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QTL identification for tolerance to fruit set in tomato by fAFLP markers

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ABSTRACT - *This study aimed to detect quantitative trait loci (QTL) by fAFLP (Fluorescent Amplified Fragment Length Polymorphism) markers associated to the trait tomato fruit set at high temperatures. A biparental cross between line Jab-95 (heat-tolerant) and cultivar Caribe (heat-susceptible) was made. A total of 192 plants of the F_2 generation were evaluated, generating 172 polymorphic markers through six primer combinations previously identified by the Bulked Segregant Analysis technique. To construct the genetic map, 106 of the 172 markers that segregated in the expected Mendelian segregation proportion (3:1) were used. The map covered 1191.46 cM of the genome. Six trait-linked QTL were identified in the analysis of simple markers and three others by the interval-mapping methodology. These results could be highly useful in improvement programs, since heat-tolerant plants can be selected rapidly, which improves tomato fruit set.*

Key words: *Lycopersicon esculentum,* stress, linkage map, molecular markers, fAFLP.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is world-wide one of the most important vegetables. China and the United States are the main producers with 25.851 and 12.275 million tons, respectively (FAO 2004). Brazil is the largest producer of Latin America, with approximately 3.5 million tons (Agrianual 2005).

Heat stress is a limiting factor to tomato yields, since it reduces the fruit set index in the more heat-susceptible species. According to Rick (1978), temperatures of over 32 ºC for longer than three hours a day provoke flower abortion in tomato. High temperatures affect the fruit set, number of flowers per bunch and number of seeds per fruit (Ahmadi and Stevens 1979).

Besides, the trait tomato fruit set is not easy to be evaluated, since it is expressed in the proportion of flowers that reach anthesis, which can only be evaluated when anthesis ends. This evaluation could be facilitated and anticipated with the support of molecular markers for this trait.

Presently, molecular markers are being used in studies of cultivar differentiation, detection of genetic variability within and among species, origin and dispersion of cultivated species, mapping of genes of interest, among others. Besides, there is the possibility of using a gene marker linked to pathogen resistance or tolerance to certain stresses, which simplifies the use in marker-assisted selection in support of improvement programs.

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To conduct an improvement program, knowledge on the genetic components of each population involved is indispensable. In the case of quantitative trait, the technology of molecular markers is an alternative that has proved quite efficient.

Most traits of economical importance, in this case fruit set in tomato, are under complex genetic control, involving the action of different genes, and are strongly influenced by environmental conditions, and consequently, of difficult manipulation. These traits vary continuously in the phenotypic expression and are denominated polygenic or of quantitative inheritance.

The study of quantitative trait loci (QTL), associated to the use of genetic maps obtained by molecular markers, allows the identification, mapping and quantification of QTL effects. The efficiency in QTL detection depends on the number of QTL, magnitude of the effects, trait heritability, interactions between the genes, type and size of the segregating population, genome size, recombination frequency between the QTL and the marker, and map saturation (Tanksley 1993, Young 1996).

QTL mapping is based on association tests between molecular markers and phenotypic traits. The simplest procedures to detect such associations are linear models (analysis of variance and linear regression), which analyze the difference between the phenotypic values for each marker separately (Edwards et al. 1987). This methodology is quite flexible and spares breeders the construction of genetic maps, since it is only used to detect marker-QTL associations. The drawbacks to this analysis are that neither the QTL can be localized nor estimates of the magnitude of its effect calculated.

The interval mapping method is an alternative to increase the detection power of the associations, which allows an estimation of the QTL effect and position. This methodology is based on information about the segregation of adjacent marker pairs as analysis units, using the maximum likelihood method to estimate the recombination frequency and magnitude of the QTL effect on the interval between two linked markers in a genetic map (Lander and Botstein 1989).

The fAFLP technique is recommended to detect fragment polymorphisms that can be used for the construction of genome maps since it is the most efficient way of constructing maps of many organisms. The AFLP marker analyzes the presence or absence of restriction enzyme sites and the polymorphic sequences adjacent to these sites. AFLP markers are obtained in three fundamental steps: 1) digestion of the genomic DNA with two different enzymes, the *Mse*I (frequent-cutting enzyme) and *Eco*RI (rare-cutting enzyme), 2) ligation of oligonucleotide adaptors to the restriction ends and 3) fragment selection by two successive PCR amplifications, using primers complementary to the oligonucleotide adaptors, with one to three additional selective nucleotides (Vos et al. 1995). The fAFLP marker is the AFLP technique with the use of primers with fluorescent dyes.

For a quick selection of QTL-linked markers, the technique denominated Bulked Segregant Analysis or BSA has found wide-spread use. This technique identifies molecular markers linked to a trait of interest and is based on in the identification of differences in the amplification products of two DNA bulks or pools, derived from segregating populations for the locus under study. Each bulk is composed of plants with genotypes that are identical in one trait or genome region, but random in the loci non-linked to the selected region. The contrasting bulks for the trait of interest are analyzed through molecular markers, which allow the differentiation. Only those markers that are polymorphic in the two bulks can be used in the population analysis to confirm their linkages and determine the genetic distances between the possible markers and the locus of interest (Michelmore et al. 1991).

This study aimed to detect quantitative trait loci (QTL) by fAFLP markers associated to the trait fruit set of tomato (*Lycopersicon esculentum*) at high temperatures, using a biparental cross between line Jab-95 (heat-tolerant) and cultivar Caribe (heat-susceptible).

MATERIAL AND METHODS

Installation site

The field experiment as well as the crosses were conducted in a greenhouse, in an experimental area of the Faculdade de Ciences Agrárias e Veterinárias - UNESP, Campus of Jaboticabal, (lat 21º 15' 22'' S, long

48º 18' 58'' W, 575 m asl). The regional climate is of the Cwa type (mesothermal, with rainy summers and dry winters), according to the Köppen climate classification.

The laboratory experiment was conducted by the Departamento de Produção Vegetal together with the Departamento de Tecnologia, of FCAV-UNESP, Jaboticabal-SP.

Installation of the experiment

Tomato seed of the segregating F_2 population, originated by crosses between the parents Caribe and line Jab-95, was sown on November 6, 2002, in polystyrene trays with 128 holes filled with substrate for vegetables (Plantmax). Only seedlings of the F_2 population were transplanted to a greenhouse on December 13, 2002, when they had three to four definitive leaves. The F_2 seedlings were transplanted to four beds (107 seedlings per bed), spaced 0.70 m between rows and 0.5 m between plants. Each plant was duly identified.

Plant evaluations

Fruit set was evaluated in individual plants of the F_2 population. The ten first inflorescences of each plant were evaluated. The number of flowers/bunch/plant was counted, and the number of set fruits/bunch/plant, with a diameter of over 4 mm. The ten inflorescences were tagged with colored ribbons, to make visualization and counting easier. The fruit set percentage was estimated by summing up the set fruits per plant of ten inflorescences, divided by the sum of flowers/plant of the ten inflorescences, multiplied by 100. The period of fruit set evaluation of the ten first bunches lasted from January 17 to February 21, 2003.

Environmental conditions

Temperature and relative humidity were measured by a thermo-hydrograph inside the greenhouse during crop development (data in Figure 1)

Collection of plant material of F_2 population

Of the 428 F_2 seedlings transplanted to the greenhouse, a total of 192 healthy plants were evaluated for fruit set percentage and collected for the molecular analyses. Leaves of the young shoots from below the secondary shoot were taken to avoid plant injuries and with regard to disease control. The leaves were immediately identified, placed in tubes and ice-cooled until arrival at the laboratory. There the leaves were washed, dried, cut with scissors, ground in liquid nitrogen and stored in closed plastic boxes. All ground leaf material was deep-frozen at -20 ºC for the following studies.

Genomic DNA extraction

The genomic DNA of the plant tissues was extracted as described by Doyle and Doyle 1991 with slight modifications. The ground samples of 100 mg of leaves were homogenized in 1.5 mL microcentrifuge tubes, with 750 µL extraction buffer (2% CTAB, NaCl 5M, 0.2% 2-β- mercaptoethanol, 0.5 M EDTA, 100 mM TRIS-HCl-pH 8.0). After 30 minutes of incubation at 60 ºC, 450 µL chloroform:isoamylic alcohol (24:1) was added and the material centrifuged at 2.000 x g, 20 ºC, for 10 minutes. The supernatant was transferred to a new tube together with 400 µL of frozen isopropanol. After another centrifugation at 12.000 x g, 0 ºC, for 10 minutes the supernatant was discarded. The so-called wash buffer solution (76% ethanol and 10mM ammonium acetate) was used followed by centrifugation at 2.500 x g, 0 ºC, for 10 minutes, to obtain the precipitate, which was dried at room temperature and enriched with 100 µL TE (10 mM TRIS-HCl, pH 7.5, EDTA 0.1 mM) and 1 µL RNAse (10 µg mL^{-1}). The tubes were incubated at 37 ºC for 30 minutes with 200 µL sterile filtered water; 100 µL ammonia acetate and 1.000 µL absolute ethanol and maintained for one hour at –20 ºC. Finally, the material was centrifuged at 12.000 x g, 0 ºC, for 10 minutes, left to dry, resuspended in 20 µL TE (10 mM TRIS-HCl, pH 7.5, EDTA 0.1 mM), and frozen at –20 ºC.

DNA Quantification

The DNA of each sample was quantified by spectrophotometry with ultraviolet light, at a ratio of 1 OD=50 ng μL^{-1} DNA (Maniatis et al. 1982).

Besides the DNA concentration, the DNA purity was verified by a ratio of 260/280 (nucleic acids / proteins). To verify the results and for quality analysis the DNA was quantified on 0.8 % agarose gel.

The DNA was then diluted in sterile filtered water for DNA standardization of each sample at 50 ng to prepare a work solution. The original DNA was diluted with TE (10 mM TRIS-HCl, pH 7.5, EDTA 0.1 mM) eight times (60 µL) and stored at -20 ºC.

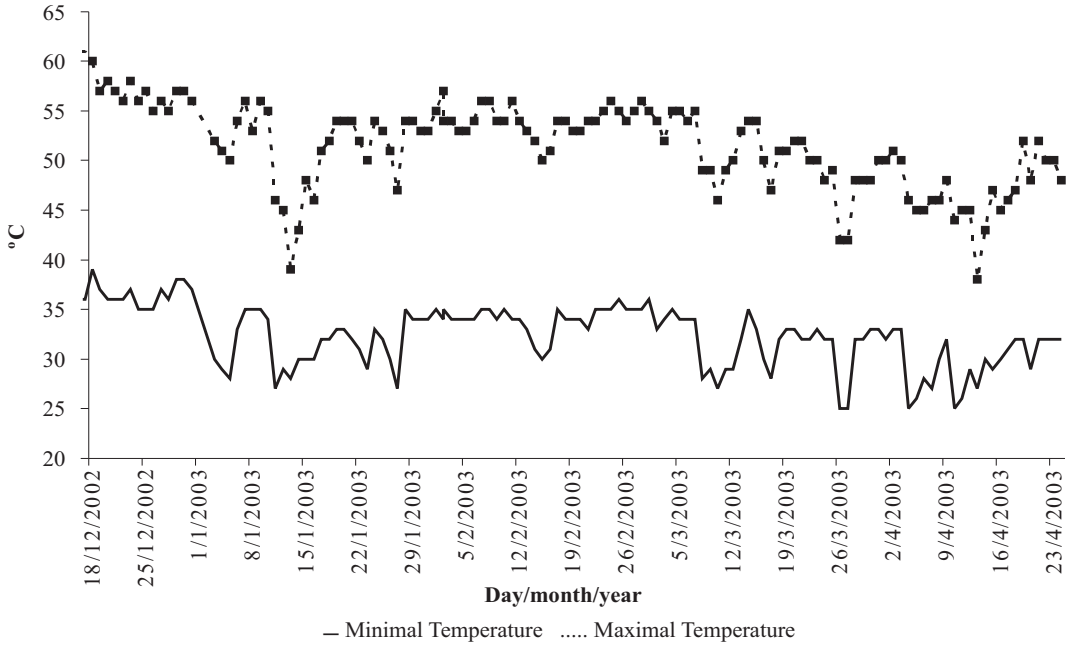

Figure 1. Maximal and minimal day temperatures during the establishment of the segregating F_2 tomato population, measured with a thermo-hydrograph in the center of the greenhouse. The evaluation period of fruit set of the ten first bunches lasted from January 17 to February 21, 2003

Use of the BSA

The BSA technique was applied to DNA samples of the ten plants with highest and of the ten with lowest fruit set, that is, the extreme phenotypes for this trait of the segregating population. The plants that set most fruits (setting percentage of over 88%) and those that set least (setting percentage below 33%) were considered.

FAFLP marker

After separating the plant by the BAS technique the fAFLP analysis was applied according to the AFLP Plant Mapping Protocol 1997, with some adaptations. The following phases were used:

DNA digestion: the genomic DNA was digested by the following reaction: 500 ng of DNA plus 1.25 µL buffer React 1 (500 mM Tris-HCl pH 8.0, 100 mM $MgCl_2$), 5 U *Eco*RI and 1.5 U *Mse*I. This reaction was incubated for 12 hours at 37 ºC and then the enzymes were inactivated for 10 minutes, at 65 ºC.

Adaptor ligation: 3.67 µL of the digestion reaction was removed and enriched with 1µL buffer T4 DNA ligase, 0.5 µL T4 DNA ligase, 0.33 µL of the *Mse*I cutting adaptor and 0.33 µL of the *Eco*RI cutting adaptor (previous annealing of both adaptors at 95 ºC for 5 minutes). Ligation occurred for two hours at 20 ºC and was diluted 8.5 times.

Pre-selective amplification: 4 µL was taken from the diluted reaction prepared based on the adaptor digestion and ligation reactions and enriched with 1 µL of the mixture of the pre-selective AFLP primers *Eco*RI and *Mse*I and 15 µL AFLP Core mix. The samples were placed in an thermocycler (Eppendorf Mastercycler gradient®) initially programmed for two minutes at 72 ºC, 20 cycles at 94 ºC for 20 seconds, 56 ºC for 30 seconds and at 72 ºC for two minutes, and finalizing at 60 ºC for 30 minutes. After verifying the pre-selective reaction on 1% agarose gel, the amplified products in the pre-selective reaction were diluted 5 times.

Selective Amplification: 1.5 µL was taken from the diluted pre-selective reaction and 7.5 µL AFLP Core mix, 0.5 µL primer-AXX of the fluorescence-tagged *Eco*RI and 0.5 µL *Mse*I primer-CXX was added. After preparing the reactions, the samples were placed in a thermocycler (Mastercycler Gradiente®, Eppendorf). The amplification started with an initial denaturing phase at 94 ºC for two minutes, followed by 10 cycles at 94 ºC for 20 seconds, then at 66 ºC (reducing one degree per cycle) for 30 seconds and at 72 ºC for 2 minutes, 21 cycles at 94 ºC for 20 seconds, 56 ºC for 30 seconds, 72 ºC for two minutes, and a final phase at 60 ºC for 30 minutes.

Electrophoresis in an ABI PRISM 377 DNA Sequencer: a mixture was prepared containing 1.5 µL

deionized formamide, plus 0.9 μL blue dextran and 0.3 μL internal molecular weight standard GeneScan-500 ROX with red fluorescence dye. 1.6 μL of this mixture was added to the selective reaction, and the reaction denatured in the thermocycler at 95 ºC for 5 minutes. 1.5 μL of each sample was applied to a 5% denaturing Long Ranger gel using TEB 1X as loading buffer. The sequencing plate was 36 cm, with a running time of 3 hours at 2500 V.

The internal molecular weight standard Gene Scan-500 ROX used in the fAFLP has 15 fragments with the following base pair lengths (pb): 50, 75, 100, 139, 150, 160, 200, 250, 300, 340, 350, 400, 450, 490, and 500.

Primer combinations of the Applied Biosystems kit

Of the 24 tested primer combinations, 10 were tagged green (JOE), 9 yellow (NED) and 5 blue (FAM), i.e., ACA-CAC (FAM), AAG-CAC (JOE), ACC-CAC (NED), ACA-CAA (FAM), AAG-CAA (JOE), ACC-CAA (NED), ACA-CAG (FAM), AAG-CAG (JOE), ACC-CAG (NED), ACA-CTT (FAM), AAG-CTT (JOE), ACC-CTT (NED), ACA-CTG (FAM), AAG-CTG (JOE), ACC-CTG (NED), AGG-CAC (JOE), AGC-CAC (NED), AGG-CAA (JOE), AGC-CAA (NED), AGG-CAG (JOE), AGC-CAG (NED), AGG-CTT (JOE), AGC-CTT (NED), AGG-CTG (JOE).

Gel analysis

The data obtained after the fAFLP gel run were analyzed using specific software:

- GeneScan Analysis: data collection of the amplified products from the fAFLP electrophoresis gel.

- Genotyper DNA Fragment Analysis: analyzes data obtained by GeneScan, generating electropherograms and binary charts of band presence (1) and absence (0).

- Gqmol: Analyzes molecular data and its associations with quantitative traits. Maps and identifies possible QTL linked to the trait of interest. In this case the simple marker and simple interval analyses were used.

RESULTS AND DISCUSSION

Of the 428 transplanted F_2 seedlings some died in the beginning of the development owing to fungal diseases causing damping-off and others with virus-related problems. In this sense, 192 healthy plants were chosen and evaluated for fruit set and sampled for the molecular analyses.

To test all 24 above cited primer combinations, DNA derived from the BAS technique was used in which each primer pair was tested in the two extreme populations for tomato fruit set, that is, the bulk of the heat-sensitive plants and the bulk of the heat-tolerant plants for this trait, previously analyzed regarding fruit set percentage. After the test six primer pairs were chosen - ACA-CAC (FAM), AAG-CAC (JOE), ACA-CTG (FAM), AAG-CTG (JOE), AGG-CTG (JOE) and ACA-CTT (FAM) - which presented a high number of polymorphic bands and were efficient for sample differentiation. Of the 192 F_2 plants evaluated in the molecular analyses 172 generated polymorphic markers with the six primer combinations cited above. According to the expected Mendelian segregation (3:1), 106 of the 172 polymorphic markers found segregate in this proportion ($p>0.01$). These markers were used to construct the tomato linkage map (Figure 2), characterized by 9 linkage groups, representing the chromosomes of the species (n=12). The genetic map covered 1191.46 cM of the genome: group 1 – 362.06 cM; group 2 – 163.11 cM; group 3 – 168.32 cM; group 4 – 36.93 cM; group 5 – 4.11 cM; group 6 – 112.16 cM; group 7 – 297.79 cM; group 8 – 16.92 cM and group 9 – 30.07 cM. The Kosambi function (Kosambi 1944) was used to convert the recombination frequency into values of map distance (centiMorgan), using a LOD value of 3.0. Of the 106 analyzed markers, 14 were not included in any linkage group. The analysis of variance and linear regression were performed considering two treatments, whose phenotypic means were associated with band presence and absence. Through the test of means for both methodologies, six QTL or genomic regions were mapped linked to tomato fruit set, distributed in the linkage groups 1, 3, 4, 6, 7, and 9. The results of the analysis of variance and linear regression for these six markers as well as the identification of each one in its linkage group are shown in Table 1. The results are consistent and the markers 202.23 ACA-FAM-CTG and 258.72 AAG-JOE-CTG with the highest values of coefficient of determination R^2 (8.78 and 7.96 %, respectively), contributed most to explain the phenotypic variation in the trait tomato fruit set. Altogether, these six markers correspond to 32.82% of the phenotypic variation of the trait. The accumulative proportion explained by the different experimentally detected QTL varied from 30 to 70%, depending on a series of factors, as well as on the cross under study (Beavis et al. 1991), the evaluated trait (Edwards et al. 1992), the experimental design, and map resolution in terms of number of markers (Edwards et al. 1987). In maize, using an F_2 population with 1700 plants and an error level of 5%, one QTL was detected that contributed with only 0.3% to the phenotypic trait variation (Edwards et al. 1987). The

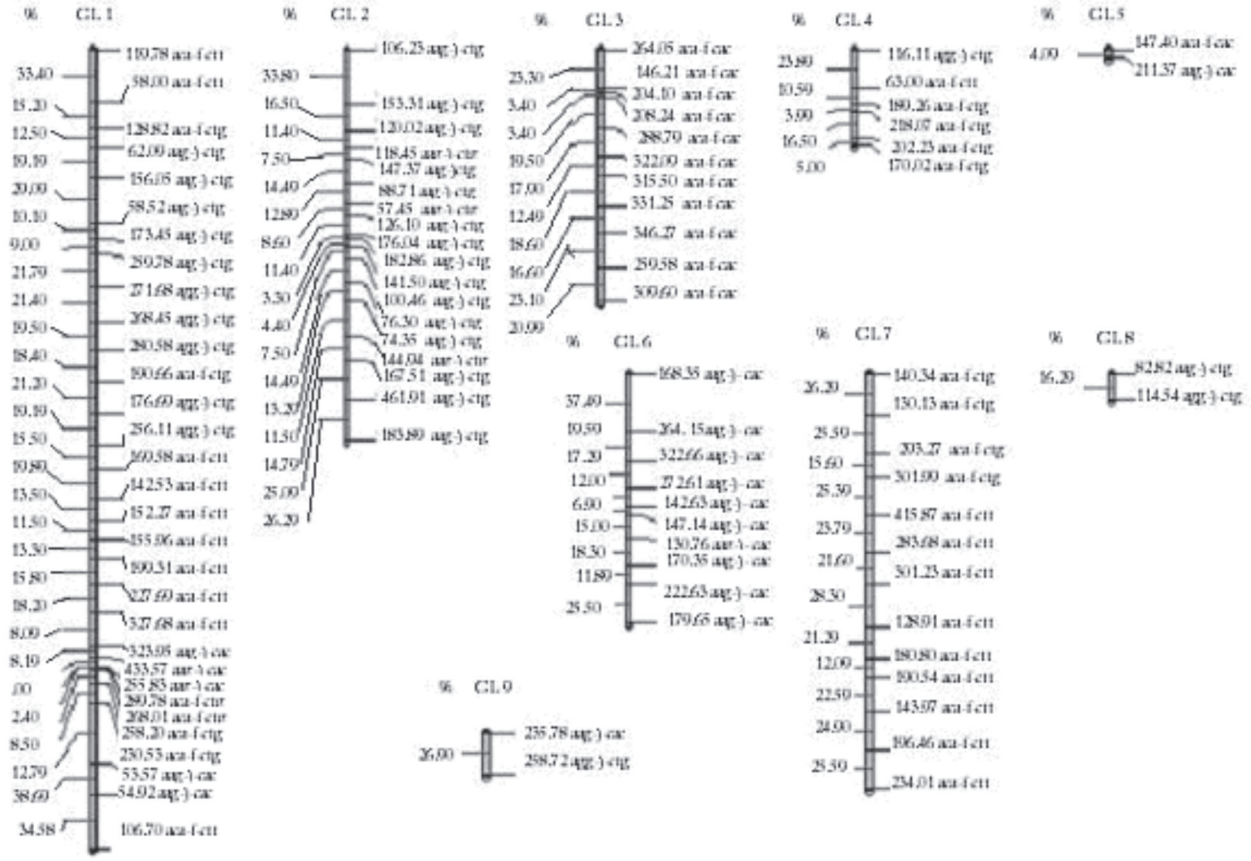

Figure 2. Genetic linkage map of tomato, based on 192 plants of the F_2 generation, derived from a specific cross between cultivar Caribe (heat-sensitive) and line Jab-95 (heat-tolerant). The recombination distances are given in centiMorgans (cM) and the markers indicate the fragment size in base pairs followed by the primer used. All markers segregated in the proportion 3:1 ($p>0.01$)

Table 1. Summary of the analysis of variance and linear regression of the fAFLP markers linked to fruit set in tomato and the respective linkage group

Marker	Group	Analysis of Variance			Regression			
		Mean Square	F	Prob. (%)	Mean Square	F	Prob. (%)	R^2 (%)
309.60 ACA-F-CAC	3	885.56	4.11	4.57	885.55	4.11	4.57	4.67
168.35 AAG-J-CAC	6	993.61	4.46	3.71	993.67	4.46	3.71	4.27
202.23 ACA-F-CTG	4	1835.99	7.86	6.20	1836.01	7.89	6.19	8.78
258.72 AGG-J-CTG	9	3522.42	16.43	0.01	3522.44	16.43	0.01	7.96
259.78 AGG-J-CTG	1	1963.29	8.82	0.34	1963.26	8.82	0.33	4.43
234.01 ACA-F-CTT	7	1124.17	4.94	2.76	1124.19	4.94	2.75	2.71

simple interval analysis is aimed to evaluate the existence of marker - QTL linkage. The maximum likelihood methodology was used for this analysis and the recombination frequency as distance unit; QTL associated with tomato fruit set were detected in the linkage groups 2 and 4 (Figure 3), and one more in group 7 (Figure 4). The presence of QTL was considered only in the cases where LOD peaks were obtained for each linkage group. The identification and detection of different expression QTL controlling fruit set indicates the polygenic nature of the trait. The identification of such QTL is of great importance, since they can be used in assisted selection in improvement programs for tomato fruit set, based on the progenies evaluated.

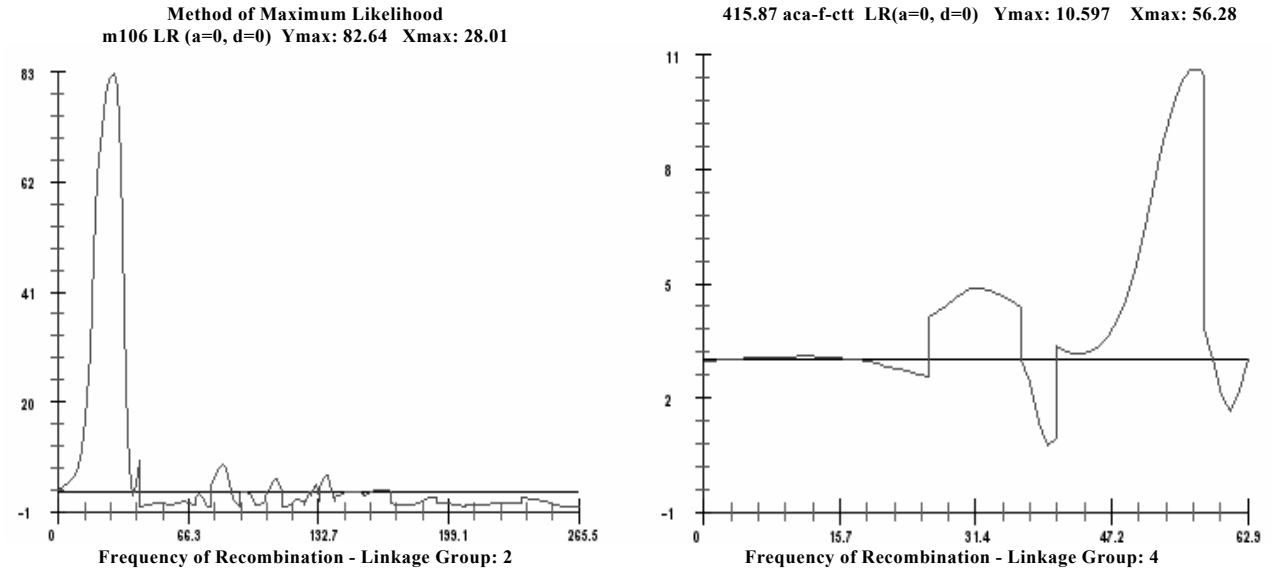

Figure 3. Analysis of the linkage groups 2 and 4 and the presence of QTL linked to fruit set in tomato. The linkage group and distances in centiMorgans are presented along the abscissa based on the left telomere. Linkage group 2 contains one QTL near marker 153.31 AAG-J-CTG and the linkage group 4, the marker 218.07 ACA-F-CTG (first peak) and 202.23 ACA-F-CTG (second peak, a marker found in the simple marker analysis as well). The scale of LOD values for each analyzed linkage group is shown on the ordinate. The horizontal line indicates the LOD value and the statistical significance limit for each linkage group (L.R.=3). The region above the LOD limit potentially contains a QTL

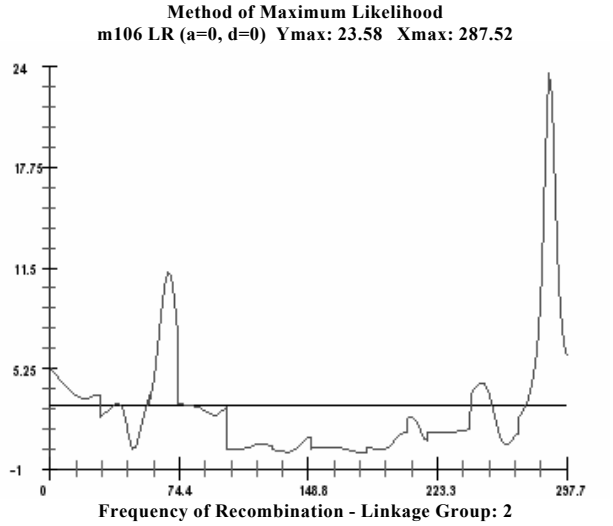

Figure 4. Analysis of linkage group 7 and the presence of QTL linked to tomato fruit set. The linkage group and distances in centiMorgans are presented along the abscissa based on the left telomere. In linkage group 7, two QTL are close to the markers 301.99 ACA-F-CTG (first peak) and 234.01 ACA-F-CTT (second peak, marker also found in the simple marker analysis). The scale of LOD values for each analyzed linkage group is shown on the ordinate. The horizontal line indicates the LOD value and the limit of statistical significance for each linkage group (L.R.=3). The region above the LOD limit could contain a QTL

Marcadores moleculares fAFLP na identificação de QTL's para tolerância a fixação de frutos em tomateiro

RESUMO - *Objetivou-se detectar, utilizando o marcador molecular fAFLP (Fluorescent Amplified Fragment Length Polymorphism), locos de características quantitativas (QTL's) associados à característica fixação de frutos de tomateiros em altas temperaturas. Realizou-se um cruzamento biparental entre a linhagem Jab-95 (tolerante) e a cultivar Caribe (suscetível ao calor). No total, 192 plantas da geração F_2 foram avaliadas, gerando 172 marcadores polimórficos utilizando seis combinações de iniciadores previamente identificados através da técnica Bulked Segregant Analysis. De acordo com a segregação Mendeliana esperada (3:1), dos 172 marcadores encontrados, 106 segregaram nesta proporção e utilizados para confecção do mapa genético. O mapa gerou cobertura de 1191,46 cM do genoma. Na análise de marca simples identificou-se seis QTL's ligados à característica, e mais três através da metodologia mapeamento por intervalo. Os resultados obtidos poderão ser de grande utilidade aos programas de melhoramento, pois permitem selecionar de maneira rápida, plantas tolerantes ao calor possibilitando maior fixação de frutos em tomateiro.*

Palavras-chave: *Lycopersicon esculentum*, estresse, mapa de ligação, marcadores moleculares, fAFLP.

REFERENCES

AFLP Plant Mapping Protocol (1997) **Applied Biosystems**. 45p.

AGRIANUAL (2005) **Anuário da agricultura brasileira**. FNP Consultoria e Comércio, São Paulo, 544p.

Ahmadi AL and Stevens MA (1979) Reproductive responses of heat-tolerant tomatoes to high temperature. **Journal of the American Society for Horticultural Science 104**: 686-691.

Beavis W, Grant D, Albertsen M and Fincher R (1991) Quantitative trait loci for plant height in four maize populations and their associations with qualitative genetic loci. **Theoretical and Applied Genetics 83**: 141-145.

Doyle JJ and Doyle JL (1991) Isolation of plant DNA from fresh tissue. **Focus 1**: 13-15.

Edwards MD, Helentjaris T, Wright S and Stuber CW (1992) Molecular-marker-facilitated investigations of quantitative-trait loci in maize. **Theoretical and Applied Genetics 83**: 765-774.

Edwards MD, Stuber CW and Wendel JF (1987) Molecular-marker-facilitated investigations of quantitative-trait loci in maize. I: numbers, genomic distribution, and types of gene action. **Genetics 116**: 113-125.

FAO (2004) **Food and Agriculture Organization of the United Nations**. Available at: http://www.fao.org. Accessed in April, 16, 2006.

Kosambi DD (1944) The estimation of map distances from recombination values. **Annals of Eugenics 12**: 172-175.

Lander ES and Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. **Genetics 121**: 185-199.

Maniatis T, Fritsch EF and Sambrook J (1982) **Molecular cloning – a laboratory manual**. Cold Spring Harbor Laboratory, New York, 545p.

Michelmore RW, Paran I and Kessely RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. **Proceedings of the National Academy of Science 88**: 988-992.

Rick CM (1978) The tomato. **Scientific American 239**: 66-76.

Tanksley SD (1993) Mapping polygenes. **Annual Review of Genetics 27**: 205-233.

Vos P, Hogers R, Bleeker M, Reijans M, Lee T. van de, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M and Zabeu M (1995) AFLP: a new technique for DNA fingerprinting. **Nucleic Acids Research 33**: 4407-4414.

Young ND (1996) QTL mapping and quantitative disease resistance in plants. **Annual Review Phytopathology 34**: 479-501.