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Use of molecular markers in breeding for soluble solids content in tomato – a re-examination

S. D. Tanksley [1] and J. Hewitt [2,*]

[1] Department of Plant Breeding and Biometry, Cornell University, Ithaca, NY 14853, USA
[2] Department of Vegetable Crops, University of California, Davis, CA 95616, USA

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Summary. Through earlier breeding efforts, portions of the genome of the wild species *Lycopersicon chmielewskii* have been introgressed into the cultivated tomato (Rick 1974). These introgressed chromosomal segments have been reported to increase soluble solids in fruit of certain tomato varieties (Rick 1974). Recently, two of the introgressed segments have been identified with RFLP markers and tested for effects on soluble solids in a single F2 population (Osborn et al. 1987). Based on results from that experiment, it was determined that one of the detected segments contains gene(s) controlling soluble solids and concluded that tomato varieties could be improved for this character by indirect selection for the linked RFLP marker (Osborn et al. 1987). In this report, we have independently tested the association between RFLP and isozyme markers and genes controlling soluble solids and other characters in the above described material. These experiments differ from the previous ones in that a set of 132 molecular markers (isozymes and DNA clones) of known chromosomal position have been used. Three introgressed chromosomal segments from *L. chmielewskii* have been identified using these markers. They map to the middle and the end of chromosome 7 (> 40 cM apart) and to the end of chromosome 10. The effects of these segments on soluble solids and other horticultural characters were tested in crosses with three different cultivars over a period of two years. Two of the three segments were found to increase soluble solids, however the effect of one of these was dependent on genetic background. Both segments were found to be associated with deleterious characters including increase in fruit pH, lower yield and small fruit.

These results confirm the utility of molecular markers for detecting genes underlying quantitative variation but demonstrate the danger in establishing breeding programs around such linkages until the effects of the quantitative genes have been tested in a variety of genetic backgrounds and for associated effects on other characters of agronomic importance.

Key words: Restriction fragment length polymorphisms – Isozymes – Tomato – Soluble solids – Quantitative traits

Introduction

Ripe tomato fruit are composed of a number of organic substances – commercially, the most important of which are the soluble components (mainly sugars and organic acids), often referred to as soluble solids. The higher the levels of soluble solids in processing tomatoes, the greater the value of the crop. The character is quantitatively inherited and genetic variation with respect to this character is fairly low among commercial tomato varieties (Lower and Thompson 1967; Ibarbia and Lambeth 1969). A wild species of tomato from Peru, *L. chmielewskii,* has been shown to have greatly elevated levels of fruit soluble solids and in 1974 Rick reported the introgression of genes from this species into a cultivated tomato variety, resulting in an increase in soluble solids (Rick 1974).

Osborn et al. (1987) used a set of random cDNA's as probes in an effort to find RFLP linkages with these "solids" genes introgressed by Rick from this wild species. With a set of 60 random probes they reported finding two chromosomal pieces from *chmielewskii* in the Rick introgression lines. In an F2 population derived from a cross with a single tomato cultivar

* Current address: Northrup King Research, P.O. Box 1827, Gilroy, CA 95021, USA

(H2038), one of those chromosomal fragments was shown to have a significant effect on soluble solids whereas the other fragment appeared to have no effect. The conclusion from this research was that the linked RFLP marker could be used in selection for high soluble solids gene(s) in tomato breeding.

In the research reported here, we have attempted to repeat and extend the results of Osborn et al. (1987). However, our approach to the problem differs in several respects. First, we have used a set of probes corresponding to known chromosomal positions over the entire tomato genome which allows any detected introgressed chromosomal segment to be mapped. Second, the effects of detected chromosomal segments have been tested in F2 and F3 populations derived from crosses with three different tomato varieties. Finally, introgressed chromosomal segments have not only been tested for their effects on soluble solids, but also on other important characters including fruit pH, fruit weight and yield.

Materials and methods

Plant material

Dr. C. M. Rick, U. C. Davis, provided five breeding lines (LA1500-1503, 1563) derived from introgression experiments between *L. chmielewskii* (LA1028) and *L. esculentum* (Rick 1974). The lines all derived from a BC2 to *L. esculentum* cv. VF36 with three subsequent backcrosses to *L. esculentum* cv. VF145 followed by several generations of selfing and pedigree selection. All lines share a common pedigree through the fifth backcross and were therefore expected to be similar if not identical with respect to any introgressed chromosomal segments from *L. chmielewskii*. VF36, VF145 and *L. chmielewskii* (LA1028) were used as controls in all experiments designed to detect RFLP fragments introgressed from *L. chmielewskii*.

Field experiments

Crosses were made between one of the introgression lines, LA1563, and three different tomato varieties – UC82B, 7879 and 78W29. Two hundred F2 progeny from each were grown in the field at Davis, CA in 1985 in a completely randomized design with multiple plantings of parents as controls. F3 families from the UC82B×LA1563 F2 were grown in the summer of 1986 in a randomized block design with three blocks and 10 F3 plants per plot.

From each individual plant in the F2 populations, 3–5 ripe fruit were harvested, homogenized and soluble solids reading obtained with a hand held refractometer. pH readings were obtained from the same extracts. From the F3, ripe fruit were harvested from each plant in every plot. All fruit from each plot were homogenized and pH and soluble solids reading obtained. For the F3 plots fruit weight, percent green fruit at harvest, green fruit yield, and red fruit yield were also determined.

RFLP analysis

DNA was extracted from each of the above tomato lines as described by Bernatzky and Tanksley (1986a). DNA from LA1563, VF36 and *L. chmielewskii* was digested with five restriction enzymes (*Dra*I, *Eco*RI, *Eco*RV, *Hind*III, and *Xba*I) and subjected to Southern analysis as described by Bernatzky and Tanksley (1986b). Filters were probed with a set of clones (cDNA and genomic) of known chromosomal position (Bernatzky and Tanksley 1986c; Tanksley et al. 1987). The clones utilized correspond to 125 loci and were selected based on map position to give maximum coverage of all regions of each of the twelve chromosomes (Fig. 1). Clones found to uncover RFLP differences between LA1563 and VF36 were further tested on DNA from the other four introgression lines (LA1500-1503) as well as additional plants of LA1563, VF36, VF145, and *L. chmielewskii*. RFLP's found in common between the introgression lines and *L. chmielewskii* but not in VF36 or VF145, were considered to mark chromosomal fragments which had been introgressed from the wild species.

DNA from F2 populations derived from crosses with LA1563 were similarly extracted and probed with clones found to mark introgressed segments of chromosomes.

Isozyme analysis

L. chmielewskii and VF36 were tested for differences in isozyme alleles at a number of previously mapped loci (Tanksley 1985). Differences were detected for 7 loci (*Prx-1, 6Pdgh-1, 6Pgdh-2, Aps-1, Aco-1, Aco-2, Pgi-1*). All introgression lines were screened for these loci to determine if any marked an introgressed chromosomal segment.

Data analysis

Effects of introgressed chromosomal segments (marked by RFLP's or isozymes) on soluble solids and pH of fruit were measured by analysis of variance (ANOVA) using sequential sums of squares with marker genotypes and populations (F2) as factors (Tanksley et al. 1982). All analyses were performed on an IBM PC/AT computer using SPSS software.

Results

Detection of introgressed chromosomal segments from L. chmielewskii

Of the 132 loci tested in the introgression lines five were found to possess alleles absent in the recurrent parents (VF36 and VF145) but present in *L. chmielewskii* donor population. *L. chmielewskii* was found to be polymorphic for several of these markers (data not shown). This result was not unexpected since previous isozyme studies have shown that most populations of this outcrossing species are polymorphic (Rick et al. 1976). In the cases where polymorphism was encountered, only one of the alleles from *L. chmielewskii* could be identified in the introgression lines whereas the allele was absent in the recurrent parents (VF36, VF145). All of the introgression lines shared the same *L. chmielewskii* alleles except for LA1563 which was still segregating for *TG13A*.

Four of the introgressed loci were detected with DNA clones (*TG64, Cab-4, TG13A* and *CD56*) and the fifth was for the isozyme locus *Aco-2*. Three of these markers, *TG64, Aco-2* and *Cab-4,* are linked and comprise a 14 cM section in the middle of chromosome

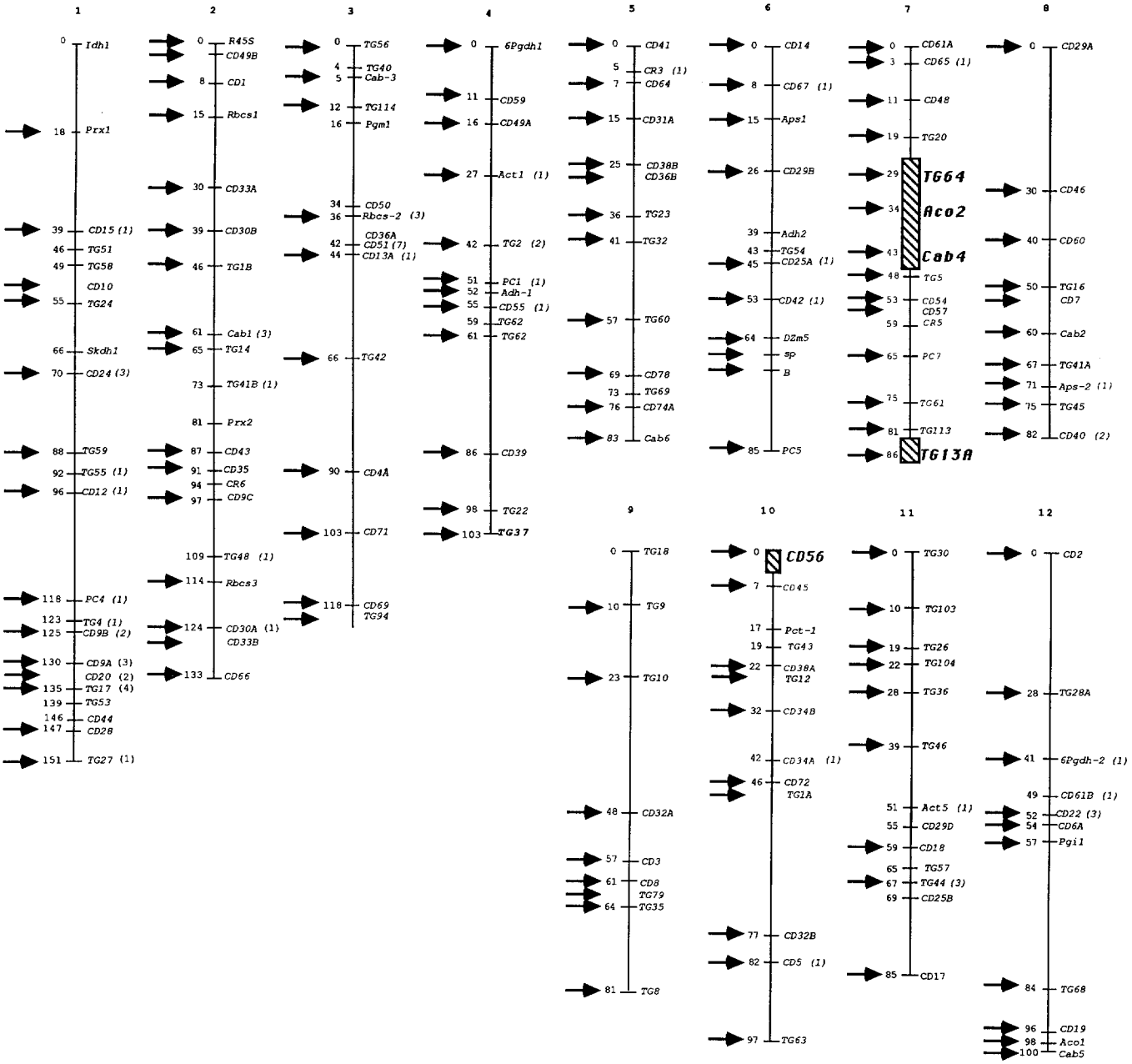

Fig. 1. Molecular map of tomato. *Arrows* point to markers evaluated on solids introgression lines. *Shaded segments* were determined to come from *L. chmielewskii*

7. Henceforth, this segment will be referred to as the *Aco-2* segment. *TG13A* is also on chromosome 7, but is at the most terminal position on the long arm, approximately 45 cM from the *Aco-2* segment (Fig. 1). Five intervening markers between the *Aco-2* segment and the *TG13A* segment were also tested and found not to derive from *L. chmielewskii* indicating that two separate fragments of chromosome 7 had been introgressed from chromosome 7 of *L. chmielewskii.* The fifth marker determined to come from the wild species was *CD56* which occupies the most terminal position of chromosome 10 (Fig. 1).

The minimum and maximum size of the *Aco-2* chromosomal segment can be estimated by examining flanking markers. This segment is marked by three loci spanning 14 cM. Thus the minimum size of the segment is 14 cM. The markers on either side of the introgressed segment (*TG20, TG5*), but not part of the segment, are 29 cM apart which represents the maximum size of the segment. An estimate of the expected

size of the piece is simply the average of the minimum and maximum which is 21 cM. The *TG13A* and *CD56* segments are at the end of chromosomes and each marked by a single locus so it is not possible to accurately estimate the size of the segment. However, *TG113,* which is 5 cM proximal to *TG13A,* tested negative indicating the maximum size of the piece extending towards the centromere is 5 cM. *CD56* was also the only marker testing positive on chromosome 10. The next proximal marker tested on this chromosome was *CD45* which is 7 cM away placing 7 cM as a maximum proximal distance that the segment extends. If one assumes that the size of the *CD56* and *TG13A* segments is 5 cM each and that the *Aco-2* segment is 21 cM, then the total amount of *chmielewskii* chromosome present in the introgression line is approximately 31 cM. The total map units in the tomato genome is approximately 1,200 cM. Thus the wild species chromosomal segments comprise approximately 31/1200 = 2.5% of the genome.

Table 1. Means and standard errors for fruit soluble solids and pH for introgression line (LA1563) and varieties used as parents in F2 populations

	Variety			
	LA1563	UC82B	7879	78W29
Soluble solids (Brix)	6.18±0.05	4.39±0.04	5.58±0.09	5.09±0.06
pH	4.68±0.01	4.17±0.01	4.30±0.01	4.31±0.01

F2 populations

Solids and pH values for each of the parental lines is given in Table 1. Distributions for the segregating F2 populations is shown in Fig. 3.

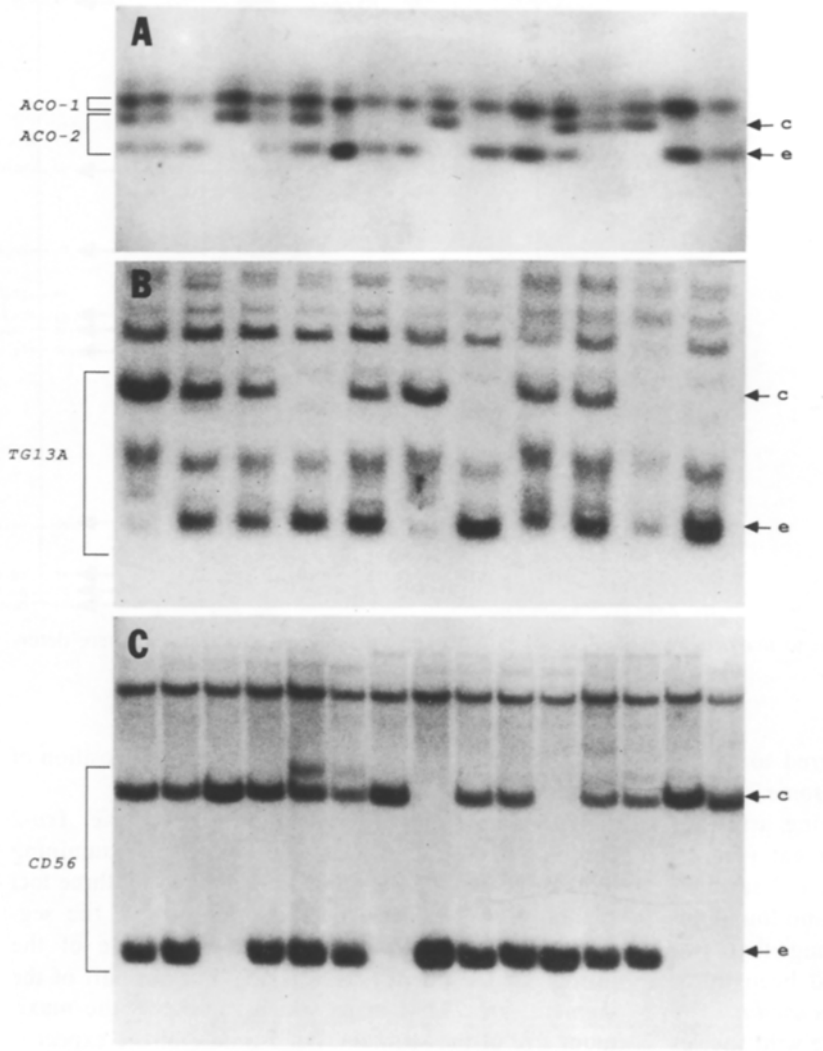

Fig. 2. Segregation of molecular markers in F2 population. *A*) *Aco-2, B*) *TG13A, C*) *CD56. e* = *esculentum* allele, *c* = *chmielewskii* allele

Table 2. Monogenetic segregation ratios for markers scored in F2 populations, e/e = homozygous *esculentum*, e/c = heterzygous, c/c = homozygous *chmielewskii*

F2 pop.	Aco-2			Cab-4			TG13A			CD56		
	e/e	e/c	c/c	e/e	e/c	c/c	e/e	e/c	c/c	e/e	e/c	c/c
78W29	60	111	41	49	82	28	44	76	41	41	79	48
7879	64	98	61	42	56	39	33	62	26	44	75	40
UC82B	72	101	49*	51	70	40	37	74	34	47	68	45

* Significant deviation from 1 : 2 : 1 ratio at 0.05 level

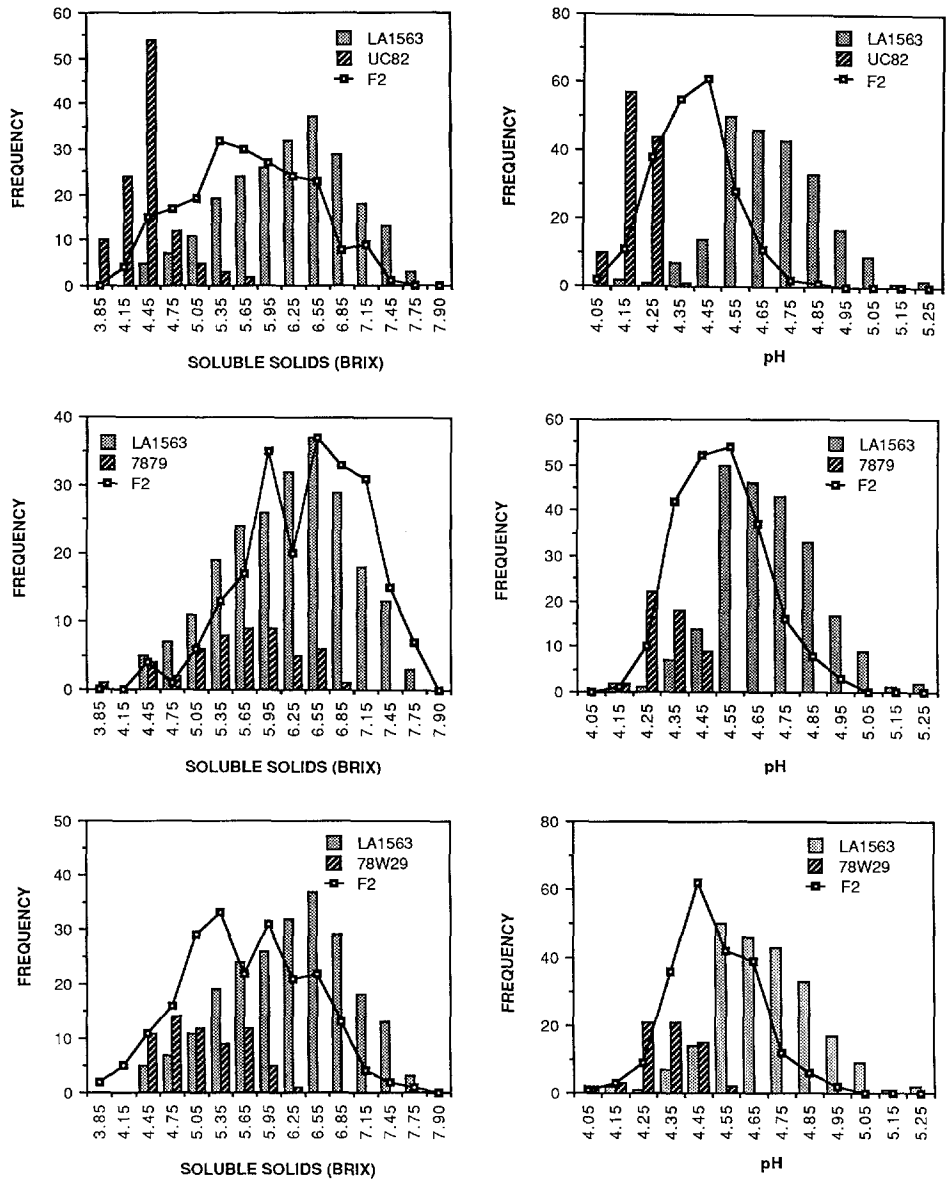

Fig. 3. Histogram showing distribution of parental and F2 populations with respect to soluble solids and pH

Table 3. Mean fruit soluble solids (Brix) for different genotypic classes in segregating F2 populations. Number in parentheses = no. of individuals examined. e/e = homozygous *esculentum*, e/c = heterozygous, c/c = homozygous *chmielewskii*

F2 population = LA1563 × 78W29						
Marker	Genotype			ms	F	Significance
	e/e	e/c	c/c			
Aco2	5.55 (60)	5.67 (111)	5.60 (41)	0.29	0.49	0.61
Cab4	5.57 (47)	5.68 (79)	5.67 (27)	0.20	0.30	0.74
CD56	5.40 (39)	5.71 (75)	5.75 (48)	1.61	2.55	0.08
TG13A	5.76 (43)	5.60 (74)	5.63 (38)	0.38	0.57	0.57
F2 population = LA1563 × 7879						
Marker	Genotype			ms	F	Significance
	e/e	e/c	c/c			
Aco2	6.18 (64)	6.53 (98)	6.50 (61)	2.64	4.84	0.01
Cab4	6.21 (41)	6.42 (55)	6.52 (39)	1.04	1.93	0.15
CD56	6.26 (42)	6.50 (75)	6.38 (40)	0.78	1.49	0.23
TG13A	6.35 (33)	6.57 (62)	6.32 (26)	0.88	1.66	0.19
F2 population = LA1563 × UC82B						
Marker	Genotype			ms	F	Significance
	e/e	e/c	c/c			
Aco2	5.63 (71)	5.76 (93)	5.44 (45)	1.66	3.14	0.04
Cab4	5.55 (49)	5.82 (65)	5.38 (38)	2.51	4.76	0.01
CD56	5.57 (45)	5.72 (65)	5.73 (41)	0.37	0.68	0.51
TG13A	5.62 (35)	5.68 (72)	5.57 (34)	0.13	0.24	0.78

Table 4. Mean fruit pH for different genotypic classes in segregating F2 populations. e/e = homozygous *esculentum*, e/c = heterozygous, c/c = homozygous *chmielewskii*

F2 population = LA1563 × 78W29						
Marker	Genotype			ms	F	Significance
	e/e	e/c	c/c			
Aco2	4.42 (60)	4.52 (111)	4.56 (41)	0.26	12.64	0.00
Cab4	4.44 (47)	4.53 (79)	4.52 (27)	0.13	5.80	0.00
CD56	4.43 (39)	4.51 (75)	4.56 (48)	0.18	8.35	0.00
TG13A	4.50 (43)	4.49 (74)	4.51 (38)	0.01	0.21	0.81
F2 population = LA1563 × 7879						
Marker	Genotype			ms	F	Significance
	e/e	e/c	c/c			
Aco2	4.44 (64)	4.51 (98)	4.58 (61)	0.27	12.72	0.00
Cab4	4.43 (41)	4.50 (55)	4.60 (39)	0.27	13.89	0.00
CD56	4.44 (42)	4.52 (75)	4.57 (40)	0.18	9.04	0.00
TG13A	4.51 (33)	4.49 (62)	4.54 (26)	0.03	1.21	0.30
F2 population = LA1563 × UC82B						
Marker	Genotype			ms	F	Significance
	e/e	e/c	c/c			
Aco2	4.34 (71)	4.39 (93)	4.43 (45)	0.11	6.43	0.00
Cab4	4.35 (49)	4.39 (65)	4.43 (38)	0.07	3.90	0.02
CD56	4.31 (45)	4.40 (65)	4.45 (41)	0.24	15.58	0.00
TG13A	4.37 (35)	4.38 (72)	4.38 (34)	0.01	0.04	0.95

DNA was extracted from each F2 plant and probed with *Cab-4*, *CD56* and *TG13A*. Isozyme genotypes were also determined for *Aco-2* from leaf tissue. Typical segregation for DNA and isozyme markers is shown in Fig. 2. Monogenic segregation ratios from each segregating locus monitored is given in Table 2. A greater number of e/e (*esculentum*) homozygotes than c/c (*chmielewskii*) homozygotes was observed for most loci in all populations. However, the skewing was statistically significant ($P \leqq 0.05$) only for *Aco-2* in the F2 derived from the cross to UC82B. Skewing toward recurrent parent alleles has been observed on many occasions when genes have been introgressed from wild species (Zamir and Tadmor 1987). While the skewing observed in these experiments was not strong, it is consistent with these previous findings.

Testing for association of markers with genes controlling fruit soluble solids and pH

Potential association of soluble solids and pH with the three segregating chromosomal segments was tested with analysis of variance procedures for each of the tree

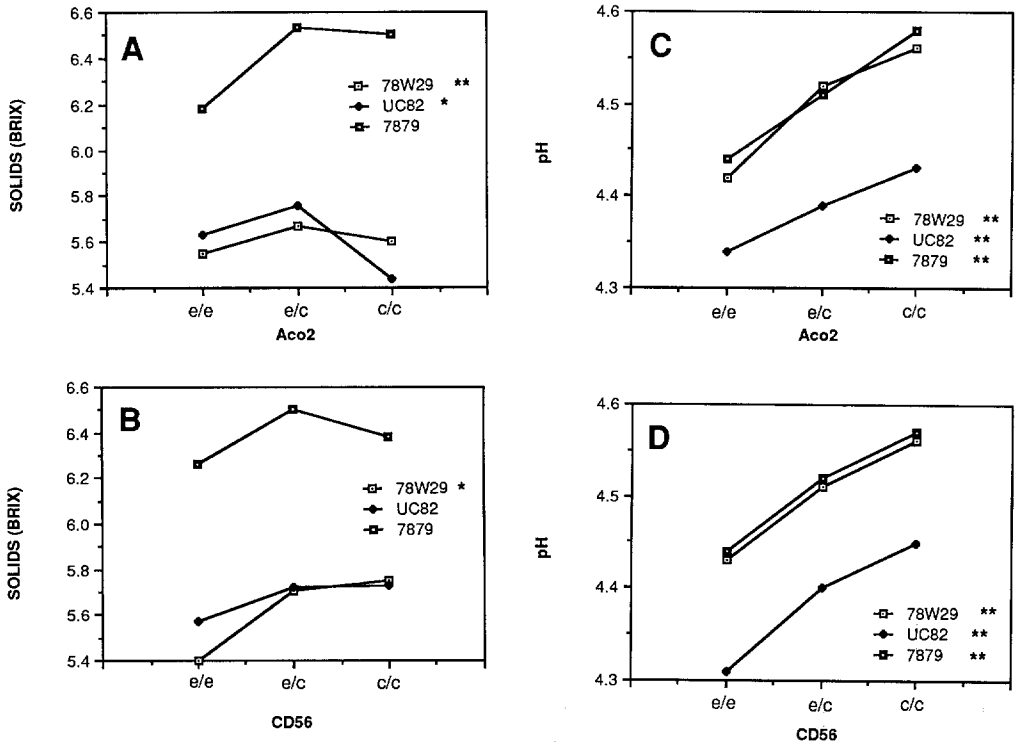

Fig. 4A–D. Plots showing genotypic effects of *Aco-2* and *CD56* in different F2 populations with respect to solids and pH. **A** *Aco-2* × solids, **B** *CD56* × solids, **C** *Aco-2* × pH, **D** *CD56* × pH. e/e = homozygous for *esculentum* alleles, c/c = homozygous for *chmielewskii* alleles, e/c = heterozygous; * significant at 0.05, ** significant at 0.01

F2 populations. Results are summarized in Tables 3 and 4 and presented graphically in Fig. 4.

Soluble solids

The *L. chmielewskii* segment marked by *TG13A* on the end of chromosome 7 had no detectable effect on soluble solids in any of the F2 populations (Table 3). This segment also appears to be neutral with respect to both soluble solids and pH (see next section), and may be a residual segment that, by chance, was maintained during the backcross program.

The effect of the *Aco-2* segment was variable, depending on the F2 population being evaluated. In the F2 with 78W29, this segment produced no detectable effect. In the UC82B cross, the segment had a significant effect, only in this case, most of the effect was due to the fact that plants homozygous for the *L. chmielewskii* segment were *lower* in soluble solids than either the *esculentum* homozygotes or the heterozygotes (Table 3). In the cross with 7879, the *Aco-2* segment was found to have a highly significant, positive effect on soluble solids ($P \leqq 0.01$). The c/c (*chmielewskii* homozygotes) and e/c (heterozygotes) genotypes were approximately the same wih respect to soluble solids and both were significantly higher than e/e genotypes (*esculentum* homozygotes). The magnitude of the difference was approximately 0.3 Brix units. These results indicate that in this genetic background, the *L. chmielewskii* segment is behaving in a dominant manner with respect to solids and is consistent with the method by which Rick originally bred the high solids lines – backcrossing where *chmielewskii* alleles would have to be either dominant or additive to have been selected (Rick 1974).

To statistically test the hypothesis that the *Aco-2* segment was having different effects, depending on the cross being examined, a 3-way ANOVA was conducted (Table 5). In this analysis the population × *Aco-2* interaction is significant ($P \leqq 0.05$) support the hypothesis that there are interactions between the *Aco-2* segment and the genetic background (variety) in which it is segregating. This result can be visualized by plotting the population by *Aco-2* genotype with respect to soluble solids (Fig. 4a).

As previously indicated, the *Aco-2* segment is also marked by two other loci, *Cab-4* and *TG64*. This segment was monitored by both *Aco-2* and *Cab-4* in the F2 populations. ANOVA with *Cab-4* produced results virtually indistinguishable to that produced using *Aco-2* as the marker (Table 3). While both loci were correlated with solids effects in certain populations, it was not possible to determine whether the gene(s) controlling the effect map closer to one or the other marker.

Table 5. Summary of analysis derived by pooling data from all three F2 populations

Source of variation	DF	Soluble solids			pH		
		ms	F	Significance	ms	F	Significance
F2 population	2	29.18	51.99	0.00	0.69	39.76	0.00
Aco-2	2	0.98	1.74	0.17	0.43	24.76	0.00
CD56	2	2.16	3.84	0.02	0.53	30.48	0.00
2-way interaction							
F2 pop × *Aco-2*	4	1.33	2.37	0.05	0.03	1.46	0.21
F2 pop × *CD56*	4	0.27	0.49	0.74	0.01	0.21	0.93
Aco-2 × *CD56*	4	0.72	1.28	0.28	0.01	0.67	0.60
3-way interaction	8	0.35	0.62	0.76	0.01	0.76	0.63

The *CD56* segment on chromosome 10 produced a small, but marginally-significant effect ($P \leqq 0.08$) on solids in the cross with 78W29 (Table 3). In this case, the e/c and c/c genotypes were similar and both higher than the e/e homozygote. While the mean values for e/c and c/c genotypes in the other crosses were slightly higher than the e/e genotypes, the effects were statistically non-significant. Combining all populations for ANOVA, the F value becomes significant ($P \leqq 0.02$) suggesting that this segment does contribute to increased soluble solids (Table 5). The interaction between populations and *CD56* was non-significant ($P \leqq 0.74$) (Table 5). In the three different populations, the difference between c/c and e/e *CD56* genotypes with respect to solids was 0.35, 0.12 and 0.16 Brix units for the 78W29, 7879 and UC82B populations, respectively. The average difference was 0.21 Brix units. The *Aco-2* × *CD56* interaction term for both within population ANOVA's as well as pooled population ANOVA was non-significant, suggesting that there is little or no interaction between these two segments with regards to control of soluble solids.

Fruit pH

The pH of processing tomato fruit is an important factor determining variety acceptability. Lower pH's are necessary to control growth of microorganisms during and after processing. Higher acid content also enhances fruit flavor. It is therefore important to monitor both variables (solids and pH) in a breeding program directed at modifying fruit characteristics. Fruit from the introgression line used in these experiments has a pH level unacceptably high for most processors (Table 1). However, it was not known if this was due to the presence of genes from *L. chmielewskii*. In an attempt to answer this question, fruit pH was monitored in all F2 populations and tested for correlations with the introgressed chromosomal segments. Results are presented in Table 4.

As with soluble solids, the *TG13A* segment was determined to have no detectable effect on pH. The other two *chmielewskii* chromosomal segments, marked by *Aco-2* and *CD56*, were highly correlated with pH ($P \leqq 0.001$) in all three F2 populations (Table 4). In all cases the pH of e/e genotypes was lower than the c/c genotypes and the heterozygotes were intermediate indicating within locus additivity. This result is depicted graphically in Fig. 4c, d. Despite a major effect of each locus on pH, no epistatic interaction could be detected based on 2-way analysis of variance (Table 5, Fig. 5). So, from both a within locus and between locus viewpoint, both of these loci are largely additive with respect to pH. Likewise, no interaction with populations was detected, further supporting the hypothesis that these segments contain non-epistatic gene(s) for pH (Table 5).

Although each of the populations had significantly different mean pH value, the magnitude of the effect of the *Aco-2* and *CD56* segments on pH was surprisingly uniform (Fig. 4b). For example with respect to the *Aco-2* locus in the 78W29 cross, pH values for the three genotypes were: e/e = 4.42, e/c = 4.52, c/c = 4.56. The increase in pH across genotypes was thus 0.10 and 0.04, respectively. For comparison, the increments of pH increase across genotypes at this locus in the 7879 cross was 0.07 and 0.07. For the UC82B cross, the increments were 0.05 and 0.04. A nearly identical uniform response across populations was also observed for genotypic effects on pH for the *CD56* segment.

By breaking the populations down into two locus genotypes for *Aco-2* and *CD56*, it was possible to estimate the combined effect of these segments on pH (Table 6). By comparing these values with the differences in pH measured in the parents of each cross, it is possible to estimate the proportion of pH difference between the two parents which can be accounted for by the two *chmielewskii* chromosomal segments. For 78W29 and 7879, 60–70% of the pH difference between these varieties and LA1563 could be accounted for by

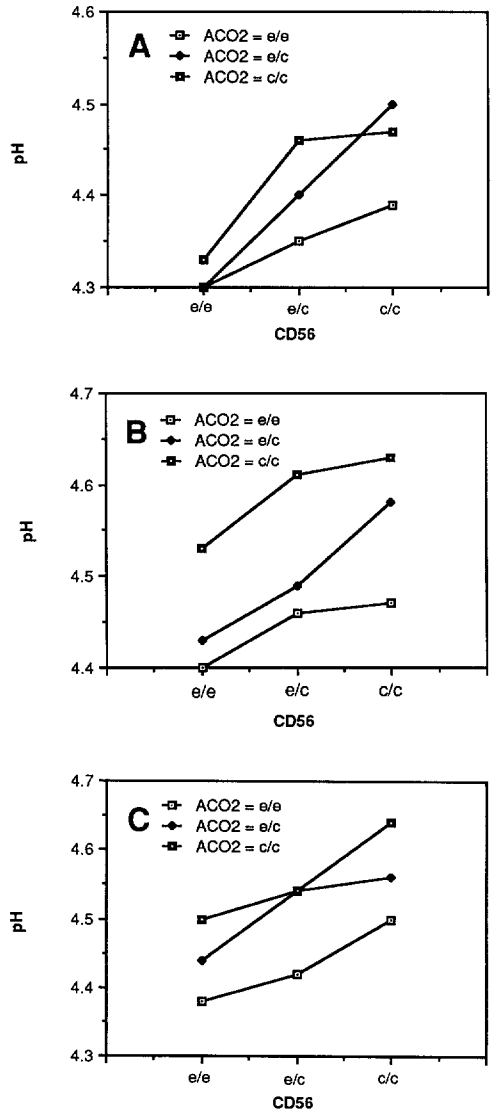

Fig. 5A–C. *Aco-2×CD56* pH interaction plots. **A** UC82B ×LA1563 F2, **B** 7879×LA1563 F2, **C** 78W29×LA1563 F2. e/e = homozygous for *esculentum* alleles, c/c = homozygous for *chmielewskii* alleles, e/c = heterozygous

the segments. For the UC82B, only 33% of the difference could be attributed to these loci. This is not surprising since UC82 has a significantly lower pH value than either 7879 or 78W29 and probably has other genetic differences, independent of the introgressed segments, which are determining pH levels in the fruit.

F3 study

UC82B is one of the most widely grown processing tomato variety in the world and many modern varieties derive from crosses to this cultivar. It was important

Table 6. Estimated combined effects of segments marked by *Aco-2* and *CD56* on fruit pH. pH values are given for 2-locus genotypes. Number in parenthesis = No. of plants of that genotype. Variety differences are derived by comparing pH values of two parents used to make F2

F2 pop	*Aco-2, CD56* genotype				
	c/c c/c	e/e e/e	Δ	var. Δ[a]	%[b]
78W29	4.64 (6)	4.38 (14)	0.26	0.37	70%
7879	4.63 (10)	4.40 (16)	0.23	0.38	61%
UC82B	4.47 (11)	4.30 (15)	0.17	0.51	33%

[a] Difference in fruit pH between LA1563 (introgression line) and corresponding variety

[b] Δ/var. $\Delta \times 100$, estimates difference in fruit pH between each variety and LA1563 that can be accounted for by *Aco-2* and *CD56* segments

then, to confirm the effects (or lack thereof) of the detected chromosomal segments introgressed from *chmielewskii*. For this reason, in the summer of 1986, 72 F3 lines, derived from the F2 population described above, were grown in replicated plots of 10 plants each as described in Materials and Methods. By repeating the field experiments a second season, it was possible to assess effects of the introgressed segments over two different years (environments). Soluble solids, pH and fruit weight were measured on all plots. On a subset of plots, green and red fruit yield were measured as well as percent green fruit at time of harvest. From the red fruit yield and soluble solids measurements, a solids yield was calculated which is simply the product of red yield × soluble solids and is a measure of the relative value of tomato varieties for commercial processing.

Effects of the introgressed chromosomal segments on each of the above described variables was estimated using ANOVA (Table 7). In addition, the means of the e/e and c/c derived F3 lines were compared using an independent two-sample T-test (Table 8). The results confirm the effect of both the *Aco-2* and *CD56* segments on pH. As with the F2, presence of the *chmielewskii* segments caused a highly significant increase of fruit pH. F3 lines derived from c/c F2 plants had a mean pH value 0.09 higher than those derived from e/e homozygotes. In the F2, from the previous year, the difference between these genotypes was also 0.09. Likewise, as in the F2, the *CD56* segment caused an additive increase in pH. The difference between the c/c and e/e derived genotypes for this marker in the F3 0.11 versus 0.14 in the F2. The *TG13A* segment again had no detectable effect on pH.

Table 7. Genotypic means and analysis of variance for effects of introgressed segments on fruit pH, soluble solids, fruit weight, percent green fruit and yield in F3 lines. e/e = homozygous *esculentum*, e/c = heterozygous, c/c = homozygous *chmielewskii*

Marker	Mean fruit pH					Significance
	Genotype			ms	F	
	e/e	e/c	c/c			
Aco2	4.41 (33)	4.47 (42)	4.50 (22)	0.06	7.71	0.00
Cab4	4.42 (20)	4.45 (34)	4.52 (17)	0.05	6.84	0.00
CD56	4.41 (18)	4.44 (31)	4.52 (22)	0.07	10.71	0.00
TG13A	4.46 (18)	4.44 (26)	4.46 (16)	0.01	0.36	0.69

Marker	Mean soluble solids					Significance
	Genotype			ms	F	
	e/e	e/c	c/c			
Aco2	5.71 (33)	5.79 (43)	5.74 (22)	0.06	0.28	0.74
Cab4	5.73 (20)	5.71 (35)	5.72 (17)	0.01	0.02	0.97
CD56	5.66 (18)	5.68 (31)	5.81 (23)	0.15	0.99	0.37
TG13A	5.73 (18)	5.78 (27)	0.02 (16)	0.10	0.10	0.90

Marker	Fruit wt					Significance
	Genotype			ms	F	
	e/e	e/c	c/c			
Aco2	71.97 (33)	69.00 (43)	72.36 (22)	118.9	0.66	0.51
Cab4	70.80 (20)	70.41 (35)	71.56 (17)	7.69	0.03	0.96
CD56	75.42 (18)	70.42 (31)	67.80 (23)	297.2	1.62	0.20
TG13A	73.48 (18)	68.65 (27)	69.36 (16)	134.9	0.66	0.51

Marker	Percent green fruit					Significance
	Genotype			ms	F	
	e/e	e/c	c/c			
Aco2	17.09 (13)	15.50 (21)	10.54 (14)	163.7	2.34	0.10
Cab4	19.51 (6)	16.14 (13)	10.67 (12)	180.2	2.16	0.13
CD56	11.21 (9)	15.72 (14)	17.17 (8)	86.4	0.97	0.39
TG13A	21.44 (6)	15.84 (12)	14.51 (9)	93.5	1.06	0.36

Marker	yield (green fruit) kg/plot					Significance
	Genotype			ms	F	
	e/e	e/c	c/c			
Aco2	6.15 (13)	4.96 (21)	2.89 (14)	37.30	2.61	0.08
Cab4	7.92 (6)	5.34 (13)	2.98 (12)	50.63	2.80	0.07
CD56	3.52 (9)	5.63 (14)	5.42 (8)	13.35	0.65	0.53
TG13A	8.25 (6)	15.38 (12)	4.25 (9)	29.50	1.46	0.25

Marker	yield (red fruit) kg/plot					Significance
	Genotype			ms	F	
	e/e	e/c	c/c			
Aco2	26.93 (13)	24.88 (21)	23.28 (14)	44.81	2.72	0.07
Cab4	28.57 (6)	25.35 (13)	23.76 (12)	46.18	3.09	0.06
CD56	26.15 (9)	25.57 (14)	24.53 (8)	5.73	0.32	0.72
TG13A	26.45 (6)	26.73 (12)	23.35 (9)	32.60	2.25	0.12

Marker	Solids yield					Significance
	Genotype			ms	F	
	e/e	e/c	c/c			
Aco2	1.83 (13)	1.73 (21)	1.51 (14)	0.75	3.95	0.02
Cab4	2.02 (6)	1.71 (13)	1.53 (12)	0.48	4.98	0.01
CD56	1.66 (9)	1.74 (14)	1.70 (8)	0.02	0.14	0.86
TG13A	1.94 (6)	1.83 (12)	1.56 (9)	0.31	2.62	0.09

None of the *chmielewskii* segments had a statistically detectable effect on soluble solids in the F3 when all genotypes were analyzed in the ANOVA. However, the mean of *CD56* c/c homozygotes was 5.81 versus 5.66 for e/e homozygotes (a 0.15 difference) which is consistent with results in the F2 which indicated that this segmet may cause a slight increase in soluble solids.

The *Aco-2* and *TG13A* segments appeared to have no effect on fruit weight. However, the means for the

Table 8. T-test for difference in means of e/e versus c/c derived F3 genotypes in F3 with respect to introgressed chromosomal segments

Marker	solids	pH	ft.wt.	% green	Yield		sol.yld.
					green	red	
Aco2	ns	0.00	ns	0.08	0.07	< 0.01	< 0.01
Cab-4	ns	0.00	ns	ns	ns	0.02	0.01
Cd56	ns	0.00	0.03	0.09	ns	ns	ns
TG13A	ns	ns	ns	ns	ns	ns	ns

CD56 segment indicated that this segment may contain genes that affect fruit weight: e/e = 75 gm/ft, e/c = 70 gm/ft, c/c = 68 gm/ft. A t-test comparing the means of the e/e and c/c derived F3 lines confirmed that this was the case ($P \leqq 0.03$) (Table 8). The difference in the mean between these two classes was 7 grams per fruit which represents a 10% reduction associated with the *CD56* segment.

Red fruit yield was significantly affected by the *Aco-2* segment (Table 7). The yield difference in the e/e versus c/c derived F3 populations was 3.65 kg/plot which represents a 14% yield reduction (Table 7). As a result of the red fruit yield reduction, the solids yield was also significantly depressed by the presence of the wild species *Aco-2* fragment (Tables 7 and 8).

Heritability of pH and soluble solids

Based on parent – offspring (F2, F3) regression analysis (Falconer 1960) it was possible to estimate narrow sense heritability (h^2) for both soluble solids and pH in the UC82 × LA1563 cross. It is estimated that for soluble solids $h^2 = 0.23 \pm 0.05$ and for pH $h^2 = 0.39 \pm 0.7$.

Discussion

Map positions of segments introgressed from chmielewskii

During the original introgression experiments from *chmielewskii,* Rick (1974) reported an association of soluble solids with *u* (gene for uniform ripening of fruit) in some breeding lines. *u* and *CD56* are approximately 10 cM apart on the short arm of chromosome 10 (S.D. Tanksley and T. Hewitt, unpublished results). It is apparent from data presented in this report that *CD56* marks a segment of chromosome 10 introgressed from *chmielewskii* which contains gene(s) affecting soluble solids. Based on Rick's report of early generation association of u^+ with soluble solids, it appears that the *chmielewskii* alleles u^+ and *CD56* were introduced together on the same chromosomal segment (i.e. in cis) and that in latter generations the linkage was broken. Breeding lines ultimately released by Rick contained only the segment of wild species chromosome marked by *CD56.*

Rick (1974) also reported an association between *B* (gene from *chmielewskii* conferring beta-carotene formation and thus orange fruit) on chromosome 6 and soluble solids. We have probed with a number of clones on chromosome 6 including ones known to flank the *B* gene (Fig. 1). We find no evidence for introgressed segments on this chromosome. This does not rule out the existence of such a segment, but if it does exist, it is undoubtedly quite short.

Comparison of results from this study with those of previous study

This marks the second time that the introgression lines described in this paper have been studied with molecular markers. In the first report, a set of 60 cDNA probes were used to detect two chromosomal segments introduced from the wild species (Osborn et al. 1987). Results from the research reported here agree with those of Osborn et al. in two ways. First, using molecular markers (isozymes and RFLP's), it is possible to detect segments of chromosomes introgressed from the wild species. Second, the number of introgressed segments is small. The previous paper reports two introgressed segments and here we report the presence of three. However, since Osborn et al. did not determine the chromosomal location of the detected segments, it is presently not possible to establish which of those segments correspond to the ones reported here. The probes used in this study were selected based on map location to cover positions on all chromosomes. Only if an introgressed segment were quite small would it likely have escaped detection. Based on the number of backcross generations used to construct the introgression lines, one can calculate that introduced segments would likely average 30 cM (based on formulas given by Hanson 1959). This estimate assumes that the segment in question is in the middle of a chromosome and that no selection for recombinant plants has occurred. If either of these assumptions are incorrect, the size of the segment would be smaller. It is interesting to note, however, that the theoretical value of 30 cM is close to the estimated size of the *Aco-2* segment (21 cM), the only segment found in the middle of a chromosome. For most positions in the genome, segments of this size and even those as small as 10 cM would likely have been detected with the probes used in this study. Based on these estimates, it seems unlikely that the total number of introgressed segments is much greater than the three reported in this study. If

this is true, the two segments detected by Osborn et al. are likely a subset of the segments reported here.

Osborn et al. (1987) monitored segregation of the two detected introgressed segments in an F2 population in a single year for one character, soluble solids. From this test it was determined that one of the segments was associated with gene(s) that increase the level of soluble solids in the tomato fruit. Based on this result, it was suggested that higher soluble solids could be bred into tomato cultivars by selection for the linked RFLP marker. In the study described here, we have tested the effects of three introgressed segments in four populations over two years. The populations represent crosses to three different cultivars of tomato. The introgressed segments were tested not only for their effects on soluble solids, but also fruit pH, fruit size, and yield. Based on the results, we conclude that two of the segments can have an effect on soluble solids. However, one of those segments (*Aco-2*) interacts significantly with the genetic background in which it is placed. In some cases, it significantly increases solids, in others it is either neutral or causes a decrease in solids. The same segment is also associated with a highly significant increase in fruit pH (a negative attribute) in all genetic backgrounds and can result in a significant decrease in total yield. The other segment affecting soluble solids, *CD56*, is more consistent in its effect. It had a tendency to cause a slight increase in soluble solids in all genetic backgrounds. However, this segment is also associated with a significant increase in fruit pH as well as a reduction in fruit weight.

If this study had been confined to a single population and a single character (soluble solids), we might also be tempted to conclude that higher soluble solids tomatoes could be bred by selection of the linked RFLP marker. However, after evaluating the effects more thoroughly, it is now apparent hat such a conclusion would be premature. Selection for the *Aco-2* segment would result in an increase in soluble solids only in some genetic backgrounds. In others it might have no effect or possible decrease in soluble solids. In addition, it would almost certainly result in an increase in fruit pH as well as a reduction in yield. Selection for the *CD56* segment would be much more likely to result in an increase in soluble solids in many different genetic backgrounds, however the fruit pH would also be increased and a reduction in fruit weight might also result.

Association of soluble solids and pH – linkage or pleiotropy

As already discussed, the two *chmielewskii* segments found to affect soluble solids also affect pH. The possibility must be considered that modification of both characters is the result of activity of a single gene (pleiotropy). An increase in sugars (major component of soluble solids) might be made at the expense of acids and thus pH would increase as solids increase. However, linkage of genes separately affecting each character can explain the results equally well. Relevant to this issue is the fact that both the *CD56* and *Aco-2* segments were found to affect pH in all genetic backgrounds whereas the effect of the *Aco-2* segment on soluble solids was variable depending on the genetic background. Thus there is not a perfect relationship between the increase in pH and soluble solids which might be expected if pleiotropy were involved. While this result weighs against pleiotropy, it does not disprove it. If linkage is causing the negative effects on pH, it might be possible to break the association by further recombination in the introduced segments which could be detected by the molecular markers that reside there. This approach would be especially valid for the *Aco-2* segment which is marked by three loci.

Use of molecular markers in quantitative genetics

There has been much discussion and debate about the role of molecular markers in quantitative genetics. Theoretical calculations have been used to both prove (Soller and Brody 1976, Soller and Beckmann 1983) and disprove (Ellis 1986) the utility of molecular markers in locating quantitative trait loci (QTL's). Until recently, there has been insufficient experimental data on which to base any firm conclusions in this regard. This is no longer the case. Quantitatively-inherited characters have now been studied with molecular markers in tomato (Tanksley et al. 1982; Osborn et al. 1987; Zamir et al. 1984), pepper (Tanksley and Iglesias-Olivas 1984), and maize (Stuber et al. 1987; Edwards et al. 1987). In all of these studies, it has been possible to detect genes controlling the character of interest. The success of these experiments give strong support to the claim that molecular markers can be used to detect, map and monitor genes controlling quantitative variation. It is no longer valid to believe that ... "RFLP markers can really only be used to follow the segregation of reasonably closely linked genes where segregating alleles confer very different phenotypes (ie classical morphological markers)" (Ellis 1986). In the study described here, molecular markers have been used to detect linked genes which control characters with moderate to low heritabilities. For pH, genes were detected which condition a change as small as 0.05 pH units. Not only could these genes be detected, but their degree of within and between locus additivity tested.

While it is no longer an issue of whether molecular markers can detect genes controlling quantitative varia-

tion, there is still a major unanswered question: What proportion of genes underlying quantitative variation function in a predictable manner across many different genetic backgrounds? It is these types of genes that could be beneficially tagged and transferred with molecular markers.

The first step toward utilizing molecular markers for improving polygenic traits is to find sufficiently tight linkage between a gene(s) controlling the characer and a molecular marker. The advent of high density RFLP maps in crops such as tomato and maize now makes that possible. Once the linkage relationship is established, the chromosomal segment containing the QTL, can be efficiently transferred into different breeding lines or varieties with a high degree of certainty. What it not certain is whether the gene will give the desired effect in a new genetic background. In order to have confidence in the effect of a QTL, it must first be tested in different genetic backgrounds. In addition, the marked segment containing the QTL must also be tested for effects (due to pleiotropy or linkage) on other characters of agronomic importance. It is only once a QTL passes these tests that one can begin predictably modifying quantitative traits via selection of linked molecular markers.

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