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Confirmation of RAPD Markers Associated with QTL for Ascorbic Acid in Melon

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ABSTRACT

Ascorbic acid is a major melon (*Cucumis melo* L.) fruit quality trait. Nine randomly amplified polymorphic DNA (RAPD) markers were previously reported to be associated with quantitative trait loci (QTL) affecting ascorbic acid in an F_2 population from the melon cross of 'TAM Dulce' x TGR1551 in a greenhouse experiment. However, the markers for the QTL were not confirmed in different populations and environments to indicate their merit in breeding. The objective was to determine if the associations of RAPD markers with QTL for ascorbic acid could be confirmed in an F_2 population from the different cross of 'Deltex' (high ascorbic acid) x TGR1551 (low ascorbic acid) in a field experiment. A continuous distribution for ascorbic acid was observed in the F_2 population indicating quantitative inheritance. Ascorbic acid was positively correlated with total soluble solids or sucrose, whereas it was negatively correlated with glucose. Of the nine RAPD markers previously identified in the 'TAM Dulce' x TGR1551 cross in the greenhouse, four were confirmed in the F_2 population of the 'Deltex' x TGR1551 cross in the field to be significantly and consistently associated with QTL for ascorbic acid based on simple linear regression (SLR). Marker OAU13.1350 amplified from 'Deltex' accounted for 14% of the phenotypic variation for the important antioxidant trait. These RAPD markers associated with the ascorbic acid QTL could be at least partially utilized in improving the level of this nutrient in new melon cultivars.

Additional Index Words: Cucumis melo, vitamin C, randomly amplified polymorphic DNA markers, quantitative trait loci

Melon fruit flesh is a significant source of ascorbic acid, folic acid, and potassium (Richter, 2000) and orange-fleshed fruit a very significant source of *beta*-carotene (Lester, 1997) as well as free sugars and water (Martyn and Miller, 1996). Ascorbic acid, also known as vitamin C, is an important nutrient for human health (Gebhardt and Thomas, 2002). It functions as a water soluble antioxidant in the human body (Lavine, 1986). It also plays a crucial role in keeping the immune system healthy (Eichholzer et al., 2001). Ascorbic acid is a highly important melon fruit quality trait due to consumer preference for healthy food. The improvement of ascorbic acid content is a significant goal of the Texas melon breeding program (Sinclair et al., 2004).

The expression of QTL may differ over environments or populations in various crops. Of 29 QTL for tomato fruit size and quality traits, only four were expressed in three environments (Paterson et al., 1991). Only two of six QTL for bacterial disease resistance were expressed in three common bean populations (Park et al., 1999). There are examples of genotype x environment interaction and genetic background affecting QTL expression. Identifying markers associated with QTL based on one environment and one population may be erroneous, especially for QTL with minor effects (Paterson et al., 1991). Park et al. (1999) emphasized the importance of confirming the marker-QTL associations in different populations and environments before using molecular markers for marker-assisted selection in breeding programs.

Nine RAPD markers, four amplified from 'TAM Dulce' and five amplified from TGR1551, were previously identified to be significantly associated with QTL for ascorbic acid by means of bulked segregant analysis in an F_2 population from the melon cross of 'TAM Dulce' (high ascorbic acid) x TGR1551 (low ascorbic acid) in a greenhouse experiment (Sinclair et al., 2004). They reported three and two markers explaining 14% and 12% of the total phenotypic variation for ascorbic acid based on stepwise multiple regression (SMR). Four markers associated with ascorbic acid were consistently associated with mature melon fruit sweetness. Marker OAW06.600 obtained from TGR1551 was associated with sugar traits including sucrose, sucrose percentage of total sugars, and glucose percentage of total sugars as well as ascorbic acid. These markers linked to QTL affecting ascorbic acid could be useful in transferring the high ascorbic acid genes into low ascorbic acid melon cultivars and lines. However, these marker-QTL associations have not been confirmed in other populations of the same cross or a different cross in different environments. Therefore, the objective of this study was to confirm the significant associations of RAPD markers with QTL for ascorbic acid in an F_2 population from the different cross of 'Deltex' (high ascorbic acid) x TGR1551 in a field experiment. Pearson correlations between ascorbic acid and seven sugar traits were also calculated in the population.

MATERIALS AND METHODS

Plant Material. Sixty-four F_2 plants were derived from the melon cross of 'Deltex' x TGR1551 in a greenhouse at the Texas Agricultural Research and Extension Center-Weslaco, Texas A&M University in Winter 2002. The high sugar 'Deltex' parent, a commercial ananas cultivar (Nunhems, Parma, Idaho), is resistant to fusarium wilt (races 0 and 2). The TGR1551 parent, originally obtained from Zimbabwe, is a wild agrestis type with low fruit quality. TGR1551 is highly resistant to cucurbit yellow stunting

Table 1. A summary of selected fruit quality characteristics of the two melon parents 'Deltex' and TGR1551.

Fruit quality trait	'Deltex'	TGR1551
Melon type	Ananas	Agrestis
Ascorbic acid (mg/100 g)	High	Low
Total soluble solids (%)	High	Low
Sucrose ($mg \cdot g^{-1}$)	High	Low
Glucose ($mg \cdot g^{-1}$)	Moderate	Moderate
Fructose ($mg \cdot g^{-1}$)	Moderate	Moderate
Sucrose percentage (%) of total sugars	High	Low
Glucose percentage (%) of total sugars	Moderate	High
Fructose percentage (%) of total sugars	Moderate	High
Andromonoecious (*a*)	Andromonoecious	Monoecious

disorder virus under natural and controlled-inoculation conditions (Lopez-Sese and Gomez-Guillamon, 2000). Important fruit quality characteristics of the two parents are indicated in Table 1.

Sixty-four F_2 plants along with 10 plants each of the parents, 'Deltex' and TGR1551, were planted on black plastic mulch with drip irrigation on sandy clay loam soil at Weslaco, Texas on 10 Mar. 2002. Single bed length and width were 31.7 m and 1.0 m, respectively, with 30.5 cm spacing between plants and 2.0 m between beds. A fertilizer containing 5N-11.3P-27.4K (Wilbur Ellis, Edinburg, Texas) was applied weekly. Irrigation was supplied as needed. Approximate day/night field temperatures were 30±3/23±3 °C. Day lengths ranged from 12 to 14 h light. The ascorbic acid content of each plant was recorded using the procedure of Hodges et al. (2001). Stamen presence and absence in female flowers were checked three different times on all plants of F_2 and parents at different times during flowering.

RAPD. Fully expanded leaves of 64 F_2 plants along with their parents were collected at 28 days after planting in the field. Total genomic DNA was extracted from the leaf tissue using the method of Skroch and Nienhuis (1995). Seven 10-mer primers (Operon Technologies, Alameda, California) that generated the nine RAPD markers associated with QTL for ascorbic acid in the greenhouse population (Sinclair et al., 2004) were tested in the F_2 population derived from the cross between 'Deltex' and TGR1551 for confirming the RAPD marker-QTL associations in the field population. Polymerase chain reactions (PCR) were performed on 96-well plates in a MJ Research thermalcycler (model PTC-0100; MJ Research, Waltham, Massachusetts). Protocols for PCR and the composition of the final volume of reactants were the same as those described by Skroch and Nienhuis (1995). A 100-base pair (bp) DNA ladder (Life Technologies, Grand Island, New York) was used to estimate the length of RAPD markers. The name of each RAPD marker is derived from an 'O' prefix for Operon primers, the letters identifying the Operon kit, Operon primer number, and the approximate length (bp) of the marker (Sinclair et al., 2004).

Linkage Analysis. To detect segregation distortion of markers and andromonoecious (*a*), the F_2 population marker data were tested for goodness-of-fit to a 3:1 ratio using the chi-square test. Due to the dominant nature of RAPD markers, the linkage analyses of two markers obtained from 'Deltex' and five markers including *a* obtained from TGR1551 were separately performed on the data for 64 F_2 plants of the 'Deletx' x TGR1551 cross using MAPMAKER version 3.0 (Lander et al., 1987). Map distances [centimorgan (cM)] between ordered loci of markers were calculated using recombination fractions and the Kosambi mapping function (Kosambi, 1944).

Detection of QTL. For confirmation of QTL for ascorbic acid in the field population, SLR was used to analyze the ascorbic acid data. Significant differences in marker-trait associations were based on F-tests ($P<0.05$) (Edwards et al., 1987). Loci with thelowest *P* value per QTL were chosen and then were added in a SMR to select the best set of markers ($P<0.05$) for prediction of the total trait phenotypic variation explained by the identified QTL (Paterson et al., 1991). Pearson correlations between ascorbic acid and seven fruit sweetness traits were also determined in this population. All statistical analyses were conducted using the Statistical Analysis System (SAS Institute, Cary, North Carolina).

RESULTS AND DISCUSSION

Inheritance. A distinct separation for ascorbic acid content between the parents was found (Fig. 1). Fruits of 'Deltex' had high ascorbic acid content. In contrast, TGR1551 plants possessed no ascorbic acid. All F_1 plants derived from the 'Deletx' x TGR1551 cross had low ascorbic acid content (Fig. 1). We noted a skewed distribution for ascorbic acid towards low values. A continuous frequency distribution for ascorbic acid content was

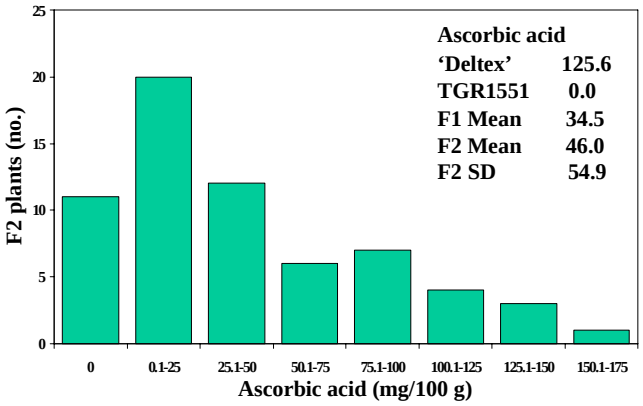

Fig. 1. A frequency distribution for ascorbic acid (mg/100 g) of F_2 plants derived from the melon cross of 'Deltex' (high ascorbic acid) x TGR1551 (low ascorbic acid) in the field experiment.

Table 2. Pearson correlations of ascorbic acid (AA), total soluble solids (TSS), and sugars in an F_2 population derived from the melon cross of 'Deltex' x TGR1551 in the field experiment.

Trait	TSS	Sucrose	Glucose	Fructose	SPTS[z]	GPTS	FPTS
AA	0.39**	0.57**	-0.47**	-0.11[NS]	0.54**	-0.62**	-0.36**

[z]SPTS=sucrose percentage of total sugars, GPTS=glucose percentage of total sugars, FPTS=fructose percentage of total sugars.
[NS],*,**Nonsignificant or significant at $P \leq 0.05$ or 0.01, respectively.

observed in the F_2 population from the cross of 'Deltex' x TGR1551 in the field (Fig. 1) indicating quantitative inheritance for the trait. The quantitative inheritance pattern of ascorbic acid found here was the same as that reported previously in a melon cross by Sinclair et al. (2004). Etienne et al. (2002) and Causse et al. (2002) also reported complex inheritance patterns of three organic acids (malic acid, citric acid, and quinic acid) in peach and two antioxidant compounds (lycopene and carotene) in tomato.

Correlation. Ascorbic acid was observed to be significantly and positively correlated with three mature melon fruit sweetness traits including total soluble solids, sucrose, and sucrose percentage of total sugars in the F_2 population in the field experiment (Table 2), indicating that selection of the four traits is feasible. The positive correlation between ascorbic acid and sucrose percentage of total sugars was similar to that reported previously in the F_2 population in the greenhouse experiment by Sinclair et al. (2004). Ascorbic acid was negatively correlated with other sugar traits such as glucose, glucose percentage of total sugars, and fructose percentage of total sugars in the field population. The negative correlation of ascorbic acid with glucose percentage of total sugars found here was consistent with the greenhouse result of Sinclair et al. (2004). We noted no significant correlation between ascorbic acid and fructose in this population.

Confirmation. Sinclair et al. (2004) identified that nine RAPD markers, four produced from 'TAM Dulce' and five obtained from TGR1551, were associated with QTL affecting ascorbic acid in the F_2 population of the 'TAM Dulce' x TGR1551 cross in the greenhouse experiment.For substantiating their merit in melon breeding as well as confirming these marker-QTL associations in different populations and environments, the nine RAPD markers were tested in the F_2 population derived from the 'Deltex' x TGR1551 cross from which the fruit quality trait was evaluated in the field experiment. Of the nine RAPD markers associated with the ascorbic acid QTL detected in the greenhouse population, six were also polymorphic between the parents 'Deltex' and TGR1551. An example of marker OAS14.800 is shown in Fig. 2. Due to no amplification (OAT03.1600 and OAW06.1100) or no segregation (OAW06.600)confirming the marker-QTL associations. All six markers also segregated in the F_2 population of the 'Deltex' x TGR1551 cross. A 3:1 goodness-of-fit ratio for band presence to band absence for each of the six markers, two amplified from 'Deltex' and four amplified from TGR1551, was observed in the field population (Table 3). Three markers (OAS14.800, OAU02.600, and OAU03.700) obtained from TGR1551 were linked within a distance of 11.3 cM on one linkage group on the basis of linkage analysis.

Four RAPD markers associated with QTL for ascorbic acid in the greenhouse experiment were confirmed in the F_2 population of the 'Deltex' x TGR1551 cross in the field experiment to be consistently associated with QTL for the vitamin C trait based on SLR (Table 4). Of the four markers confirmed in the field, one was generated from 'Deltex', while three were produced from TGR1551. Marker OAU13.1350 from 'Deltex' was more associated with QTL for the fruit quality trait than the other three markers from TGR1551 in the field population, and accounted for 14% of the phenotypic variation for the trait. The three markers OAS14.800, OAU02.600, and OAU03.700 from TGR1551 were closely linked and were associated with QTL for the vitamin C content in the greenhouse and field. However, the two markers OAT03.250 from 'Deltex' and OAW10.400 from TGR1551,in the parents, the remaining three markers were not tested for associated with the fruit quality trait identified in the greenhouse F_2 population, were not confirmed in this field F_2 population. These four RAPD markers associated with QTL for the important antioxidant trait identified and confirmed here could be at least partially utilized in improving the level of this nutrient in new melon cultivars.

Table 3. Chi-square analyses for segregation of six RAPD markers and andromonoecious (*a*) in an F_2 population derived from the melon cross of 'Deltex' x TGR1551.

		F_2 plants (no.)				
Marker	Source	Presence	Absence	Ratio	χ^2	*P*
OAU13.1350	Deltex	51	13	3:1	0.52	0.47
OAT03.250	Deltex	44	20	3:1	1.01	0.31
OAS14.800	TGR1551	52	12	3:1	1.01	0.31
OAU02.600	TGR1551	51	13	3:1	0.52	0.47
OAU03.700	TGR1551	50	14	3:1	0.18	0.66
OAW10.400	TGR1551	42	22	3:1	2.52	0.11
a	TGR1551	45 (monoecious)	19 (andromonoecious)	3:1	0.52	0.47

Table 4. Confirmation of RAPD markers associated with QTL for ascorbic acid in an F_2 population derived from the melon cross of 'Deltex' (high ascorbic acid) x TGR1551 (low ascorbic acid) in the field experiment on the basis of simple linear regression and stepwise multiple regression analyses.

			Simple linear regression		Stepwise multiple regression	
Marker	Source	Linkage	*P*	R^2 (%)	*P*	R^2 (%)
OAU13.1350	Deltex	Unlinked	0.000	14	0.000	14
					Cumulative R^2=14	
OAS14.800	TGR1551	Linked[z]	0.012	6	0.012	6
OAU03.700	TGR1551	Linked	0.014	6		
OAU02.600	TGR1551	Linked	0.036	4		
					Cumulative R^2=6	

[z]Three RAPD markers, amplified from TGR1551, were linked within a distance of 11.3 cM on one linkage group.

Of six QTL for resistance to common bacterial blight found in a common bean population, only two were consistently expressed in three common bean populations (Park et al., 1999). Among 29 QTL affecting fruit size, soluble solids concentration or pH in a tomato cross, only four were expressed in three environments and ten were expressed in two environments (Paterson et al., 1991). These results show the importance of confirming the marker-QTL associations in other populations and environments before using markers for marker-assisted selection in a breeding program. Therefore, our confirmation of the identified marker-QTL associations in different populations and environments is an essential step for universal use of our linked markers in melon breeding. The RAPD markers linked to QTL for the vitamin C trait identified and confirmed in two populations and environments here should be more reliable for marker-assisted selection than those evaluated in a single population and environment.

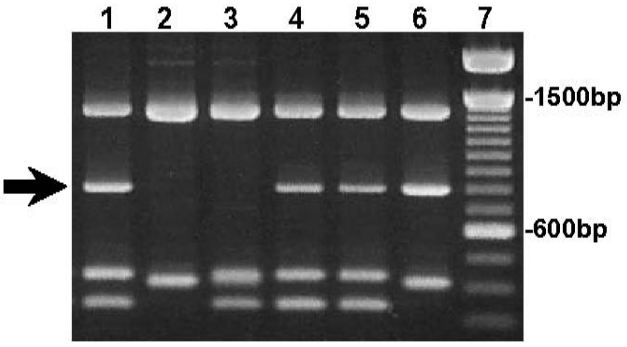

Fig. 2. RAPD marker OAS14.800 expressing polymorphism between the low ascorbic acid parent TGR1551 and the high ascorbic acid parent 'Deltex', and between low and high ascorbic acid F_2 plants. 1=TGR1551, 2='Deltex', 3 to 6=F_2 plants, and 7=a 100 bp DNA marker ladder.

Common Marker. The RAPD marker OAU13.1350 from 'TAM Dulce' associated with the ascorbic acid QTL was reported by Sinclair et al. (2004) to be consistently and highly associated with QTL for three mature melon fruit sweetness traits including sucrose, sucrose percentage of total sugars, and glucose percentage of total sugars in the greenhouse population of the 'TAM Dulce' x TGR1551 cross. According to the result of Park et al. (2006), the OAU13.1350 marker from 'Deltex' was also found to be significantly associated with QTL for the three mature fruit sweetness traits in the field population of the 'Deltex' x TGR1551 cross, and accounted for 4% to 9% of the phenotypic variation for the sugar traits (Table 5). The andromonoecious locus (*a*) on linkage group 4 of the classical melon map regulating stamen absence or stamen presence in female flowers was not associated with the vitamin C trait in the field population. The lack of association between the *a* marker and the fruit quality trait found here confirms the finding of Sinclair et al. (2004), who reported no significant association of the andromonoecious locus with the vitamin C trait in the greenhouse population. The RAPD marker OAU13.1350, consistently expressed in two different melon crosses under greenhouse and field environments, could be useful in melon breeding for enhancing the levels of the mature melon fruit sweetness as well as the vitamin C content.

Table 5. A single common marker associated with sugars as well as ascorbic acid (AA) in an F_2 population from the melon cross of 'Deltex' x TGR1551 in the field experiment.

RAPD marker	Source	AA	Sucrose	Glucose	Fructose	SPTS[z]	GPTS	FPTS
OAU13.1350	Deltex	***	*	NS	NS	*	***	NS

[z]SPTS=sucrose percentage of total sugars, GPTS=glucose percentage of total sugars, FPTS=fructose percentage of total sugars.

[NS],*,***Nonsignificant or significant at $P \leq 0.05$ or 0.001, respectively.

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LITERATURE CITED

Causse, M., V. Saliba-Colombani, L. Lecomte, P. Duffe, P. Rousselle, and M. Buret. 2002. QTL analysis of fruit quality in fresh market tomato: A few chromosomal regions control the variation of sensory and instrumental traits. J. Expt. Bot. 53:2089-2098.

Edwards, M.D., C.W. Stuber, and J.F. Wendell. 1987. Molecular marker-facilitated investigations of quantitative trait loci in maize. I. Numbers, genomic distribution, and types of gene action. Genetics 116:113-125.

Eichholzer, M., J. Luthy, F. Gutzwiller, and H.B. Stahelin. 2001. The role of folate, antioxidant vitamins and other constituents in fruit and vegetables in the prevention of cardiovascular disease: The epidemiological evidence. Intl. J. Vitamin Nutr. Res. 71:5-17.

Etienne, C., C. Rothan, A Moing, C. Plomion, C. Bodenes, L. Svanella-Dumas, P. Cosson, V. Pronier, R. Monet, and E. Dirlewanger. 2002. Candidate genes and QTLs for sugar and organic acid content in peach [*Prunus persica* (L.) Batsch]. Theor. Appl. Genet.105:145-159.

Gebhardt, S.E. and R.G. Thomas. 2002. Nutritive value of foods. U.S. Department of Agriculture, Home and Garden Bulletin 72.

Hodges, D.M., C.F. Forney, and W.V. Wismer. 2001. Antioxidant responses in harvested leaves of two cultivars of spinach differing in senescence rates. J. Amer. Soc. Hort. Sci. 126:611-617.

Kosambi, D.D. 1944. The estimation of map distances from recombination values. Ann. Eugenics 12:172-175.

Lander, E.S., P. Green, J. Abrahamson, A. Barlow, M.J. Daly, S.E. Lincoln, and L. Newburg. 1987. MAPMAKER: An interactive computer package for constructing primary genetic linkage maps with experimental and natural populations. Genomics 1:174-181.

Lavine, M. 1986. New concepts in the biology and biochemistry of ascorbic acid. New England J. Med. 314:892-902.

Lester, G. 1997. Melon (*Cucumis melo* L.) fruit nutritional quality and health functionality. HortTechnology 7:222-227.

Lopez-Sese, A.I. and M.L. Gomez-Guillamon. 2000. Resistance to cucurbit yellowing stunting disorder virus (CYSDV) in *Cucumis melo* L. HortScience 35:110-113.

Martyn, R.D. and M.E. Miller. 1996. Monosporascus root rot/vine decline: An emerging disease of melons worldwide. Plant Dis. 80:716-725.

Park, S.O., D.P. Coyne, N. Mutlu, G. Jung, and J.R. Steadman. 1999. Confirmation of molecular markers and flower color associated with QTL for resistance to common bacterial blight in common beans. J. Amer. Soc. Hort. Sci. 124:519-526.

Park, S.O., K.M. Crosby, and K.S. Yoo. 2006. Identification and confirmation of loci for sucrose percentage of total sugars in melon. 27th International Horticultural Congress:235.

Paterson, A.H., S. Damon, J.D. Hewitt, D. Zamir, H.D. Rabinowitch, S.E. Lincoln, E.S. Lander, and S.D. Tanksley. 1991. Mendelian factors underlying quantitative traits in tomato: Comparison across species, generations, and environments. Genetics 127:181-197.

Richter, H. 2000. Fresh produce guide: Nutrition, selection, preparation, storage, handling, cooking. Try-Foods Intl. Inc., Apopka, Florida.

Sinclair, J.W., S.O. Park, and K.M. Crosby. 2004. Identification of QTL affecting vitamin C in melon. Subtropical Plant Sci. 56:10-15.

Skroch, P.W. and J. Nienhuis. 1995. Qualitative and quantitative characterization of RAPD variation among snap bean genotypes. Theor. Appl. Genet. 91:1078-1085.