Relationship of Tomato Fruit Sugar Concentration with Physical and Chemical Traits and Linkage of RAPD Markers

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ABSTRACT. Small-fruited cherry tomato accession PI 270248 (Lycopersicon esculentum Mill. var. cerasiforme Dunal) with high fruit sugars was crossed to large-fruited inbred line Fla.7833-1-1-1 (7833) that had normal (low) fruit sugar. Sugars in the F2 were positively correlated with soluble solids, glucose, fructose, p$\text{H}$, and titratable acidity, and inversely correlated with fruit size. Earliness was not significantly correlated with sugars but was negatively correlated with fruit size. Thus, the lack of a sugar–earliness correlation indirectly indicates a trend for early tomato plants to be lower in sugars than later maturing plants. Sugars were not correlated with yield or pedicel type. Fruit from indeterminate plants had significantly more sugars than from determinate plants. Six random amplified polymorphic DNA (RAPD) markers linked to high sugars were found, five dominant (OPAE 4, UBC 731, UBC 744, UBC 489, UBC 290) and one co-dominant (UBC 269). Five of the markers were also linked to small fruit size and one of these also was linked to low yield (UBC 290). The sixth marker (UBC 269) was linked to indeterminate plant habit. UBC 731, UBC 489, and possibly OPAE 4 were in one linkage group, while UBC 744 and UBC 290 were in another linkage group. Combinations of all the markers together explained 35% of the sugar variation in the F2 grown in Spring 2002.

Consumers often are dissatisfied with the flavor of fresh-market tomatoes (Lycopersicon esculentum) (Bruhn et al., 1991). In tomato breeding, there are priorities other than flavor that are important, such as high yield, disease resistance, and shipping ability, and it is difficult to breed for improved flavor (Scott, 2002). Indeed, flavor is a complicated trait influenced, mainly, by taste (sweetness, sourness) and aroma. Taste is basically determined by sugars and acids (Kader et al., 1977; Malundo et al., 1995; Stevens et al., 1977a, 1977b). Sugars in L. esculentum are mostly comprised of glucose and fructose with trace amounts of sucrose (Davies and Hobson, 1981; Stevens, 1972), and Petro-Turza (1987) attributed the sweet taste of tomato to reducing sugars. Fructose and glucose are found in almost equal quantities in tomato fruit with fructose being a little higher, while sucrose usually does not exceed 0.1% (Davies and Hobson, 1981; Davies and Kempton, 1975; Petro-Turza, 1987). Citric and malic acids are the primary acids in tomato fruit with citric predominating and determining sourness (Davies and Hobson, 1981). Another important flavor component is aroma, which is determined by volatiles (Baldwin et al., 1998, 2000; Kazeniac and Hall, 1970; Krumbein and Auerswald, 1998). In tomato, more than 400 volatiles have been reported, but less than 30 are above the odor threshold (Tandon et al., 2000).

Part of the problem of poor flavor is that most commercial cultivars of fresh-market tomatoes have a low level of sugars [total soluble solids (SS) from 4% to 5%] (Kavanagh and McGlasson, 1983; McGlasson et al., 1983), including two most prominent cultivars in Florida, ‘Florida 47’ and ‘Sanibel’ (SS = 4% to 5%) (N. Georgelis, unpublished data). Malundo et al. (1995) found that an increase of sugar level (glucose and fructose) higher than most large-fruited commercial cultivars enhanced the aroma intensity and made the overall flavor more acceptable within a certain acid concentration range. Additionally, Baldwin et al. (1998) showed that sugars were positively correlated with overall flavor acceptability. Hence, if the sugar level of the existing large-fruited fresh market cultivars was increased, it is likely that the overall flavor would be improved.

There are some accessions from wild tomato species with high fruit sugar levels, like L. chmielewskii Rick et al., L. hirsutum Humb. & Bonpl., and L. pimpinellifolium Mill., which could be used as potential sources of sugars. However, some of them have sucrose as the predominant sugar with just small amounts of glucose and fructose. So, a possible transfer of genes from these species would probably change the type of sweetness in large-fruited tomatoes that usually have fructose and glucose as predominant sugars. Additionally, interspecific crosses usually entail a host of prebreeding tasks (Jones and Qualset, 1984).

Apart from the high sucrose level, L. hirsutum has been used as a source of the gene Fgr, which modulates the ratio of fructose to glucose (Levin et al., 2000). More specifically, L. hirsutum has a fructose:glucose ratio of more than 1.5:1, which contrasts with L. esculentum where glucose and fructose are almost equimolar. This increased ratio can be transferred to L. esculentum without changing the overall sugar level, which means that the amount of fructose is increased and that of glucose is equally reduced. Biester (1925) showed that fructose is almost twice as sweet as glucose, which means that tomatoes with the Fgr gene should taste sweeter compared to tomatoes with an equal sugar level.
However, the effect of that gene on the overall flavor remains unknown.

Other sources of high sugars are cherry tomatoes with elevated sugar levels. Their high sugar level is primarily determined by glucose and fructose and a transfer of these to large-fruited tomatoes would not be expected to change the type of sweetness perceived by consumers. In this study, the small cherry accession PI 270248 was studied as a potential source of high sugars. Apart from the sweet taste, its fruit was resistant to bacterial spot incited by Xanthomonas campestris pv. vesicatoria (Doidge) Dye (Scott et al., 1989).

A potential problem in the transfer of high sugars from all the above sources to large-fruited tomatoes is the transfer of undesirable traits such as low yield and small fruit size along with high sugar. Another problem is that sugar level is under polygenic control (Causse et al., 2001; Fulton et al., 2002), and is influenced by the environment (Saliba-Colombani et al., 2001). Backcrossing these genes into large-fruited tomatoes that have low sugar content would be difficult, as many generations would be required and errors would be encountered in phenotypic selection. Marker-assisted selection (MAS) could improve backcrossing efficiency. One objective of this research was to evaluate the relationship of high sugars derived from PI 270248 with other traits, such as yield, fruit size, and plant habit, to discern which ones may be important to consider in the transfer of high sugars into large-fruited tomato lines. Another goal was to find molecular markers linked to high sugars derived from PI 270248 that would facilitate selection in breeding programs.

There have been several cases where molecular markers (almost entirely RFLPs) linked to sugars were found. However, most of these studies used a wild species in the initial cross (Bernacchi et al., 1998; Tanksley et al., 1996). These markers may be useful in an attempt to transfer sugar from a wild species to large-fruited tomatoes, but they are not useful when the high sugar plant is the same species. Many of these markers do not show even a single polymorphism in an intraspecific cross (Foolad et al., 1993). Therefore, it was decided to try to find other markers linked to high sugars from PI 270248.

In this research, RAPD markers were chosen because they are inexpensive and give rapid results. RAPDs linked to high sugars would be very convenient to use in an attempt to select for high sugar phenotypes, especially when hundreds of individuals would have to be screened. One major problem with RAPDs linked to high sugars could be that some polymorphisms might also be linked to genes controlling undesirable traits. This could be due to tight linkage of genes controlling these traits with genes controlling sugar level, or due to pleiotropic effects. These kinds of polymorphisms may cause problems if MAS is used to incorporate high sugars from PI 270248 into large-fruited tomatoes. Traits such as plant habit, pedicel type, yield, earliness, and fruit size were measured along with sugars in this research. Thus, polymorphisms linked to high sugars and also linked to traits such as indeterminate plant habit (sp+), low yield, or small fruit size should be excluded or used with caution. Polymorphisms linked to sugar level and at least some of the traits above are likely because there has already been evidence about correlations between sugar level or SS with traits such as fruit size and plant habit. Emery and Munger (1970) showed that indeterminate tomato plants produced more SS in fruit than isogenic determinate plants. McGillivray and Clemente (1956) showed that smaller fruits had more solids content than larger fruits in ‘San Marzano’. Also, Stevens and Rudich (1978) showed that there was an inverse relationship between yield and SS.

Materials and Methods

**Fall 2001.** Accession PI 270248 was crossed to Fla.7833-1-1-1 (7833) and subsequently F1 seeds were obtained. Line 7833 was used as a parent because it had low SS typical of most commercial cultivars, it is determinate, and it has a jointless pedicel that allowed us to determine if plant habit or pedicel type affect fruit sugar levels. Selections were then made to produce F1, F2, and F3 lines with high or low sugars. The parents, F1, F2, and F3 were grown in a randomized complete-block design (RCBD) with 3 blocks and 10 plants per plot. However, samples were taken from only 21 plants of PI 270248, as some plants were lost. Seeds were sown in the greenhouse in Black Beauty spent coal (Reed Minerals Div., Highland, Ind.) medium on 27 July, and transplanted into Todd planter flats (3.8 cm cell size) (Speedling, Sun City, Fla.) on 6 Aug. The plants were transplanted to the field on 30 Aug. on 20-cm-high, 81-cm-wide beds of EauGallie fine sand that had been fumigated with 67% methyl bromide : 33% chloropicrin at 392 kg ha⁻¹ and covered with white polyethylene mulch 2 weeks before transplanting. Plants were spaced 46 cm apart within plots that were 91 cm apart in rows, with 152 cm between rows. Recommended fertilizer and insecticide programs were followed (Hochmuth et al., 1988). Plants were grown with stake culture and irrigated by seepage from ditches adjacent to the six experimental beds.

**Spring 2002.** The parents and F1 were grown in a RCBD with 4 blocks with 10 plants per plot for the parents and 50 plants per plot for the F1. However, samples were taken from 30 plants of PI 270248, 38 of 7833 and 198 of F1, as some plants were lost. Seeds were sown, as before, in the greenhouse on 11 Jan. and transplanted on 25 Jan. They were transplanted to the field on 6 Mar. on fumigated beds covered with black polyethylene mulch 2 weeks before transplanting. Other growing procedures and techniques were the same as in Fall 2001.

**Both seasons.** The habit of each plant (determinate, indeterminate) was noted. Earliness and yield were rated subjectively when the earliest plants had almost 100% table-ripe fruit. For earliness, plants were rated on a 1–5 scale, where 1 = very late fruit ripening and 3 = very early fruit ripening. Yield was determined by taking into account the number and the size of fruits per plant and using a 1–5 scale, where 1 = very low yield and 5 = very high yield. The pedicel type (jointed, jointless) and fruit size were also determined when some fruit were ripe in each plant. For fruit size, a Craftsman caliper was used to measure the average diameter of three to five table-ripe fruits per plant.

On 10 Dec. 2001 and 23 May 2002, ≈500 g of table-ripe fruit per plant were harvested. Fruits from each plant were ground using a Waring blender and the fruit homogenate was stored in plastic bags at −20 °C prior to analysis. Part of that pulp was used to measure sugars and part of it was used for measuring pH, titratable acidity (TA), and SS.

Sugars and acids were extracted using the following procedure: Fruit homogenate (40 g) was added to 70 mL of 8% ethanol, boiled for 15 min (with a loose-fitting cover), cooled, and vacuum-filtered through Whatman #4 filter paper. The resulting extract was brought up to 100 mL with 8% ethanol; and 12 mL was then passed through a C-18 Sep Pak (Waters/Millipore, Milford, Mass.) and a 0.45-μm Millipore filter. There was 5–8 mL of filtered extract per plant available for high-performance liquid chromatography (HPLC) analysis (Baldwin et al., 1998).

Sugar measurements were conducted at the USDA Citrus and Subtropical Products Lab in Winter Haven, Fla. The filtered

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extract was injected using a Bio-Rad AS-100 HPLC autosampler (Bio-Rad, Richmond, Calif.), fitted with a 20-µL sample loop, into a Perkin Elmer Series 410 HPLC system (Perkin Elmer, Boston, Mass.). Sugars were analyzed using a Waters Sugar Pak column at 90 °C with a mobile phase of 100 µm ethylenediaminetetraacetic acid disodium-calcium salt (CaEDTA) and a flow rate of 0.5 mL·min⁻¹. A Perkin Elmer LC-25 Refractive Index detector was used to measure sugars (Baldwin et al., 1998). Filtered analytical grade reagents were used for standard preparation to establish HPLC retention times and calibration. Determination of purity of individual peaks was accomplished by absorbance index (all wavelengths monitored simultaneously) on a Perkin Elmer LC-235 Diode Array Detector.

The pH, TA, and SS were measured using the following procedure: Tubes (50 mL) were filled up with tomato pulp from each plant and were centrifuged at 4000 g, for 5 min. The aliquot was filtered through filter paper (Fisher Brand) into 50 mL beakers and was used to measure pH with a Corning pH meter 340. Two to three drops of filtered aliquot were also used to measure SS content with an Atago-101 digital refractometer. Then, 10 mL of filtered aliquot were pipetted into 50-mL flasks and five to six drops of phenolphthalein were added. Next 0.1 mL NaOH was slowly poured into the flasks until phenolphthalein changed from transparent to slightly purple and the amount of NaOH added was determined. Titratable acidity was measured using the formula: TA (% citric acid) = (mL 0.1 N NaOH) × 0.064 × 100.

Correlations between sugar level and some other traits were tested with all 198 F2 plants from PI 270248 and 7833. Those that did not yield products or have polymorphic bands were excluded from further analysis. The polymorphic markers were tested with five F2 plants that had very high sugar level vs. three F2 and two F1 plants from lines homozygous for very low sugar levels. Their homozygosity was expected and needed to distinguish high from low sugar plants using dominant RAPD markers. Primers that did not seem to correlate with sugar level were excluded from further analysis. The remaining primers were tested with all 198 F2 plants from Spring 2002.

Data analysis. The genetic mapping of the markers was conducted using MAPMANAGER (QTXb15) software (Manly et al., 2001) and confirmed by MAPMAKER 3.0 (Lander et al., 1987). QTL analysis was conducted by MAPMANAGER (QTXb15) software. The purpose of using those programs was to find linkages among the markers, determine which of them were linked to high sugars (in coupling or repulsion), find how much they contribute to the phenotype, and determine which of them were linked to other traits. Linkage of each marker to high sugars was also tested using SAS (SAS Institute, Cary, NC). Markers linked to high sugars were tested whether they fit a 3:1 ratio (dominant markers) in the F2 using chi-square goodness of fit test. The size of the markers was approximated using Alpha Imager 2000 v4.03 software. Finally, the percentage of sugar variation explained by all the markers found was estimated using SAS.

**Results and Discussion**

PI 270248 was significantly earlier and had significantly higher sugars, SS, TA, and pH than 7833 (Table 1). Additionally, PI 270248 had lower yield and fruit size than 7833. Since PI 270248 and 7833 were significantly different for all traits measured, it was expected that all of them would segregate in the F2 generation. The relationship of sugar level with these traits and some information about relationships between other important traits in the F2 population are in Table 2. Sugars were strongly correlated (r > 0.9) with SS as expected since they constitute a significant part of them (Davies and Hobson, 1981). Thus, SS measurements are a good estimate of sugars.

In this experiment, total sugars (primarily reducing sugars) were positively correlated to pH (0.306) and TA (0.395) and both correlations were highly significant (P < 0.001). The correlation between sugar and TA confirms data from previous research where cherry tomato ‘Cervil’ was used (Saliba-Colombani et al., 2001). Reports on correlations between sugar and pH have varied widely and they have been positive, negative, or insignificant (Bernacchi et al., 1998; Saliba-Colombani et al., 2001; Tanksley et al., 1996). A positive correlation of tomato fruit sugar with both TA and pH has not yet been reported. However, Anderson (1957) suggested that there were lines with both high pH and TA, and cases where pH and TA were not always inversely correlated. Stevens (1986) suggested that both of them should be measured in tomato to determine the organoleptic quality of the fruit. The positive correlation of sugar with pH in this experiment may be an obstacle in an effort to transfer sugars from PI 270248 to cultivars with suitable acidity for processing. The positive correlation between sugars and pH and between sugars and TA means that plants with high sugars generally have more free organic acids (citric and malic acid) and less hydrogen ion concentration (pH) than plants with low sugars. Thus, it is not known whether the transfer of high sugars from PI 270248 will also transfer a more sour flavor.

In the future, taste panels may be help answer this question.

Indeterminate plants had higher mean sugar concentration than determinate plants (Table 3). This is in agreement with what Emery and Munger (1970) have shown. They suggested that their results may be explained by the fact that indeterminate plants have more leaves per fruit than do determinate plants. In Florida, determinate plants are the predominant type grown. Therefore, breeders may face an additional problem trying to transfer high sugars from the indeterminate PI 270248 to determine large-fruited tomatoes.

Fruit size was negatively correlated to sugars (–0.468, P < 0.001) and this confirmed what other researchers have shown (Goldman et al., 1995; McGillivray and Clemente, 1956; Saliba-Colombani et al., 2001). However, it has not been delineated if this is mostly due to linked genes or genes with pleiotropic effects. There is a tendency to accept the second possibility as the
ratio of fruit/leaf tissue did not seem to be a problem. This is encouraging to breeders as it shows that it may be possible to uncouple high sugars from low yield to some extent. However, once high sugars are moved into more advanced high-yielding backgrounds, a negative association of high sugars and yield might yet be encountered.

It might be expected that plants that ripen their fruits earlier have less time to provide them with carbohydrates, and thus sugars, than plants that ripen later. Although earliness did not show a significant correlation with sugars, there was a significant negative correlation between earliness and fruit size (Table 2) indicating plants with smaller fruit tended to be earlier than those with larger fruit. Since there was a significant correlation between small fruit and high sugars (Table 2), the lack of a correlation between high sugars and earliness might indicate that there is a tendency for lower sugars in earlier maturing tomato plants. Finally, there was no relationship between pedicel type and sugar level ($P = 0.258$) (Table 3).

It is not known if plant habit and fruit size will be insurmountable obstacles to transferring high sugars to determinate, large-fruited tomatoes. The transfer would appear to be difficult but it may not be necessary to transfer the entire high level [5 $g/100$ g fresh weight (FW)] of sugars from PI 270248 for flavor improvement. An increase up to 3.5 $g/100$ g FW would likely noticeably increase sweetness over most existing cultivars and may be easier to achieve.

Of 800 UBC primers (Univ. of British Columbia) and 200 OPERON primers tested with PI 270248 and 7833, 148 of them did not produce any visible band. The rest gave a total of 5964 polymorphic bands between the parents. Six primers produced bands (seven bands per primer) while 235 primers gave 303 bands produced by UBC 269 primer segregated as allelic in the F2 generation as they fit a 1:2:1 ratio (Table 4). Thus, the two bands were considered as one co-dominant marker. The rest of the markers yielded bands that segregated in the F2 population of spring 2002 fit the 3:1 ratio as expected (Table 4). Figure 1 depicts the bands linked to high sugars while Table 5 shows the sugar means of the plants with and without the markers. Three markers (OPAE 1, UBC 731, UBC 489) were in one linkage group, two (UBC 744, UBC 290) were in another linkage group, while one (UBC 269) was unlinked. The linkages between UBC 744 and UBC 290, and between UBC 731 and UBC 489 were significant at $P < 0.001$, while the linkage between OPAE 4 and UBC 731 was significant only at $P < 0.01$. The former indicates linkage while the latter is just suggestive. Hence, OPAE 4 may not be linked to UBC 731 and UBC 489. Identification of markers in at least three regions suggests polygenic control of sugars. The polygenic control of sugars has also been reported with other high sugar sources (Fulton et al., 2002; Saliba-Colombani et al., 2001). However, an estimate of the number of these genes would be very speculative at this point. It is possible that there were more genes affecting sugars, but markers linked to them were not found. In a recent study, Saliba-Colombani et al. (2001) used a cross between the cherry tomato ‘Cervil’ and the large-fruited tomato ‘Leovil’. They constructed a genetic map using mainly RFLPs with someRAPDs and about four QTLs for sugar were found. One of the markers significantly linked to high sugars in that study was OPERON.

Table 1. Comparison of tomato inbreds PI 270248 and Fla. 7833 for sugars, soluble solids, titratable acidity, pH, earliness, yield, and fruit size in Spring 2002 at Bradenton, Fla.

| Genotype | Sugar (g/100 g fresh wt) | SS (%) | TA (%) | pH | Earliness | Yield | Fruit size (cm) |
|----------|------------------------|--------|--------|----|-----------|-------|---------------|
| PI 270248 | 5.00*                  | 8.03*  | 0.60*  | 4.36 | 3*        | 2.0*  | 2.0*          |
| Fla.7833  | 2.17                   | 3.80   | 0.31   | 4.22 | 1         | 4.5   | 10.0*         |

*SS = soluble solids content, TA = titratable acidity.

Table 2. Correlation coefficients ($r$) of total sugars with soluble solids (SS), glucose, fructose, fructose/glucose, titratable acidity (TA), pH, yield, earliness, and fruit size from an F2 population between tomato inbreds PI 270248 and Fla. 7833 in Spring 2002 at Bradenton, Fla.

| Trait       | Sugar |
|-------------|-------|
| SS          | 0.923*** |
| Glucose (Glu) | 0.995*** |
| Fructose (Fru) | 0.995*** |
| Fru/Glu     | -0.387*** |
| pH          | 0.306*** |
| TA          | 0.395*** |
| Yield       | -0.043  |
| Earliness   | -0.124  |
| Fruit size  | -0.468*** |

*Indicates significance at $P < 0.001$. Other significant correlations at $P < 0.001$ were pH-SS = 0.263, TA-SS = 0.577, TA-pH = -0.445, and earliness-fruit size = -0.28.

Table 3. Effect of plant habit and pedicel type on fruit sugar levels for an F2 population between tomato inbreds PI 270248 and Fla. 7833 at Bradenton, Fla., in Spring 2002.

| Trait        | Plants (no.) | Sugar (g/100 g fresh wt) |
|--------------|--------------|--------------------------|
| Plant habit  |              |                          |
| Indeterminate| 144          | 3.68*                    |
| Determinate  | 54           | 2.98                     |
| Pedicel type |              |                          |
| Jointed      | 160          | 3.51                     |
| Jointless    | 38           | 3.42                     |

*Indicates a significantly greater value for plant habit by $t$ test at $P ≤ 0.05$.

Negative correlation has been observed in many cases. Stevens (1986) suggested that large fruits tend to have more water in the cells, which dilutes sugars and lowers their concentration. The negative correlation was quite strong in this experiment and it will probably be difficult to transfer high sugars from PI 270248 to large-fruited inbreds.

The correlation between sugars and yield was insignificant in contrast to the negative correlations previously reported between yield and sugars or SS (Bernacchi et al., 1998; Saliba-Colombani et al., 2001; Stevens and Rudich, 1978). The negative correlation, shown in previous research, may be explained by the fact that plants with high yield may also have a high ratio of fruit/leaf tissue and thus, they cannot provide fruit with as much carbohydrate as plants with a lower ratio. However, in our research the
The primer OPAE4. This primer was also linked to high sugars in the present experiment, where it explained 18% of the phenotypic variation in the F2 generation. Another marker that explained a fair amount of variation (16%) was UBC 744. The rest of the markers, although significantly linked to high sugars, explained no more than 4% to 5% of the phenotypic variation. Unfortunately, the chromosomal location of the markers was not identified. Saliba-Colombani et al. (2001) showed that OPAE 4 marker was found on chromosome 2. In this experiment, OPAE 4 was located 45.3 centimorgans (cM) away from UBC 731, which was, in turn, linked to UBC 489 (34.3 cM). Since 45.3 cM is very close to independent assortment and the linkage between OPAE 4 and UBC 731 was only significant at $P < 0.01$, it cannot be definitely concluded that UBC 731 and UBC 489 were located on chromosome 2.

The markers identified could also be used in fine mapping programs in tomato. Apart from sugars, most of them were linked to fruit size, yield, SS, pH, or TA (Table 6). So, they can contribute along with other markers to find a more precise location of QTLs affecting all these traits. This could clarify if some of the traits that are correlated to each other are closely linked genes or genes with pleiotropic effects.

One of the goals of this research was to find markers useful for MAS to transfer high sugars to large-fruited tomatoes. It would be desirable that these markers be closely linked to genes controlling high sugars and not linked to genes controlling other undesirable traits. Unfortunately, two markers (OPAE 4, UBC 744) explaining 18% and 16%, respectively, of the sugar variation in the F2 population were also linked to small fruit size and explained 21% and 22% of the fruit size variation. This means that plants with the markers had generally high sugars, but also small fruit size. Marker UBC 290

Table 4. Band size of RAPD markers linked to high sugars in an F2 population between tomato inbreds PI 270248 and Fla. 7833 grown at Bradenton, Fla., in Spring 2002 and chi-square tests for goodness of fit for control by single genes.

| Primer (Marker) | Band size (kilobases) | PI 270248 X Fla.7833 pattern Expected ratio $\chi^2$ | $P$ |
|----------------|-----------------------|-------------------------------------|-----|
| OPAE4 (OP4)   | 0.90                  | 144 --- 54 3:1 0.545 0.460          |     |
| UBC269 (269)  | 1.76                  | --- 98 48 1:2:1 $\chi^2$ 0.182 0.913 |     |
| UBC731 (731)  | 1.07                  | 50 --- 148 1:3 $\chi^2$ 0.007 0.933 |     |
| UBC744 (744)  | 1.20                  | 153 --- 45 3:1 $\chi^2$ 0.545 0.460 |     |
| UBC489 (489)  | 0.59                  | 152 46 3:1 $\chi^2$ 0.330 0.566    |     |
| UBC290 (290)  | 0.83                  | 159 --- 39 3:1 $\chi^2$ 2.969 0.085 |     |

$^a$Ratio is for the three patterns in the row, respectively.

Table 5. Mean sugar concentration for plants from an F2 population between tomato inbreds PI 270248 and Fla. 7833 with or without various RAPD markers at Bradenton, Fla., in Spring 2002.

| Sugars plants (g/100 g fresh wt) | Plants (no.) |
|----------------------------------|--------------|
| Marker and Group                  |              |
| 744(c) (+) 3.60                  | 153          |
| 744(c) (–) 3.15                  | 45           |
| 731(r) (–) 3.70                  | 50           |
| 731(r) (–) 3.40                  | 148          |
| 269 (A) 3.69                     | 52           |
| 269 (H) 3.49                     | 48           |
| 269 (B) 3.32                     | 98           |
| 489(c) (+) 3.63                  | 144          |
| 489(c) (–) 3.13                  | 54           |
| OP4(c) (+) 3.56                  | 159          |
| OP4(c) (–) 3.32                  | 39           |
| 290(c) (+) 3.56                  | 152          |
| 290(c) (–) 3.33                  | 46           |

$^a$For marker abbreviations see Table 4, in addition: (c) = coupling, (r) = repulsion, (P1) = PI270248, and (P2) = 7833. Arrows point to the polymorphic bands.
explained just 4% of sugar variation, but it was linked to small fruit size (18% of variation) and low yields (7%). Markers UBC 731 and UBC 489 were also linked to small fruit size, while the co-dominant marker 269, which was not linked to small fruit size, was linked to indeterminate habit (9%). The question remains whether all these markers can be used in a MAS program without transferring low yield, small fruit size or indeterminate habit to the recurrent parents. It is recommended that UBC 290 should be avoided. It was either closely linked to a weak gene or loosely linked to a strong gene for high sugars because it only explained 4% of the phenotypic variation. Additionally, it seemed to have a closer linkage to small fruit size (18%) and low yield (7%). Finally, all the markers were linked to pH or TA or both (Table 6). Selecting for high sugars using these markers would likely increase TA that is highly correlated to free acids (Paulson and Stevens, 1974). A change in pH is not clear but it also seems to be increased by most markers. A balance between sweetness and sourness has been shown to be important for a good overall flavor (Jones and Scott, 1984; Malundo et al., 1995). The simultaneous transfer of the sourness along with sweetness to a large-fruited tomato may not disturb the balance and promote, in this way, a more acceptable flavor. Sourness depends on both pH and TA (Harvey, 1920). Thus, it is not clear if a simultaneous transfer of sweetness and sourness might be achieved using the markers found in this research.

All the markers together explained 35% of sugar variation in the F2 (data not shown). Almost 96% of the sugar variation was explained by genetic variation in Spring 2002 (Georgelis, 2002). Thus, 51% of the genetic sugar variation was not explained by the markers. There are several possible explanations for this. First, there were more genes affecting sugars, but no markers linked to them were found. Second, some of the markers were not very closely linked to genes affecting sugars. Finally, five of six markers linked to high sugars were not co-dominant, thus heterozygous plants with non-dominant sugar control are grouped with one of the homozygous classes increasing experimental variability.

None of the markers by themselves explained much sugar variation. Thus, combinations of markers may be necessary for modified backcrossing in breeding programs. All the possible combinations of markers were tested, and the ones with a high sugar average and a reasonable population size were selected (Table 7). The population sizes used could give breeders the opportunity to select against small fruit size, low yield and indeterminate plant habit. High sugars could be confirmed by measuring SS, as the correlation between the two was very high ($r = 0.92$). In almost half of the best combinations of markers, UBC 731 is found (linked to sugars in repulsion). This marker may be more useful in breeding than anticipated by the 5% of the sugar variation it explained. For UBC 731, the heterozygotes that may have relatively high sugars were incorporated into the homozygotes with low sugars, raising the sugar mean. Thus, the sugar difference between the classes with and without the marker may be greatly reduced and thus the explained phenotypic variation would also be reduced. However, by selecting against the UBC 731 marker, some heterozygotes with high sugars would be eliminated leaving plants homozygous for high sugars. The sugar mean of plants remaining after selecting against UBC 731 was 3.7 g/100 g FW compared to plants selected to OP4E 4 and UBC 744 (3.63 and 3.6, respectively).

Another disadvantage of many RAPDs was their poor reproducibility. Often, the results were extremely sensitive to changes in factors affecting PCR. It would be much easier to use the markers found in this experiment in a breeding program if they were converted to sequence characterized amplified region (SCAR) markers, which would be fairly reproducible. SCARs could also verify that 269 is a co-dominant marker. Our markers also need to be tested with different low sugar parents. In this research, they were tested with 7833, but they would only be useful to breeding programs if they were converted to sequence characterized amplified region (SCAR) markers, which would be fairly reproducible. SCARs could also verify that 269 is a co-dominant marker. Our markers also need to be tested with different low sugar parents. In this research, they were tested with 7833, but they would only be useful to breeding efforts if they worked with different low sugar lines.

To summarize, it seems that most of the markers linked to high sugars were not linked to low yield or indeterminate habit and this means that sugars can be uncoupled to some extent from these traits. However, the question about whether high sugars could be uncoupled from small fruit size remains unanswered since five out of six markers of this research with linkage to high sugars were also linked to small fruit size. Our results corroborate previous research that has shown a negative correlation between sugars and fruit size (McGillivray and Clemente, 1956). This correlation has also been illustrated by co-localization of QTLs of these traits on chromosomes 2, 3, and 11 (Goldman et al., 1995; Paterson et al., 1991; Saliba-Colombani et al., 2001).
Since these co-localizations have been found in many cases, it may be assumed that part the negative correlation between sugars and fruit size is due to pleiotropic effects of some genes (physiological relationship), since other research supports pleiotropy (McGillivray and Clemente, 1956). However, there may be genes like the one linked to the co-dominant marker UBC 269, found in this research, that affect sugars without an effect on fruit size. These genes may be suitable for transferring a significant part of sweetness from small-fruited to large-fruited tomatoes and markers like UBC 269 may be useful. However, markers like OPAE 4, UBC 744, UBC 489, and UBC 731 should not be excluded from MAS programs, because they may help to transfer enough sweetness to enhance the overall flavor without significantly reducing the fruit size.

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