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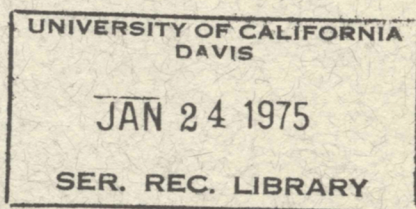
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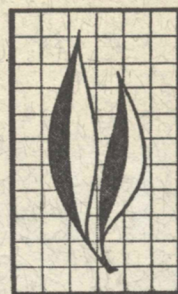
High Soluble-Solids Content in Large-Fruited Tomato Lines Derived from a Wild Green-Fruited Species

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An attempt was made to combine the high soluble-solids content of ripe fruits of the small, green-fruited *Lycopersicon minutum* with the horticulturally desirable characteristics of a standard *L. esculentum* cultivar. By backcrossing from the former to the latter, and by subsequent pedigree selection, pure-breeding lines in which soluble-solids content was elevated to 7–7.5 per cent—at least 2 percentage points above that of the recurrent parent—were synthesized. The new lines are comparable with the recurrent parent in size, color, and shape of fruit, plant habit, and yield, although they suffer from fruit softness, perishability, and other defects. The recombination of high soluble-solids content and large fruit size was achieved despite a moderately strong negative correlation ($r = -0.46$ in BC_1) between these characters. Genetic linkages between soluble solids and a dominant delta-carotene character (possibly *Del*) as well as with the uniform ripening of unripe fruit (*u*) were also detected and broken by the backcross selection procedure. Additionally, soluble solids tended to be associated with several severe fruit-cracking defects. The continuous nature of distributions of soluble solids in the segregating generations, the linkage of solids content with several genetically independent characters, and the recapture of only part of the *minutum* genotype determining high soluble solids point to a complex, polygenic inheritance of this character. Several of the derived lines carry *Ip*, a dominant gene for intense fruit pigmentation evidently derived from *L. minutum*. A foliage variegation character of undetermined inheritance, and *rv-4*, a gene for reticulated virescence, also appeared *de novo* in the segregating generations, probably originating from mutation as deduced from genetic criteria. Appropriate genetic tests proved the existence of the *r* and *y* fruit pigmentation genes or similar alleles at the same loci in the *minutum* parent. The genetic difference between the parental species for certain fruit quality characteristics is thereby characterized.

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High Soluble-Solids Content in Large-Fruited Tomato Lines Derived From a Wild Green-Fruited Species¹

INTRODUCTION

THE PRIMARY OBJECTIVE of this investigation was to attempt to transfer genes for high soluble-solids (SS) content of ripe fruits from a wild, green-fruited species to large, red-fruited lines comparable with modern cultivars. SS, comprising mainly fructose, glucose, and other sugars, are of prime importance to the processing tomato industry. The bulk of California cannery tomatoes are manufactured into products that are sold on the basis of solids content. As a consequence, relatively slight changes in SS have considerable economic significance.

Substantial differences have been measured in SS amongst the red-fruited species *Lycopersicon esculentum* Mill. and *L. pimpinellifolium* (Jusl.) Mill. (Goldenberg and Pahlen, 1966; Ibarbia and Lambeth, 1969, 1971; Lower and Thompson, 1967; Stoner and Thompson, 1966). Whereas determinations in this group reach relatively high values, these maxima are considerably exceeded by certain green-fruited species, which tend to be avoided as sources of germ plasm in tomato breeding programs because they engender severe problems of sterility and generally introduce more undesirable genes than do the more closely related red-fruited species. However, the literature is replete with examples of the transfer

from the former to acceptable *esculentum* cultivars of desirable new traits—mostly resistance to diseases and other pests—often of enormous economic value. As this area has been reviewed recently (Rick, 1967), examples need not be listed here.

Inasmuch as the nature of inheritance of SS has been the goal of competent research by Goldenberg and Pahlen (1966), Ibarbia and Lambeth (1969, 1971), and Stoner and Thompson (1966), it was not a major objective of this investigation, although any pertinent data obtained were analyzed. Attention was also paid to the inheritance of other traits, particularly those of the fruits, and to their relationships with SS.

For various reasons the selection of the wild parent was narrowed to a single accession of the unofficial species "*L. minutum*," an undescribed yet fully qualified tomato species (Chmielewski, 1962, 1968a). High SS values can be found in the other green-fruited highly variable *L. chilense* and *L. peruvianum*, but formidable compatibility problems are encountered in mating these species with *L. esculentum* and in immediate subsequent generations. High SS and good compatibility are characteristic of *L. minutum*. Although much unfruit-

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fulness was encountered in later generations, this problem is less serious than with the other aforementioned green-fruited species. Two distinct races of *L. minutum* are known—a smaller flowered, smaller fruited one (the biotypes studied by Chmielewski) and a more recently acquired, more robust

type. The latter was selected for this study because its fruits have a higher SS content and because it is free of *Df*, the dominant defoliator character of the diminutive race, which is usually lethal to approximately three-quarters of the F_2 and half of the first backcross to *L. esculentum* (Chmielewski, 1968b).

MATERIALS AND METHODS

The materials for this project consisted of various accessions of tomato species. The leading roles were played by the recurrent parent, *L. esculentum*, and the wild, non-recurrent parent, *L. minutum*. For the latter, a single accession of the more robust race was used: LA1028, collected by Iltis and Ugent (their #832) at Hacienda Casinchiua, along the drainage of Río Cachachaca, Dept°. Apurimac, Perú. A highly inbred line of cv VF36 (LA490) served as the *esculentum* parent in the original cross and as recurrent parent for the first two backcrosses. VF36 was chosen because it was then a leading California cultivar and because it serves as the standard *esculentum* genotype for our cytogenetic program. For BC₃₋₅ it was replaced by VF145-22-8 in order to approximate more closely the genotype of this cultivar, which had by that time replaced VF36 in popularity as the principal cannery tomato. These two cultivars are similar in many respects, having small determinate (*sp*) habit, prolific fruit set—owing in no small measure to the low position of their stigmas (Rick and Dempsey, 1969)—and resistance to Fusarium and Verticillium wilts, as well as other characters. They differ considerably in fruit characters: size, shape, firmness, and presence vs absence of *u*, the gene for uniform ripening. It should be observed here that in recent years VF145-22-8 has been largely replaced by the strain VF145-7879, because the latter has higher SS, tends to yield better in cer-

tain areas, and is otherwise superior. Backcrosses were not made to the latter because restoration of the *esculentum* characters had been essentially achieved by BC₅, and an additional change in the recurrent parent was deemed inadvisable.

The following additional lines of *L. esculentum* were used for incidental purposes. The accession LA13 was used as a source of the genes *r* and *y*, and LA876, an accession originally received from Dr. Anson Thompson, as a source of *hp*. Twenty accessions of small-fruited primitive cultivars of *L. esculentum* and of the wild var. *cerasiforme* of the same species were planted for a preliminary comparative study of fruit size vs SS.

All hybridizations were made in the greenhouse, utilizing standard methods of emasculation, pollination, and notation. From the ripe fruits thus produced seeds were extracted by means of fermentation or low-speed blending (Boynton, 1962).

With the exception of winter generations grown for two successive years in the greenhouse, the studies (including all testing for SS) were conducted on field plantings made according to standard practices of the industry in respect to distance between rows, cultivation, irrigation, and pest control. One exception to these methods was our transplanting all lines from nursery flats to the field—nearly all commercial plantings are currently direct seeded.

The yearly plantings were not massive: generally no more than 2,000 plants were grown and tested in a single season. After selected lines had reached relative uniformity, they were tested with VF145 controls under the aforementioned conditions in replicated plantings.

SS was determined by use of a Zeiss hand refractometer under field conditions at temperatures of 27° C or higher. Samples consisted of a few drops of juice squeezed from fresh fruits. At least two independent readings were made on each plant; in case they disagreed substantially, as a result of real fruit-to-fruit variation or

of errors, more determinations were made and an arithmetic mean calculated. The single instrument, which was routinely recalibrated against distilled water, functioned well for the entire period of the tests—furthermore, it yielded repeatable values on the same extracts.

Standard statistical procedures were used for the t and χ^2 tests and analysis of variance. Ideal conditions for randomization prevailed in comparisons made on segregating lines: at the time of transplanting seedlings to the field, their genotypes were indeterminable, hence order of plants in the rows was not biased.

RESULTS

Preliminary tests of small, red-fruited lines

The success of efforts to breed lines with high SS and large fruit size must depend to some extent on the genetic-physiological relationships between these two characters. Thus a strong inverse genetic regression of SS upon size, as detected by Goldenberg and Pahlen (1966), would constitute a serious obstacle to attaining such a goal. My earlier, scattered observations of large differences in SS between different tomato accessions, all with similar small fruit size, suggested that the lines differ in genetic determination of this trait. It was therefore considered important to make a preliminary test of SS in a collection of unrelated small-fruited lines.

For the aforementioned purposes, observations were made on a series of 21 accessions of small-fruited primitive cultivars of *L. esculentum* and the wild or feral *L. esculentum* var. *cerasiforme* from Ecuador and Perú. Single row plantings were made of each line and repeated measurements were made in midseason of fruit weight (mean of

10 fruits), SS (mean of six independent samples), and estimates of fruitfulness. The extremes for mean fruit weight were 2.4 and 15.9 g; for mean SS, 5.9 and 10.0 per cent; and for fruitfulness, less than 2 per cent of the flowers setting fruit (grade 1) to nearly perfect fruit set (grade 9).

Figure 1 summarizes the observations. The relationship between fruit size and SS appears to be nearly random, the correlation being $r = -0.054$ —far below statistical significance. Likewise, the relationship between fruitfulness and fruit weight, and that between fruitfulness and SS are indeterminate. Clearly, the regression of fruit size on SS amongst these tested lines having more than a 6-fold difference in fruit size is so trivial that it should not obstruct attempts to attain new genetic combinations between them. Degree of fruitfulness, within the limits of the test, would also not appear to be a limiting factor to attaining high SS. Admittedly, the range of fruit size tested here is relatively narrow, and the results do not preclude a strong inverse relationship when the range is extended

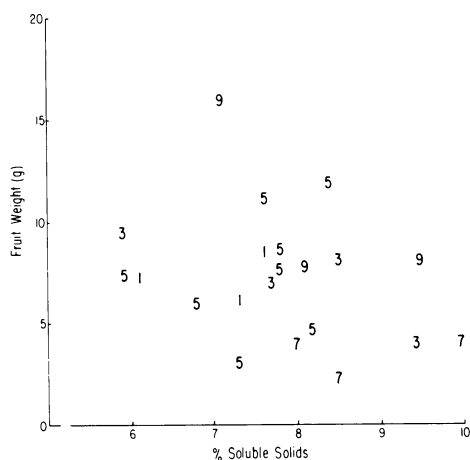


Fig. 1. Relationship between fruit weight, per cent soluble solids, and fruitfulness in 21 accessions of *L. esculentum* var. *cerasiforme* and primitive cultivars of *L. esculentum*. Numbers in the grid symbolize the level of fruit setting from 1 (lowest per cent set) to 9 (highest per cent set). Position of the numbers indicates mean values of the respective populations for fruit weight and per cent soluble solids.

to include the large fruit size of commercial cultivars. This loophole might permit reconciliation between these results and those of Goldenberg and Pahlen (1966). Nevertheless, the marked differences maintained by the different lines must have reflected underlying genetic differences and encouraged our efforts to incorporate high SS from small-fruited wild species into large-fruited tomato lines.

The first backcross generation

The parents, F_1 , F_2 , and first backcross to VF36 (BC_1) were grown in the same plot in the summer of 1966. It was soon learned that replicate refractometer readings of fruits in all of these lines except VF36 were subject to unusual fluctuations. A large share of this variability could be traced to stage of maturity, as demonstrated by the following figures from a single segregate of BC_1 (66L1407-74). A large quantity of fruit was harvested

from this plant and sorted into maturity classes consisting of eight fruits each. Mean SS values for each were: slight color flush—6.60 per cent; strong color flush—7.35; over-all orange color—8.30; over-all orange red—8.90; dead ripe, firm—9.95; overripe, slightly shrivelled—15.85. Differences in the most advanced stages of maturity were no doubt affected to some extent by moisture loss. For this single sample it is evident that stage of maturity can account for more than a two-fold difference in SS of the *minutum* derivatives. This experience permitted us to minimize the effect of maturity by making determinations only on fruits that had recently reached full color—a judgment that can be facilitated by comparisons of color between all ripening fruits of a single plant and position of the fruits on their respective inflorescences and branches. Success in coping with this and other sources of environmental variance is evident in the genetic improvement in SS reported below. Maturity determinations are more difficult in genotypes that never develop appreciable carotenoid pigments and in which very few fruits are set; in fact, determinations in the F_2 were thereby impeded to such an extent that, aside from a preliminary survey, analysis of this generation had to be abandoned.

Figure 2 summarizes results of the determinations. Mean SS readings were: VF36—5.0; *minutum*—9.9; F_1 —8.5; BC_1 —8.5 per cent. Aside from the typically quantitative nature of inheritance exhibited by these data, the BC_1 segregates widely, reaching SS levels of both parents, and having a surprisingly high mean, approximating that of the F_1 . This extensive array of values, including many in the very high range, as well as the indications of additive inheritance, encouraged further research on the material. Accordingly, plants with best combinations of SS

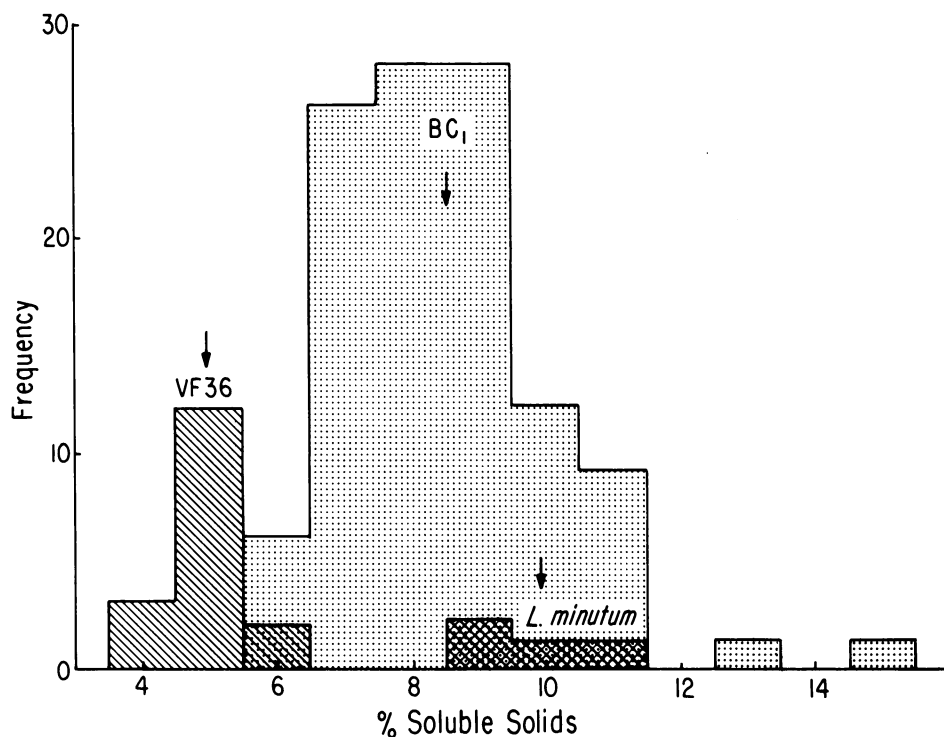


Fig. 2. Frequency distributions of per cent soluble solids for the parents—*L. esculentum* cv VF36 and *L. minutum*—and the first backcross of the F₁ hybrid to VF36.

and other traits were selected for the next round of backcrosses. Eight such selected segregants were backcrossed to VF36, but only four (66L1407-7, 12, 73, and 88) yielded sufficient seed for further studies.

Other data pertinent to the genetic analysis were also collected from BC₁. Qualitative monogenic segregation could be detected for determinate habit (*sp*) characteristic of VF36 versus the normal indeterminate *sp*⁺ habit characteristic of *minutum* (56 + : 59 *sp*). A differentiation between red and orange fruit pigmentation could also be detected, but classification of a few plants having intermediate color was problematic, the tally of classifiable plants being 55 red and 53 orange. A strong association was observed between segregation for the observed pigment and

habit traits, thus validating observations on the former character:

	Indet.	Det.	
Red	7	48	$\chi^2 = 53.6^{***}$
Orange	45	8	

Of more direct bearing on the main topic of this report are the relationships between SS and the aforementioned characters, as well as fruitfulness and fruit size. Comparisons with plant habit yielded the following results: mean SS of the indeterminate (*sp*⁺) plants—8.75 per cent; determinate (*sp*)—8.25 per cent. For the color segregation: red—8.55 per cent; orange (presumably *B*)—8.47 per cent. The

differences are not significant in either comparison. The correlation between SS and fruitfulness, expressed as per cent of flowers that set fruit, is -0.14 , far from significant, whilst that for SS and fruit weight is -0.46 ($p < 0.01$). The latter relationship appears to be curvilinear (fig. 3), but when log, square, or cube root transformations are made, the strength of the correlation is markedly decreased. It therefore

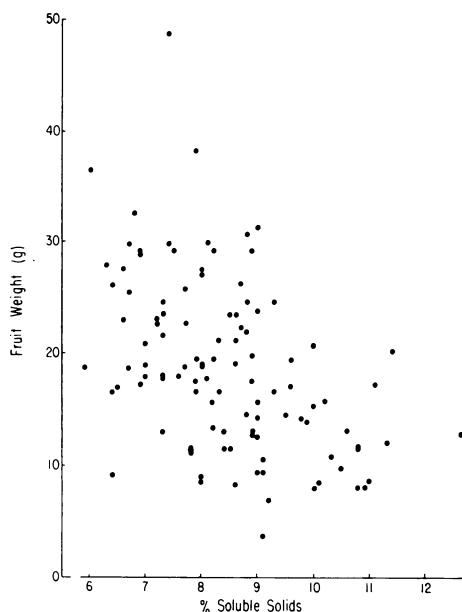


Fig. 3. Relationship between fruit weight and per cent soluble solids in the first backcross—VF36 \times (F_1 VF36 \times *L. minutum*). Each dot represents the value for a single plant. $r = -0.46$.

appears that a moderately strong negative correlation exists between fruit size and SS. The later experiments revealed that this relationship, whether genetic or developmental, is not strong enough to prevent the selection of genotypes with large fruits and high SS.

Subsequent backcross generations

The presentation of detailed accounts of the results for each subsequent generation of the program is not justified.

Instead, the progress is summarized briefly, and, where appropriate, exemplified. The BC₂, grown in 1967, also displayed much unfruitfulness, wide segregation for many traits, and a marked improvement in fruit size and color. The mean SS of all BC₂ lines was 5.40 per cent, only 0.60 per cent higher than that of the VF36 controls (4.80 per cent). Despite the low means, SS segregated considerably and superior plants were again selected for siring the next BC.

In the ensuing generations, BC₃ lines were grown and tested along with controls and progenies from three selfed BC₂ parents. Since the results of these tests are instructive, they are presented in graphic form (fig. 4). Again, the BC lines have mean SS values (5.08 per cent) only slightly higher than those of the VF36 controls (4.46 per cent); nevertheless, as figure 3 shows, the range of segregation amongst the

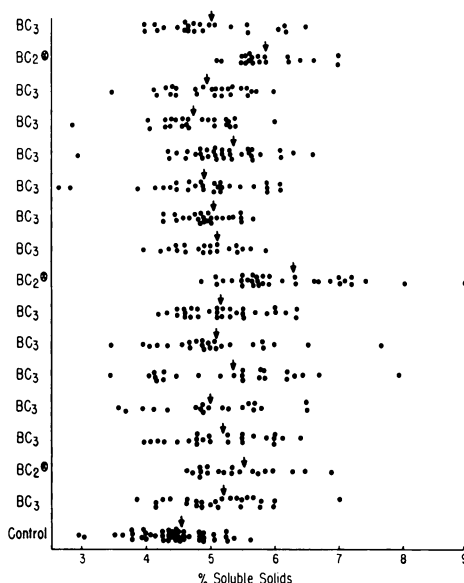


Fig. 4. Arrays of soluble solids values for populations of the third backcross and selfed progenies of the second backcross. Each row represents a different population. Each dot represents mean value for a single plant. Arrows indicate population means.

former is extensive, many segregants falling well above the range of VF36 values. More encouraging were the results of the three BC₂ selfed lines, whose means exceeded those of any BC₃, and the mean of the three lines (5.88 per cent) lay far above that of the BC₂ and control lines. A difference of the same degree is also found between the mean of these three lines and that (5.13 per cent) of the three BC₃'s to which they correspond. Segregation within these BC₂ derivatives was likewise promising, ranging from 4.7 to 9.0 per cent. Selfing evidently resulted in fixation of certain *minutum* genes determining high SS content.

Experience in the next two generations of backcrossing to VF145 was of a similar character. Improvement in fruit size was so rapid that by BC₄ and BC₅ many segregants had essentially the same dimensions as those of the recurrent parent. Progress in attaining good red color was similar in certain lines but hampered in others by a tight association with off-colors (an aspect to be elaborated below). More persistent problems were encountered in low fruitfulness and fruit cracking; all lines exhibited a tendency for high SS to be associated with both of these undesirable traits. A high selection intensity was maintained to reduce or eliminate these flaws and to retain high SS values.

Following BC₄ and BC₅, selection was continued in selfed pedigree progenies for two to five generations, depending upon the desirability and promise of certain lines for continued improvement. Derivatives from all groups tracing to the four original BC₁ lines were maintained through two to three generations of pedigree selection; thereafter, for reasons of defects to be detailed below, selection was narrowed to derivatives from only one such line. The major 'break' occurred in the winter generation of 1970-71, when in the

second pedigree generation several plants from this line with exceptionally improved characteristics were found. Further selection was largely limited to the descendants of these plants. The next generation, grown in the field in 1971, maintained these features and provided good opportunity for observing performance and making further selections. Two more generations of autogamy, bringing the total to five since BC₅, followed in the winter of 1971-72 and summer of 1972. The latter culture provided the best opportunity for comparisons between these lines and between them and controls.

Final products

The results of tests between lines of the aforementioned generation (fifth generation of pedigree selection following BC₄ and BC₅) are summarized in table 1 and figures 5, 6. Analysis of variance of the mean SS of seven replicated lines and the VF145 controls (table 1) reveals a highly significant variance for lines, thereby proving a high degree of genetic differentiation. Many of the differences between lines and all differences between lines and

TABLE 1
ANALYSIS OF VARIANCE OF MEAN
SOLUBLE SOLIDS IN VARIOUS LINES
DERIVED FROM *L. ESCULENTUM*
× *L. MINUTUM* AND CONTROLS

Item	df	SS	MS	F
Lines	7	35.35	5.05	84.17***
Error	29	2.87	0.06	
.....				
Lines Control	961	960	965	952
	964	968	979	
Means	5.24	6.08	6.11	7.00
	7.18	7.24	7.53	7.65
Comparisons*				

* Tests of significance based on Duncan's multiple range criterion as extended by Kramer (1945) to group means with unequal numbers of replications. Mutually underscored lines do not differ from each other significantly; differences between 952 and 979 and between 965 and 968 are significant at the 0.05 probability level; differences in all other comparisons are significant at the 0.01 level or less.

controls are highly significant. In figure 5, which further depicts the extent of these differences, the pooled values of the descendants of a single plant selected in S_2 are compared with the interplanted controls. The extent of the differences is indicated by the facts that, despite environmental variance, the two distributions are mutually exclusive and the mean SS values of controls (5.52 per cent) and the selected lines (7.74 per cent) differ by 2.22 per cent. Differences of the same degree were observed again in the 1973 season.

The other characteristics of these lines are summarized as follows. Yields per plant of the selections are comparable with those of the controls (figs. 5, 6); the same applies to comparisons of fruit size (fig. 7), mean weight of controls being 53.4 g, that of the se-

lected lines, 74.3 g. The considerably larger size of the latter may owe to genes for larger fruit size inherited from VF36, the first recurrent parent, or to a load effect on the VF145 controls. The new lines also compare favorably in respect to exterior as well as interior color, and to shape and smoothness of fruit. Flavor has not been extensively assayed, but in the opinion of several tomato experts, the fruits have a markedly sweet taste and otherwise seem acceptable. It should also be noted that analyses have not yet been made of the kind and quality of sugars affected to elevate SS and of the impact of such changes on fruit quality. Possible defects include softness, low consistency, and high perishability. The plants have essentially the same determinate habit as VF145, and the season of fruiting is

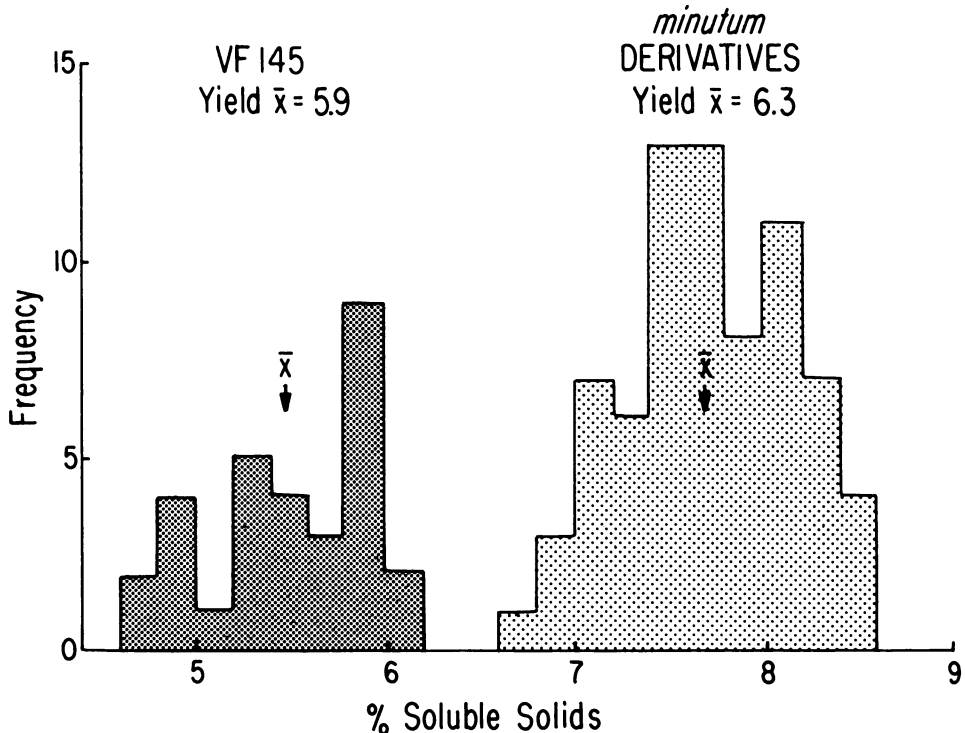


Fig. 5. Frequency distributions of soluble solids values of pooled controls (VF145) and derivatives after five selfed generations following five backcrosses. The derivatives, pooled from the five families shown in figure 6, trace to a single plant selected in S_2 after five backcrosses. Population means are indicated by arrows.

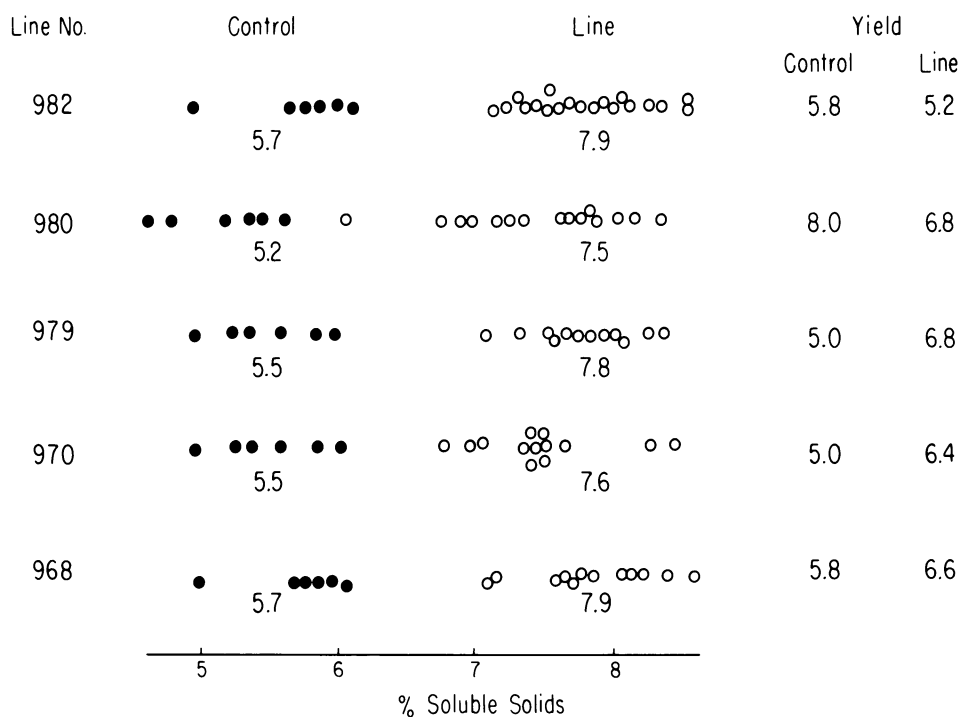


Fig. 6. Arrays of soluble solids values in five populations of the fifth selfed generations after five backcrosses. Each population is flanked in the same row by its corresponding VF145 control. Each circle represents the mean value of a plant: the black circles for controls, the open circles for backcross derivatives. Means are specified by numbers situated below each array. Mean total fruit yields are listed at the right.

the same or slightly retarded. One of the derivative lines has a detectably more curly leaf than *esculentum* cultivars. Five lines of the aforementioned origin have been released as sources of high SS germ plasm for the use of plant breeders. In essence, a vast improvement has been effected in SS content without substantially degrading most other horticultural characteristics; nevertheless, the aforementioned persistent defects preclude acceptance of the lines as cultivars in their present condition.

Associated characters

This section is devoted to a summary of experience with other traits, chiefly those of the ripe fruits, and their relations with SS.

Fruit size. As indicated above, a moderate correlation between fruit size and SS was observed in BC₁. To some extent this relationship could be detected in the subsequent backcrosses and in a few of the pedigree lines. Whatever the nature of this correlation, it proved relatively simple to break by intentional selection, as manifest in achieving the combination of high SS and large fruit size in the selected lines.

Fruit cracking. Cracking, which tends to be characteristic of certain green-fruited species, was manifest in various degrees and types in fruits of most of the lines. It tended to be concentric, although certain lines showed persistent radial cracking; much pheno-



Fig. 7. Representative samples of ripe fruits of VF145 controls (left), derivatives of the fifth selfed generation following five backcrosses (right), and *L. minutum* (bottom, just above identification sign). Fruits placed in various positions to display shape and other external features. Label at bottom is 12 cm long.

typic variation was also observed in the texture of cracking—i.e., whether fine or coarse; in the most extreme situations, the whole fruit surface was covered by a fine network, reminiscent of Lyall's (1965) corky mutant. In view of these variations in cracking, its phenotype must be of a complex nature and affected by several *minutum* genes. It also follows that these genes can interact with others of the *esculentum* background to result in far more extensive cracking than that observed in either parent. The persistence of cracking despite numerous generations of disruptive selection reveals a tight association between the genetic propensity for cracking and high SS. Success in breaking this association proves that it must be determined by linkage, not pleiotropy.

Delta-carotene. Although this program was not primarily directed at the inheritance of fruit color, certain use-

ful information was obtained concerning segregation of orange carotenoid traits and their relations with SS. The observations presented above on red vs orange segregation in BC_1 suggest that *B* or a very similar allele at the same locus is present in the *minutum* parent and that it segregates clearly in Mendelian fashion in the early backcrosses. Of all traits, delta-carotene was the most tightly associated with high SS (except in BC_1). It persisted to late generations in high SS selections in three of the four original BC lines. Attempts to break the association failed in two, but succeeded in one, of the lines.

Since disruptive selection succeeded in dissociating high SS from the delta-carotene character, pleiotropy could be ruled out and linkage identified as the factor responsible for the association. Information concerning the nature of this association is presented in table 2,

TABLE 2
COMPARISONS OF SOLUBLE-SOLIDS PERCENTAGES BETWEEN RED- AND
ORANGE-FRUITED SEGREGANTS IN VARIOUS GENERATIONS

Generation	Year	Normal		Orange		Difference (Or. - Nor.) (% SS)	t
		n	\bar{x} (% SS)	n	\bar{x} (% SS)		
BC ₃	1968	14	4.99	10	5.82	0.83	2.06*
		14	5.00	12	5.84	0.48	1.83*
S ₁ ex BC ₂	1968	8	5.81	24	6.38	0.57	1.47
		5	5.04	14	5.67	0.63	2.09*
S ₂ ex BC ₅	1971	9	6.12	36	6.82	0.70	1.99*
		13	5.41	34	5.67	0.26	1.06
		16	5.02	30	5.59	0.62	2.45†
		6	7.22	8	7.89	0.67	1.57

* Difference significant at 10% level.

† Difference significant at 2% level.

in which mean SS values are compared between red- and orange-fruited segregants within six representative pedigree families and two BC families. Any bias in the refractometer readings was averted by one worker extracting juice and another making the readings independently. In every family the orange derivatives exceeded the red ones, the difference between means varying between 0.26 to 0.83 per cent, the mean difference being 0.59 per cent. The difference was significant in only one family ($P < 0.02$), but in all families the associated P was 0.3 or less and < 0.1 in four. Clearly, differences of such a sizeable and consistent nature must be highly significant in the total consideration. Thus it must be concluded that the delta-carotene gene and at least one of the major *minutum* genes for high SS are tightly linked and pleiotropy, as in the case of fruit size, can be ruled out by the recovery of red-fruited lines having SS equivalent to that of the orange segregants. This linkage clearly does not involve all of the determination for high SS, because the difference

associated with red vs orange is 25 per cent or less of the total gain in SS realized in these tests.

Uniform ripening. The well-known gene *u*, controlling uniform pigmentation of the unripe fruit, was contributed to the progenies by the VF145-22-8 parent. Association of SS with this character in segregating progenies provides an interesting comparison with the aforementioned relations ascertained between SS and fruit color. Experience in 18 families is summarized in table 3, in which the same statistical advantages prevailed as in the tests with the delta-carotene character. In contrast with the latter, the pattern of relationships between SS and *u* is variable—a highly significant association of large differences in mean SS values being observed in some families and no significant association encountered in others, the break between the two groups being pronounced. In the former group the differences are considerably greater than in the comparisons between red and orange segregants. The difference is highly significant in six

TABLE 3
COMPARISONS OF SOLUBLE-SOLIDS PERCENTAGES BETWEEN NORMAL AND
UNIFORM-RIPENING (*u*) SEGREGANTS IN ADVANCED GENERATIONS

Year	Normal		<i>u</i>		Difference (<i>u</i> - Nor.) (% SS)	<i>t</i>
	<i>n</i>	\bar{x} (% SS)	<i>n</i>	\bar{x} (% SS)		
1971	14	6.92	10	6.75	-0.17	0.79
	17	7.31	6	6.42	-0.89	3.11**
	15	6.65	4	5.58	-1.07	4.00**
	11	6.58	7	6.46	-0.12	0.36
1972	14	6.81	4	6.08	-0.73	2.05*
	10	7.82	6	7.37	-0.45	1.70
	10	7.57	5	7.32	-0.25	1.18
	11	7.95	5	7.16	-0.79	3.57**
	10	7.61	5	7.62	0.01	0.04
	25	7.66	15	7.55	-0.11	0.91
1973	33	6.82	15	6.19	-0.63	3.06**
	26	6.24	14	5.56	-0.68	2.51*
	9	6.89	4	7.23	0.34	1.45
	8	6.96	7	7.03	0.36	0.44
	12	7.00	6	6.67	-0.33	1.03
	12	6.68	4	6.00	-0.67	1.73
	9	6.99	5	6.82	-0.17	1.01
	11	6.87	7	6.36	-0.51	1.42

* Difference significant at 5% level.

** Difference significant at 1% level.

families, varying from 0.63 to 1.07 per cent. For families in which the difference is not significant, it varies between -0.67 and +0.36 per cent. Interpretation of these results is obscured by the fact that the normal (*u*⁺) allele may have been derived from either *L. minutum* or VF36, the recurrent parent of the first two backcrosses. The instances of significant association might trace to the former, and those of no effect of *u* on SS to the latter—a hypothesis supported by the fact that SS in VF36 is only slightly higher than in VF145-22-8. It is clear from the data, however, that selection repeatedly broke the asso-

ciation: in several instances families with no association were derived by selfed selection from those in which a highly significant association was found. Furthermore, pure breeding *u* lines were obtained with SS values equivalent to the best *u*⁺ lines. Thus the linkage between a major gene affecting SS and *u* (on chromosome 10) could be easily broken.

Intense fruit pigmentation. An intriguing dark pigmentation of the fruit, in both unripe and ripe stages, is associated with nearly all lines derived from the single BC line derived from 66L1407-73, which was divested of the

delta-carotene character. This new character remained undetected until S_3 , when we became aware of its presence in several sub-lines. Chlorophyll pigmentation of the unripe fruit, particularly of the normally darker shoulder area, is more intense and (as in *hp*, the high pigment mutant [Thompson, 1955] and other dark-green genotypes) the carotenoids of the ripe fruit are more concentrated. Expression of this trait in immature fruits seems to be more sensitive to the environment than that of the normal type, the intensity often varying markedly between fruits on the same plant. It is very difficult to detect in combinations with *u*. Similar phenotypic variation and epistatic interaction with *u* have been observed by Stevens (unpublished) in *hp*. When segregation is well-defined, it behaves as a Mendelian dominant (63 dark : 26 normal in all classifiable F_2 's). It is accordingly symbolized *Ip* (Intense pigmentation). *Ip* resembles *hp* more than any other known fruit-color mutant, but differs in its dominance, good germination, and normal plant vigor in contrast to the subnormal behavior of *hp*. In an attempt to ascertain the genetic relationships between the two variants, a cross was made between true breeding stocks of each. In keeping with the known dominance of the new trait, the F_1 showed intense coloration and therefore gave no critical information. An F_2 was subsequently grown, which, despite its small size (25 plants), yielded the following critical data: four had normal coloration, 15 had intense coloration equivalent to that of either parent, and six were pigmented even more intensely than either parent, the shoulders being essentially black. The recovery of the normal and very intense classes establishes that the determining genes cannot be allelic.

The relationships of *Ip* trait with SS are not clear. Its persistence in lines selected for high SS suggests that they

might be interrelated pleiotropically or by genetic linkage, but the useful segregating material tested has been too limited for an adequate test. In the small sample tested, the mean SS values for normal and dark classes do not differ appreciably.

Other pigmentation characters. An attempt was made to ascertain the nature of alleles at the *r* and *y* loci in the *minutum* parent by making crosses between it and our LA13 line (*r/r*, *y/y*) and by subsequently backcrossing the hybrid to the latter. All 20 plants of this BC progeny had both the non-red fruit color characteristic of *r* and the colorless fruit epidermis characteristic of *y*, thereby establishing that both of these recessive genes are present in *L. minutum* (LA1022).

Other characters

1. **Variegation.** A unique variegation appeared in a sub-line in S_3 from BC_5 derived from tracing to the single progenitor 66L1407-88. The leaves are irregularly streaked with bands of several intensities of green and are greatly distorted. Plants are variably stunted, especially when grown under cooler conditions. In its extreme manifestations this variant produces only malformed flowers, which fail to set fruit. One true-breeding line has been established. Segregation indicates a recessive condition, but is too irregular and expression too variable to permit a satisfactory classification. The nature of genetic determination, though probably simple, remains unknown. The few variegated plants that produced ripe fruits under field conditions registered high SS values (7.0–9.4 per cent), well above values of normal segregants in the same family.

2. **reticulate virescent-4 (*rv-4*) (LA-1496).** Another mutant that appeared *de novo* in a later generation is *rv-4*, a very sharply defined virescence in

which the yellowish color of the immature leaves is strongly contrasted with the dark green network of the veins. The seedlings are somewhat retarded but with care will grow to maturity and will flower and fruit satisfactorily. The mutant segregates in a well-defined fashion as a recessive, the pooled F_2 progenies thus far scored yielding 1269 normals : 294 (18.8 per cent) *rv-4*. No tests have been made of SS because the

slow growth of *rv-4* prevents testing of interplanted normal controls, thus obstructing satisfactory evaluation. This mutant, like the variegated one, is a derivative of 66L1407-88 and appeared only once, in S_2 ex BC_5 .

The pattern of origin of these two variants is nearly identical: they appeared only after several generations of inbreeding following BC_5 in only one of the four original BC lines.

DISCUSSION

Relationships with fruit size and plant yield

This investigation has demonstrated that even in a relatively modest back-cross program it is feasible to transfer, from a green-fruited species, genes that condition an increase of 2.2 per cent SS ($^{\circ}$ Brix)—a gain of more than 40 per cent over that of the recurrent parent. This objective has been accomplished without degrading total yield, fruit size or color. Until the fruit volatiles have been analyzed it would be premature to make any positive statements concerning aroma characteristics. On the other hand, the derived lines suffer from softness and a tendency of the fruit to deteriorate rapidly.

The relationships between SS and both fruit size and yield deserve further consideration. Goldenberg and Pahlen (1966) detected a strong negative correlation (r values as high as -0.83) between SS and fruit size in their extensive studies of several hybrids between parental extremes. The regression of SS on fruit weight was so pronounced in certain hybrid progenies—for example, the F_4 of the cross (Yellow Cherry \times Magnif Potente) \times Yellow Cherry—that it could discourage attempts to select disruptively for geno-

types of high SS combined with large fruit size. On the contrary, Ibarbia and Lambeth (1971) concluded from their studies that fruit weight and SS are poorly correlated. The basis for this apparent paradox probably lies in the rather different approaches followed by the two groups: Goldenberg and Pahlen based their studies largely on segregating generations whilst Ibarbia and Lambeth restricted theirs to a group of pure-breeding lines and diallel hybrids. The former analysis affords a less biased, hence superior, measure of the genetic relationships and more closely approximates the anticipated experience of the breeder. Nevertheless, despite the limitations anticipated from the work of Goldenberg and Pahlen, the present experience demonstrates that linkage and/or development correlations are not so restrictive that they cannot be overcome by selection, at least to a certain extent. Although this investigation did not succeed in recovering all the SS measured in the wild parent, it did result in a substantial gain in a character in which relatively slight improvement is greeted with enthusiasm by the tomato industry.

This program has apparently changed the genetic determinants of

solids assimilation, but the available data do not reveal the basic nature of these changes. It therefore remains for future research to determine whether the efficiency of CO₂ fixation, translocation of photosynthates, reduction of respiratory losses, and other processes or a combination thereof have been modified.

It is interesting to note also that the gain in SS was made without disturbing plant yields. Other workers have reached the conclusion that a maximum limit is imposed on total SS production by the amount of foliage arrayed on a plant. Thus, in a comparison of lines approaching isogenicity for *d*, *j*, and *sp*, Emery and Munger (1970) could relate gains in SS with an increased number of leaves and decreased SS with a reduced leaf number between inflorescences. In the circumstances of such tests, SS may have been highly correlated with fruit size because the latter is a direct component of yield. These limitations may be valid and the total metabolite production by a plant must inevitably be limited by its total leaf area, but apparently they were not reached in the research materials reported here.

Inheritance of SS

Although estimation of the various parameters affecting inheritance of SS in the present material was not a primary objective, certain data were obtained which are enlightening concerning this matter. They point toward complex genetic control. A consideration of the linkage relations *per se* reveals that several loci must be active in determining the SS levels. Thus, in each of the four backcross lines tracing to four different segregants of BC₁, high SS tended to be associated with different, and often genetically independent, characters. Such associations were found with the delta-carotene character, uniform ripening, and various cate-

gories of fruit cracking. Further, the amount of difference in SS associated in a relatively tight linkage situation, as with the delta-carotene trait, never constituted more than one-quarter of the total SS gain. These results would demand a minimum of three loci with relatively large effects on SS; the total number of loci is probably higher.

The orange fruit character

As noted in the section on Results, clearly defined segregation for dominant orange fruit color was observed in BC₁ and subsequent generations (table 2). Since *B*—a gene determining beta-carotene formation and, consequently, orange fruit color—is also known to be tightly linked with *sp* on chromosome 6 (Mackinney *et al.*, 1954), *B* might logically be suspected as the responsible gene in these *minutum* derivatives. This notion is supported by the discovery by Chmielewski and Berger (1966) of *B*, or a similar allele, in *L. minutum* and its tight linkage with *sp* in backcrosses to *L. esculentum*. Carotene determinations did not assist in attempts at verification, primarily because total colored carotenoid levels are low and highly variable in BC₁, being strongly modified by other pigmentation genes.

In later generations delta-carotene was detected as the major pigment of the orange segregants. At this stage of introgression, sufficient *minutum* fruit-color modifiers were evidently eliminated to permit unambiguous identification of the major carotenoid constituents. A question thus arises as to the relationship between this character, which resembles Tomes' (1969) *Del* in respect to its dominance, phenotype, and biochemistry, and the one observed in BC₁. Two additional bits of evidence suggest that they might indeed be different: (1) The delta character was derived from both red and orange segregants of BC₁. (2) The delta character was

tightly associated with high SS, whereas the BC₁ character showed no such association.

On the other hand, certain observations contradict the hypothesis of two dominant *minutum* genes coding for orange carotenoids. Actually, the aforementioned linkage with *sp* argues *per se* against the segregation of *B* in BC₁; although *detection* of the linkage seemingly argues in favor of *B*, the *intensity* of linkage disproves its presence. The observed recombination between orange and *sp* in BC₁ was 13.9 per cent—significantly very much higher than the 1 per cent or less (0.12 per cent in Chmielewski and Berger's studies) previously obtained between *B* and *sp*. The data thereby reveal another locus on chromosome 6 for a major carotenoid gene—thus adding another example to the growing list of tomato genes that have the same or similar phenotypic effects and tend to be associated on the same chromosome (Rick, 1971). Another aspect of this relationship is the apparent discrepancy between the present results and those of Chmielewski and Berger (1968) regarding the *minutum* gene coding for orange fruit color. The linkages indicate that the respective loci are different in the two investigations. No serious contradiction is engendered, however, because the genes were derived from different *minutum* stocks—the tiny flowered race (LA734) in the research of Chmielewski and Berger, the more robust form (LA-1028) in the present study.

That *B* apparently did not segregate in BC₁ does not *per se* rule out the possibility that the delta-carotene gene was segregating instead, nor does it discriminate between the one or two gene hypotheses. The available information does not permit any firm conclusion. Until further experiments prove otherwise, it is in keeping with the law of parsimony of assumptions to

consider a single locus responsible for all observed orange vs red segregations despite some evidence to the contrary.

Novel variants

Three inherited characters—*Ip*, *rv-4*, and the variegated trait—which are unknown in the parental lines, appeared as segregants in late generations. In the case of *Ip* (Intense pigmentation), it is conceivable that this gene, like the one coding for delta-carotene, is fixed in *L. minutum* but fails to express itself because the background genotype of this species prevents the development of any colored carotenoid pigments. For the other two variants, such an interaction appears less applicable because they appeared only once, and then only in selfed derivatives of BC₅. The criteria proposed by Rick (1967) to distinguish between various genetic mechanisms to explain the origin of such novel variants in the progenies of species hybrids can be applied to these examples. Clearly, neither can be considered as a latent recessive trait carried by either parent because both parents have been inbred and progeny tested for several generations before the crosses were made. It is not likely, either, that they owe their appearance to recessive genes of *L. minutum*, which gain expression when combined with the cytoplasm or background genotype of *L. esculentum*, for in both instances the characters should appear after one generation of selfing. Gene mutation, possibly induced in some fashion by the hybrid origin, remains as the most plausible mode of origin.

Gene mutation induced by species hybridization is by no means a new phenomenon. The literature is in fact replete with examples, including, among tomato species, novel mutants in the progenies of *L. esculentum* × *Solanum pennellii* (Rick, 1967), the classic cases in hybrids of maize and teosinte (Mangelsdorf, 1958), as well as many others.

Although some interesting new crosses, the experience does not shed
 amplexes are thus added to the list of new light on the mechanisms responsi-
 mutations probably stimulated by wide ble for the phenomenon.

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