also, there was negative correlation between fruit weight, fruit length and resistance. Therefore, we identified some morphological markers linked to *Ty-3a* which can be used in selection for TLYCV resistant breeding programme.

Keywords: biological test; MAS; morphological traits; tomato; *ty-3a*, resistance

Introduction

Tomato (*Solanum lycopersicum* L.,) is one of the most economically important vegetables in the world. The world tomato production is over 163 million tons from an area of 4.83 million hectares (FAO, 2017). Yield, fruit quality, shelf-life, and nutritional content are reduced when tomato crops can be infected by disease-causing bacterial, fungal, and viral pathogens (Oerke, 2006; Hanson *et al*., 2016). Among the virus diseases, the tomato yellow leaf curl virus (TYLCV) is the most destructive disease and causes loss of yield between 85% and 100% (Pico *et al*., 1996; Czosnek and Laterrot, 1997; Polston and Anderson, 1997). TYLCV is transmitted by

Received: 19 Nov 2019. Received in revised form: 18 Mar 2020. Accepted: 20 Mar 2020. Published online: 31 Mar 2020.

the whitefly *Bemisia tabaci* (Zamir *et al*., 1994; Ghanim and Czosnek, 2000). Domestic *S. lycopersicum* species were found to be susceptible to TYLCV by (Hassan *et al*., 1991). But some wild tomato species have been discovered as resistant source for TYLCV (Pico *et al*., 1996; Scott, 2007). Among the identified six loci (*Ty-1* to *Ty-6*) for TYLCV resistance, *Ty-3*, *Ty-3a* and *Ty-1* genes mapped on chromosome 6 provide a very large part of the resistance. *Ty-3*, *Ty-3a* genes were obtained from *S. chilense* accessions such as LA2779 and LA1932, respectively (Ji *et al*., 2007), *Ty-1* gene was obtained from LA1969 (Zamir *et al*., 1994).

Although *Ty-3* gene provides wide resistance, *Ty-3a* gene is mostly preferred in breeding program because of many undesirable morphological traits that are related with *Ty-3* gene. There is no effective chemical treatment to control viral diseases that results in severe damage, such as TLYCV, therefore, the use of resistant varieties is the best and environmentally friendly method for pathogen management (Cho *et al*., 1995; Oerke, 2006; Mutlu *et al*., 2015). Furthermore, the gene pyramiding that combines multi-resistant genes in a variety via molecular markers, has been an important component of modern tomato breeding programme. Tomato is very rich in the number of available molecular markers (Foolad, 2007), thus marker assisted selection (MAS) in tomato breeding programme have been effectively used for transferring of many disease resistance genes. It has been shown that some of resistant genes were in the linkage with other genes, having epistatic properties (Gómez *et al.,* 2004; Mejía *et al*., 2005; Rani *et al*., 2008; Consuegra *et al*., 2015). Therefore, the aim of this research is to determine whether there is a relationship between morphological traits and *Ty-1* and *Ty-3a*, resistant genes against to TYLCV. Thus, it is possible to make a selection via morphological markers linked to the TLYCV genes, as an alternative MAS in resistance breeding.

Materials and Methods

Plant material

Segregation populations (F1, F2 and F3) were obtained by crossing a susceptible line with a heterozygous resistant commercial variety ('TayfunF1'). 'Tayfun F1' having *Ty-1* and *Ty-3a* genes was obtained from Antalya Seed Company. Susceptible line called BATEM1 from Bati Akdeniz Agricultural Research Institute (BATEM), had fruit weight of 180 g. *Ty-1* and *Ty-3a,* genes via molecular markers were screened in F1, F2 and F3 population.

Molecular markers and PCR amplifications

Genomic DNA of the parents and F1, F2, F3 individuals was isolated from the fresh leaves according to CTAB method (Doyle and Doyle, 1987). The presence of the resistance genes *Ty-1* and *Ty-3a* was investigated essentially, using CAPS JB1 (De Castro *et al*., 2007) and SCAR P6-25 (Ji *et al*., 2007) primers, respectively. Amplifications were performed in thermalcycler in a 20 µL final volume, containing 25 ng of genomic DNA, 1X reaction buffer, 0.5 mM of $MgCl_2$, 0.6 mM of each dNTP, 0.5 µM of each primer and 1 U of Taq DNA polymerase. For the marker of *Ty-1*, the PCR programme was as follows after initial denaturation for 2 min at 95 °C. 19 cycles of 10s at 94 °C, 30 s 55 °C s, 1 min 10 s 72 °C and 9 cycles of 10 s 94 °C, 30 s 53 °C, 1 min 10 s 72 °C and a final extension of 5 min at 72° C.

After PCR amplification, 5 µl of the PCR products were digested with the restriction enzyme Taq1 following the manufacturer protocol (Thermo Fisher Scientific). In the case of the marker of *Ty-3a*, named P6-25, the PCR reaction was as follows: denaturation for 4 min at 94 °C, 34 cycles each consisting of 30 s at 94 °C, 60 s at 53 °C, 60 s at 72 °C and a final extension of 10 min at 72 °C.

All PCR products were separated on a 1.5% agarose gel (Sigma, St. Louis, MO), visualized with ethidium bromide under UV light.

TYLCV inoculation and disease screening

The seedlings were grown in equal volume of steam-sterilized perlite: peat mix. The experiment was conducted in a completely randomized block with two replicates and ten plants were tested each replicate. 'Tayfun F1' (as resistant hybrid), Batem Ozcelik F1 (as susceptible hybrid) and F3 individuals, were inoculated with Whiteflies (*Bemisia tabaci*, Biotype B =*Bemisia argentifolii*) collected from cotton plants (*Gossypium hirsutum*). Inoculation test was carried out in insect-proof wooden cages at 24-27 °C in a controlled temperature room provided with 16 h illumination per day (Zeidan and Czosnek, 1991). To transmit the virus, whiteflies were grown on TYLCV-infected tomato plants. TYLCV was acquired by whiteflies during a 48-h acquisition access period on TYLCV-infected tomato plants. The plants were scored at 56 days after inoculation based on symptoms or no symptoms on the leaves (Scott and Schuster, 1991).

Morphological traits

95 F3 individuals which were selected by molecular markers, were also evaluated based on 43 morphological traits according to UPOV criteria (Table 1).

Table 1. Morphological traits

Plant growth habit	determinate, indeterminate, semi determinate
Length of plant	short, medium, long
Plant stem thickness	weak, medium, strong
Stem: length of internode	short, medium, long
Length of stem at first inflorescence	short, medium, long
Stem hairs	weak, medium, strong
Leaf: attitude	erect, horizontal, drooping
Plant habitus	weak, medium, strong
Leaf: length	short, medium, long
Leaf width	narrow, medium, broad
Leaf: intensity of green color	light, medium, dark green
Flower sepal color	yellow, white
Fruit set on Inflorescence	weak, medium, strong
Fruit green shoulder (before maturity)	absent, present
Fruit: intensity of green color (before maturity)	light, medium, dark green
Length of inflorescence	short, medium, long
Inflorescence type	uniparous, forked, multiparous or irregular
Number of fruits on second inflorescence	average 5 plants
Uniformity of fruit on plant	bad, medium, good
Fruit weight	average of 10 fruits
Status of calix	horizontal, medium, vertical
Thickness of calix	weak, medium, strong
Colors of petal	light, medium, dark green
Length of fruit stalk	short, medium, long
Presence of jointless pedicel	flattened, slightly flattened, strong flattened
Size of dry section around the fruit	small, medium large
Predominant fruit shape (After the fruit turns color)	flattened, oblate, circular, oblong, elliptic, or obovate
Fruit width	average of 5 fruits
Fruit length	average of 5 fruits
Green shoulder (at maturity)	absent, present
Fruit: shape at blossom end	indented, indented to flat, flat to pointed, pointed

Size of blossom end	small, medium, large
Blossom end shape	indented, flat, pointed
Fruit color intensity at maturity	dark, light
Fruit: firmness	very soft, very firm
Transvers section	round, angular, irregular
Fruit: thickness of pericarp	average 5 fruits
Size of fruit locules	small, medium, big
Fruit number of locules	average 5 fruits
Fruit puffiness at maturity	absent, present
Fruit concentric cracks at maturity	absent, present
Fruit radial cracks at maturity	absent, present
Cat face at maturity	absent, present

Data analyses

The correlation between the disease resistance gene and 43 traits were analysed by using Pearson correlation analysis in SPSS 14.0 program.

Results and Discussion

Segregation of Ty-1 and Ty-3a by molecular markers

F1 plants which were obtained by crossing Tayfun F1 and BATEM1 pure line, were screened with molecular markers linked to *Ty-1* and *Ty-3a*. F2 and F3 can be obtained by selfing F1 and F2 plants. Individuals of F2 and F3 population were screened via molecular markers to determine segregation of the *Ty-1* and *Ty-3a* genes.

Ty-1 gene

Ty-1 gene had two allelic forms based on molecular marker (JB1). The susceptible pure lines had susceptible allele (ty1:ty1) at 400 bp and commercial hybrid was heterozygous form having band at 400 bp for susceptible allele and 500 bp for resistant allele. The susceptible individuals of F2 and F3 had allele at 400 bp and resistant individuals had alleles at both 400 and 500 bp (Figure 1).

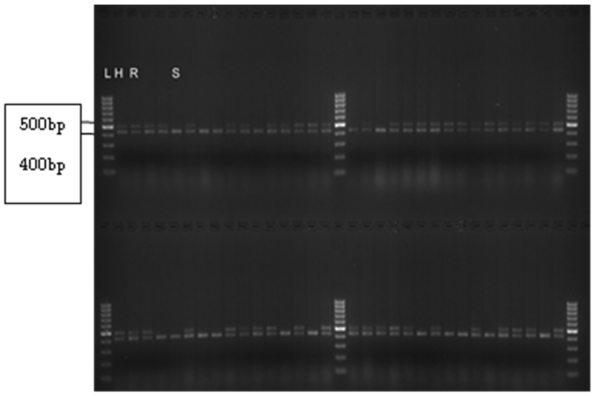

Figure 1. PCR fragments represent CAPS JB1 marker for *Ty-1* gene. Susceptible individuals of F2 and F3 had alleles at 400 bp and resistant individuals had alleles at both 400 and 500 bp.L: 100 bp DNA ladder, R: homozygous resistant, H: heterozygote resistance, S: Susceptible genotypes

Ty-3a gene

Two different allelic forms for Ty3a were observed in F1 individuals, that susceptible ones had allele at 320 bp and heterozygous individual carried alleles at 320 and 630 bp. The individuals of segregating populations carried three different allelic forms at 320 bp for susceptible or at both 320 bp and 630 bp for heterozygous resistant or at 630 bp homozygous resistant (Figure 2). The *Ty-3a* gene is more preferred due to the fact that the *Ty-3* gene provides a wider durability, and many other features are undesirable with the gene.

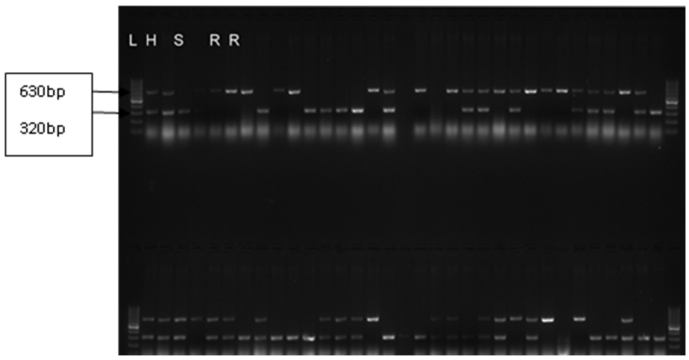

Figure 2. PCR fragments represent SCAR P6-25 marker for *Ty-3a* gene. The individuals of segregating populations carried three different allelic forms at 320 bp for susceptible or at both 320 bp and 630 bp for heterozygous resistant or at 630 bp homozygous resistant. L: 100 bp DNA ladder, R: homozygous resistant, H: heterozygote resistance, S: Susceptible genotypes

Segregation ratios for resistant and susceptible individuals for F1 were checked with Chi-square test. The resistant Tayfun F1 was crossed with the susceptible line to obtain F1 plants which segregated resistant and susceptible plants in a 1:1 ratio (Table 2). According to χ^2 (Chi-square) analysis, *Ty-1* and *Ty-3a* based on molecular marker show equal segregation at statistical significance level of 0.05.

Table 2. The segregation analysis of reaction of F1 plants resistance to *Ty-1* and *Ty-3a* genes

Gene	Phenotypic segregation		
	Observed F1 plants	Expected F1 plants	Chi-square (χ^2)
Ty1ty1	190	200	1.0
ty1ty1	210	200	
Ty3aty3a	190	200	
ty3aty3a	210	200	

χ^2 (0.05)=3.841

The segregation of the *Ty-3a* gene in F2 population tested by molecular marker was homozygous resistant in 99 individuals and heterozygous resistant in 194 individuals and homozygous susceptible in 107 individuals. Moreover, the 107 susceptible individuals for *Ty-3a* were also found to be susceptible for the *Ty-1* gene (Table 3).

Table 3. The segregation analysis of reaction of F2 plants resistance to *Ty-1* and *Ty-3a* genes

	Observed F2 plants			Expected F1 plants			Chi- square
	RR	Rr	Rr	RR	Rr	rr	
Ty3	99	194	107	100	200	100	0,68
Ty1	293		107	300		100	0,65

X^2(0.05)=3.84, RR: Homozygot resistant, Rr: heterozygote resistant, rr: susceptible

Chi-square analysis was used to determined segregation ratio for *Ty-1* and *Ty-3a* in F2 population. According to results segregation ratio was evaluated the same distribution at statistical significance level of 0.05 (Figures 3 and 4).

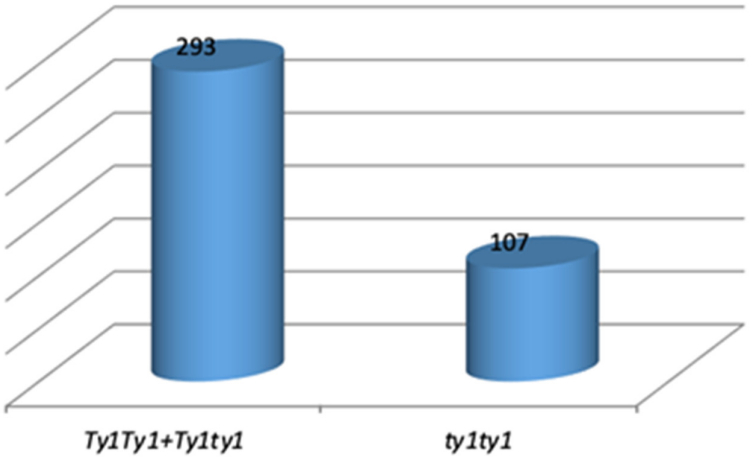

Figure 3. Segregation of F2 genotypes in terms of *Ty-1* gene

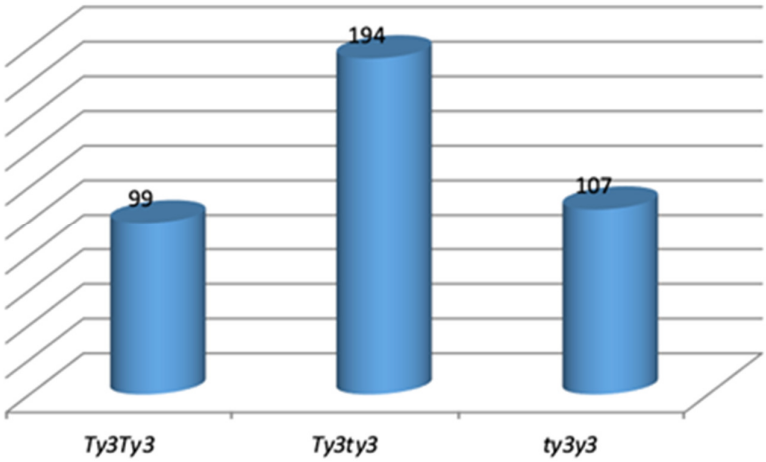

Figure 4. Segregation of F2 genotypes in terms of *Ty-3a* gene

Ty-1 gene

Although three different alleles (allele 1 at 400 pb, allele 2 at slightly larger than 400 pb and allele 3at 500 pb) for *TY-1* gene; were identified by De Castro *et al*. (2007), in our research the individuals of segregating populations and parents had two alleles. Also, allele at 500 bp were dominant over allele at 400 bp.

Ty-3a gene

The individuals of segregating populations carried three different allelic forms at 320 bp for susceptible or at both 320 bp and 630 bp for heterozygous resistant or at 630 bp homozygous resistant (Ji *et al*., 2007). The Ty-3a gene is more preferred due to the fact that the *Ty-3* gene provides a wider durability, and many other features are undesirable with the gene.

Segregation ratios for resistant and susceptible individuals for F1 were checked with Chi-square test. In this research it was determined that *Ty-1* and *Ty-3a* genes did not segregate in the segregating populations and all individuals had both alleles together. These results also provided that *Ty-1* and *Ty-3a* were alleles of the same gene (Ji *et al*., 2007; Verlaan *et al*., 2013).

Disease assessment

In the biological test 'Tayfun F1' (as resistant hybrid), 'Batem Ozcelik' F1 (as susceptible hybrid) and F3 individuals, inoculated with Whiteflies. There were no symptoms of resistant control varieties while susceptible hybrid showed disease symptoms. Ten F3 individuals which were determined as homozygous resistant by molecular marker for *Ty-1* and *Ty-3a*, were also found to be resistant by biological test. Disease symptoms were not observed in the resistant F3 individuals. In contrast, F3 individuals which were evaluated as susceptible with molecular marker, showed the disease symptoms. There were no disease symptoms in only one plant of each susceptible DT-27, DT-37, DT-224, DT-246 individuals and 2 plants in susceptible DT-109 individual.

The individuals determined as heterozygous via marker test in the F2 generations segregated as 3:1 ratio based on biological test in F3 generations. According to the chi-square analysis, it was determined that the distribution at 0.05 level was 3:1 ratio. Some breeders could obtain false data in negative and positive results because they can't calculate the distance of markers P6-25 stated (Jensen *et al*., 2007).

Especially; emphasized that faulty results could be obtained from the sources transferred from different wild species of tomato to culture tomato. However, our results of the marker and biological testing were found to be compatible with each other.

The relationship between the characteristics of the lines and disease resistance

In the total 95 F3 individuals were evaluated based on both morphological traits and disease resistance. The 56 individuals were determined as a susceptible 9 individuals were heterozygous resistance and 30 individuals were homozygous resistant by molecular markers.

There was statistically significant positive correlation between *Ty-3a* and *Ty-1* genes and lengths of stem at first inflorescence, stem length of internode, leaf attitude, plant habitus, length of inflorescence and status of calix position of petal (Table 4). The length of stem at first inflorescence and stem length of internode directly affects the yield of tomato production. According to (Balliu and Hallidri, 2002), the length of internode was determined by the addition effect. Indeterminate varieties have less cluster than determinate varieties so the yield is decrease in indeterminate varieties. For this reason, breeders have preferred the varieties which have short length of stem at first inflorescence and short stem length of internode.

However, in this research we determined that as the stem length of internode and the length of inflorescence in tomato increased, the tomato individuals carried homozygous *Ty-3a* gene. Also, the leaf size and the length of internode were determined to be significant in reduction at susceptible tomato plants (Padidam *et al*., 1995). Therefore, it can be stated that the length of internode can be used as selection criteria for resistant breeding programme in tomato.

Leaf attitude was evaluated at three different position (erect, horizontal, drooping) according to UPOV. There was a significant positive correlation between resistance to TLYCV and leaf attitude (Table 4).

The individuals having leaf at dropping position was resistant to TLYCV indicating that these genotypes carried *Ty-3a* gene. The producers and breeders generally prefer the tomato variety with horizontal leaf position to prevent the transmission of fungal diseases. Because it believes that the horizontal leaf position can allow the plants to get better air. In contrast to this, we determined that the resistant tomato individuals had drooping leaf position.

Plant habitus was evaluated as weak, medium and strong type. The plant habitus of the resistant tomato individuals were weak type. Also, the resistant tomato individuals had long length of inflorescence. Although

not statistically significant, there was a negative correlation between resistance to TLYCV and fruit characteristics such as fruit weight and fruit length (Table 4).

Table 4. The correlation coefficient value between disease resistance genes and morphological traits

Traits	*Ty-3a and Ty-1*	Traits	*Ty-3a and Ty-1*
Plant growth habit	0.11	Colors of petal	0.06
Length of plant	-0.12	Length of fruit stalk	-0.33
Plant stem thickness	-0.14	Presence of jointless pedicel	-0.08
Stem: length of internode	**0.23***	Size of dry section around the	0.06
Length of stem at first	**0.23***	Predominant fruit shape	0.00
Stem hairs	0.00	Fruit length	**-0.20**
Leaf: attitude	**0.26****	Fruit width	-0.17
Plant habitus	**0.30****	Green shoulder (at maturity)	-0.02
Leaf: length	-0.09	Fruit: shape at blossom end	0.11
Leaf width	-0.08	Size of blossom end	0.06
Leaf: intensity of green colour	-0.07	Blossom end shape	-0.17
Flower sepal colour	0.00	Fruit colour intensity at	-0.15
Fruit set on Inflorescence	-0.13	Fruit: firmness	0.09
Fruit green shoulder (before	-0.13	Transvers section	-0.17
Fruit: intensity of green colour	-0.01	Fruit: thickness of pericarp	-0.14
Length of inflorescence	**0.40****	Size of fruit locules	-0.18
Inflorescence type	0.16	Fruit number of locules	0.13
Number of fruits on second	-0.14	Fruit puffiness at maturity	-0.14
Uniformity of fruit on plant	0.00	Fruit concentric cracks at	0.00
Fruit weight	**-0.18**	Fruit radial cracks at maturity	-0.01
Status of calix Position of petal	**0.24***	Cat face at maturity	-0.13
Thickness of calix	-0.06		

As the fruit weight decreased, the resistance to TYLCV increased. It was also determined that fruit size was affected with resistance gene and homozygous resistant gene resulted in the middle-small fruit group (Consuegra *et al*., 2015). Two QTLs for fruit weight were identified on chromosomes 2 and the wild alleles were associated with a reduction in fruit weight (Chen *et al*., 1999; Grandillo *et al*., 1999; Doganlar *et al*., 2002). Five QTLs were identified for fruit length and similarly the most significant QTL for fruit length was detected on chromosome 2 (Doganlar *et al*., 2002).

The transfer of resistance genes from wild genotypes to culture forms were affected both the fruit and the morphological structure of the genotypes (Tanksley, 2004). As (Gómez *et al*., 2004; Mejía, 2005) pointed, we determined that the resistant individuals had undesirable plant traits such as reduction of the number of fruits per plant and fruit traits. (Consuegra *et al*., 2015) also found a statistically significant and negative correlation between resistance to TLYCV and the number of fruits in the first 4 clusters, the number of branches per plant, the number of fruits per plant and yield per plant.

It can be stated that in breeding programs for resistance which don't use marker technology, resistant genotypes can be eliminated due to their undesirable fruit and plant characteristics such as small sized fruit, weak plant habitus, a smaller number of fruits per plant.

Conclusions

Molecular Marker Assisted Selection (MAS) has been successfully used to select resistant genotypes in the breeding study. In this research, we identified 95 individuals of F3 population based on their morphologic traits and their resistance to TLCV by molecular markers linked to *Ty-1* and *Ty3-a* and by biological test. It was determined the 30 individuals having homozygous resistant alleles, the 9 individuals having heterozygous alleles for both *Ty-3a* and *Ty-1* genes. The 56 individuals were susceptible against to TLYCV.

In our study, it was found that the *Ty-1* and *Ty-3a* genes did not segregated in any individuals and were together in all individuals. Thus, it can be concluded that *Ty-3a* and *Ty-1* genes could be allele genes. The 43 morphological traits were observed in the selected individuals of the F3 population by molecular marker to correlate with resistant allele, *Ty-3a*. It was found that there was statistically significant correlation between Ty-3a and length of internode, length of stem at first inflorescence, status of calix position of petal, leaf: attitude, length of inflorescence, plant habitus.

Also, there was negative correlation between fruit weight, fruit length and *Ty3-a* gene. Consequently, we identified some morphological markers such as stem at first inflorescence, stem length of internode, leaf attitude, plant habitus, length of inflorescence, status of calix position of petal, fruit weight and fruit length linked to *Ty-3a* which can be used in selection for TLYCV resistant breeding programme.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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