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Changes in ripening-related processes in tomato conditioned by the *alc* mutant

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Summary. The *alc* mutation affects the ripening and storability of tomato fruit. The alteration of fruit color in *alc* lines is due to a reduction in total pigment and a reduction in lycopene relative to total carotinoids. Polygalacturonase (PG) activity is reduced to less than 5% of normal, and the isozymes PG2a and PG2b are absent in *alc* fruit. The level of anti-PG precipitable proteins is also reduced to less than 5% of normal. Total polyA +mRNA is not significantly reduced in ripening *alc* fruit, but hybridization of polyA+mRNA to different ripening-related cDNA clones showed that specific mRNAs are present at reduced levels in the mutant. Specific mRNA levels were reduced to 10%–80% of normal levels, depending on the cDNA clone used as the probe. PG mRNA was present at 5%–10% of the normal level.

All effects of *alc* on fruit ripening are relived in the line Alcobaca-red, which arose spontaneously from the original *alc* line, Alcobaca. The Alcobaca-red trait segregates as a single dominant trait at or very near the *alc* locus, and it is probably the result of a reverse mutation at the *alc* locus.

The chromosomal locations of regions homologous to 5 ripening-related cDNA probes were determined. Regions homologous to 4 of these probes map to chromosomes other than chromosome 10, indicating that the effects of *alc* are transactive. A cDNA clone for PG was homologous to only one chromosomal region. This region is located on chromosome 10, which is also the chromosome on which *alc* and *nor* are located.

Key words: *Lycopersicon esculentum*, Alcobaca, mRNA, fruit ripening

Introduction

Tomato fruit ripening (reviewed in Brady 1987; Grierson 1985; Grierson et al. 1985a) is a complex developmental system consisting of several coordinated processes. These processes result in such diverse changes as the solubilization of cell wall materials, the disappearance of tomatine and chlorophyll, the appearance of the pigments carotene and lycopene, and a climacteric increase in CO_2 and C_2H_4 evolution.

The results of several physiological and biochemical studies indicate that ripening involves changes in gene expression (Rattanapanone et al. 1977, 1978; Grierson 1985; Grierson et al. 1985a). Protein profiles change at the onset of ripening (DeSwardt et al. 1973; Speirs et al. 1984; Grierson et al. 1985b). Activities of the enzymes invertase (Iki et al. 1978) and polygalacturonase (PG) (Brady et al. 1982; Tucker et al. 1980; Hobson 1964) increase during ripening. These changes in protein profile and amount are due to alterations in mRNA population present in ripening fruit (Speirs et al. 1984; Grierson et al. 1985b). For example, the fruit-softening enzyme polygalacturonase is not present in green fruit, but accumulates rapidly during ripening as the result of de novo synthesis (Tucker and Grierson 1982). Of the three forms of PG, PG1 appears first during fruit ripening, but PG2 (PG2a plus PG2b) accounts for more of the total PG activity in nearly ripe normal fruit (Tucker et al. 1980; Brady et al. 1983). PG1, PG2a, and PG2b are structurally related and all contain the same polypeptide of 46,000 molecular weight (Ali and Brady 1982; Tucker et al. 1980). In in vitro assays, PG2 can be converted to a form very similar to PG1 by addition of a heat stable non-dialyzable factor isolated from green fruit (Tucker et al. 1981). The increase in PG activity during ripening is accompanied by an increase in the amount of PG protein present and an

increase in the level of mRNA coding for PG (Grierson et al. 1985b, 1986b; Maunders et al. 1987; DellaPenna et al. 1986). The differences in mRNA profile during ripening has permitted the production and selection of cDNA clones of messages specific to or enhanced during ripening (Grierson et al. 1985b; Mansson et al. 1985; Speirs et al. 1984). cDNA clones of PG have been identified by several groups (Grierson et al. 1986a; DellaPenna et al. 1986).

The landrace Alcobaca possesses a mutation (*alc*) that affects many of the processes involved in ripening, including fruit softening and the production of CO_2, ethylene and pigments (Mutschler 1984a, b). On the vine, *alc* fruit ripen to a light, rather than a deep, orange-red color, and ripe *alc* fruit can be picked and stored at room temperature for about 3 times longer than fruit of the standard line, Rutgers. The increased storability of *alc* fruit is not due to extreme firmness of the harvested fruit, but an attenuation of the over-ripening process, including a slower rate of fruit softening. If the fruits are picked mature green and held in darkness at 20 °C, they fail to ripen although detached fruit of normal tomato varieties will ripen. Fruit heterozygous for *alc* will ripen off the vine and have an intermediate reduction in softening rate and increase in fruit storability. F_2 linkage studies showed that *alc* is a recessive gene on the short arm of chromosome 10 distal 14 map units from *hy* and 21 map units from *u* (Mutschler 1984a).

We decided to use the pairs of lines Rutgers vs Rutgers-*alc* and Alcobaca vs Alcobaca-red in conjunction with ripening-specific cDNA clones as hybridization probes to study the effects *alc* on fruit ripening and on the mRNAs homologous to ripening-specific clones. In addition, the number and locations of some of the loci homologous to the ripening-specific genes were determined.

Materials and methods

Plant materials

The line Rutgers-*alc* was created at Cornell by backcrossing the *alc* allele into the cultivar Rutgers. All of the work was done using stock of Rutgers-*alc* created by 5 backcrosses, unless stated otherwise.

After growing several generations of self progeny of Alcobaca, one distinctive plant of Alcobaca was found from one self progeny. Fruit of this plant ripened on or off the vine to a very intense red color. It is very unlikely that this plant was produced by an accidental outcross since it retained the recessive morphological markers (*y*: colorless skin, and *c*: potato leaf) and the distinctive growth habit and disease susceptibility of Alcobaca. Progeny of the original red-fruited plant segregated for red-fruited and *alc*-like plants. After two more selfed generations a non-segregating red-fruited line was identified. This line was named "Alcobaca-red".

Tissue preparation

Flowers of all four genotypes were tagged at anthesis. Fruit not harvested at 30 d were tagged again at mature green. Fruit were harvested and pericarp was isolated from fruit at the following stages: 30 d after anthesis, mature green, mature green plus five days on the vine, and ripe. The tissue was rapidly chopped coarsely, frozen in liquid nitrogen, and stored at −80 °C.

Clones used for probes

Fifteen of the non-homologous ripening-related cDNA clones created using polyA+mRNA from ripening fruit (Slater et al. 1985) were used as probes (pTOM 4, 5, 6, 13, 25, 31, 36, 38, 41, 88, 92, 96, 99, 111, and 114). Of these clones, pTOM 6 has been identified as being homologous to the gene coding for PG (Grierson et al. 1986a). pTOM 5, 36, and 99 have been classified as ripening-specific (Grierson et al. 1986b; Maunders et al. 1987). pTOM 13 is expressed in fruit during ripening and in wounded leaves (Holdsworth et al. 1987).

Pigment assays

Pigment was extracted from equal weights of ripe fruit pericarp of Rutgers, Rutgers-*alc*, Alcobaca and Alcobaca-red by the method of Grierson et al. (1985b) and scanned from 350–680 nm.

Polygalacturonase extraction and assays

PG was extracted from equal weights of tomato pericarp from each genotype using the method of Tucker et al. (1980), except that after the final pellet was resuspended the solution was dialyzed overnight at 4 °C. Activities of the PG extracts were assayed as described by Tucker et al. (1980), and relative levels of PG protein present were tested by immunodiffusion (Ouchterlony 1962). PG extracts were run on 10% acylamide gels and stained as described by Crooks and Grierson (1983), except that 0.03% methylene blue in 10 mM ammonium acetate pH 8.6 was used to stain the gels.

RNA and poly A+mRNA purification

Total RNA and polyA+mRNA samples were prepared by the method of Grierson et al. (1985b) with the following modifications. Pericarp tissue (75 g) was chilled in liquid nitrogen, rapidly ground fine in a domestic coffee grinder (Krups) before homogenization in a mortar and pestle in 100 mL cold homogenization buffer, and methylene chloride was used instead of chloroform for phenol/chloroform extractions. Yields of polyA+RNA ranged from 0.4–1 μg/gm pericarp.

Slot blots and northern blots

Slot blots were prepared using a BRL Hybrislot apparatus and Genescreen membrane using glyoxal/DMSO. Each slot was loaded with 0.25 μg of polyA+mRNA isolated from the 4 ripening stages of Rutgers or Rutgers-*alc*, or 1.0 μg of polyA+mRNA from Alcobaca or Alcobaca-red. Slot blots were also made in which total RNA samples prepared from Rutgers and Rugers-*alc* fruit of the 4 ripening stages were fixed to nylon filters on a per gram fruit weight basis.

Northern transfers were prepared using Hybond N membrane and hybridized using the MOPS protocol provided by the manufacturer (Amersham).

Probe preparation and hybridization

Either whole plasmid DNA or the inserts of the ripening-specific clones were isolated, labelled by nick translation, then the DNA

Table 1. Segregation for Alcobaca-red trait in populations derived from Alcobaca × Alcobaca-red crosses

Population	No. of plants	Fruit type		Ratio tested	*P*
		red	*alc*-like		
Alcobaca	24	0	24	–	–
Alcobaca-red	48	48	0	–	–
F_1	10	10	0	–	–
F_2	166	131	35	3:1	$0.25 > P > 0.10$
BC to Alcobaca	60	31	29	1:1	$0.90 > P > 0.75$
BC to Alcobaca-red	58	58	0	–	–

Table 2. Linkage analysis of *alc* and flanking markers in a BC population derived from Alcobaca-red[a]

Loci			No. of plants
hy	*alc*	*h*	
+	+	+	181
+	+	–	73
+	–	+	16
+	–	–	8
–	+	+	8
–	+	–	14
–	–	+	76
–	–	–	153
0.50	0.69	1.03	

χ^2 for 3:1 ratio

[a] Population derived from the cross (*hy-alc-hy* × Alcobaca-red) × (*hy-alc-h*)

was purified from unincorporated nucleotides by chromatography over G100 Sephadex and used to probe the slot blots and Northern blots of poly A + mRNA. (Maniatis et al. 1982). The slot blots made with total RNA were probed with dT15 end-labelled using T4 kinase. X-ray films from the northern gels were scanned using a densitometer to determine the relative densities of the bands representing the RNA species homologous to each probe.

Mapping loci homologous to ripening-specific clones

A preliminary determination of the number and location of loci homologous to some of the ripening-specific cDNA clones was done by the restriction fragment polymorphism method of Bernatzky and Tanksley (1986). Southern blots of genomic DNA from plants in the F_2 population (*L. esculentum* × *L. penellii* LA716) were probed with nick-translated insert DNA from the ripening-specific clones. The *esculentum* parent used was either VF36 (blots kindly provided by Dr. Tanksley) or New Yorker.

Results and discussion

Alcobaca-red

Alcobaca-red performs like a normal tomato on all assays run to date. Fruit from plants of Alcobaca-red, Alcobaca, and their F1 stored for 12.1 ± 0.9, 29.0 ± 0.7 and 15.3 ± 0.6 days, respectively. These are very similar to the 1983 storage results for Rutgers, Alcobaca and their F1 (9.0, 32.9, and 13.6, respectively, Mutschler 1984b). Fruit of Alcobaca-red can also ripen in the dark at 21 °C, as do normal fruit. Studies of F_1, F_2, and BC populations of crosses of Alcobaca and Alcobaca-red indicated that these lines differ at a single locus, with the red trait dominant (Table 1). Data from the backcross population (*alc-hy-h* × Alcobaca-red) × (*alc-hy-h*) including 529 plants show that the Alcobaca-red trait is on chromosome 10, 9 mu proximal to *hy* and 31 mu from *h* (Table 2). This suggests that Alcobaca-red arose as the result of a reversion at *alc*, but does not eliminate the possibility of a suppressor of *alc* near *alc* on chromosome 10. The backcross population (Alcobaca-red × Rutgers) × Alcobaca including over 450 plants did not include any plants with an *alc* phenotype, but did result in the expected 1:1 segregation for the unlinked control markers, *c* and *y*. These data support the hypothesis that Alcobaca-red is the result of a reversion at the *alc* locus.

Fruit pigment

The fully ripe *alc* fruit is paler than a normal fruit. Pigment profiles show three major peaks at approximately 445, 470, and 502 nm and a shoulder at 420 nm (Fig. 1). The major pigments in tomato are beta-carotene and lycopene (Thomas and Jen 1975). Since beta-carotene in hexane absorbs at 420, 450 and 477 nm, and lycopene absorbs at 448, 473 and 504 nm (Davies 1965), the peaks at 445 and 470 are due to both pigments, but the 502 peak reflects lycopene content. There is less total pigment in Rutgers-*alc* tissue than in an equal weight of Rutgers tissue (Fig. 1). In addition to the reduction in pigment content, the relative proportions of pigments, as reflected by the relative area under the three absorbance peaks, is also affected. The area under peak 3 is 32% of the total area under all three peaks in the scans of the pigment from the normal genotype, Rutgers, but is only 26% of the total area in the scans of the pigment from Rutgers-*alc* pericarp. Since peak three is an absorbance peak of lycopene, and peaks 1 and 2 are absorbance peaks of both lycopene and beta carotene, the change in relative areas under the peaks suggests a decrease in lycopene relative to beta-carotene in Rutgers-*alc*. This has since been confirmed by more detailed pigment studies (D. Law, personal communication). Alcobaca pericarp shows a reduction in total pigment and in the proportion of total area which is under the lycopene peak (24%) similar to those in Rutgers-*alc* pericarp, except that the reduction in total pigment is greater in Rutgers. This difference in total pigment in the two *alc* lines follows a tendency for large fruited *alc* lines to be poorer in color than smaller fruited lines (Mutschler, unpublished). The

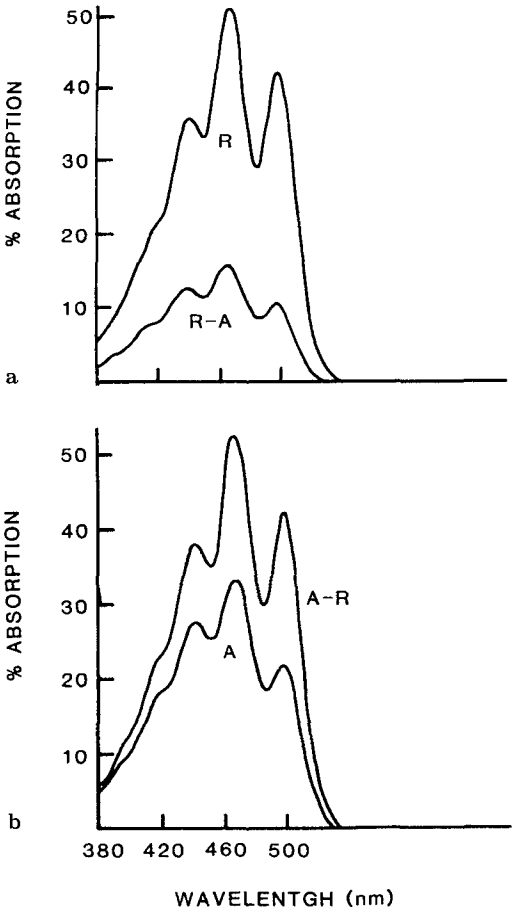

Fig. 1 a and b. Absorbance spectra of pigments extracted from equal weights of pericarp of **a** Rutgers (R) and Rutgers-*alc* (RA), and **b** Alcobaca (A) and Alcobaca-red (AR) pericarp

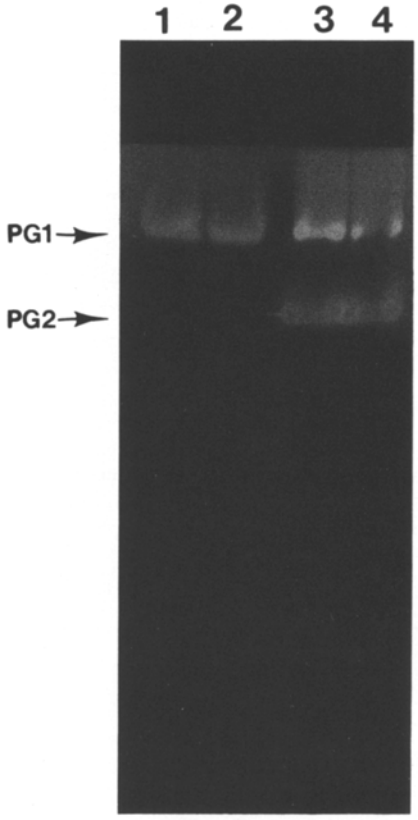

Fig. 2. PG isozymes present in protein extracts from pericarp of Rutgers-*alc*, Alcobaca. Rutgers, and Alcobaca-red (lanes 1–4, respectively)

alteration which gave rise to Alcobaca-red restored normal pigment production; pigment content and profile in pericarp of Alcobaca-red is nearly identical to that of Rutgers, with 32% of the area under the curve being in peak 3.

Polygalacturonase

PG activities in extracts from ripe pericarp of Rutgers and Rutgers-*alc* average 29.1×10^{-3} and 0.9×10^{-3} μmoles galacturonic acid produced min^{-1} gm fresh $weight^{-1}$. The lower PG activity is not due to the formation of an inactive form of PG, since immunodiffusion tests demonstrated that PG extracts from Rutgers-*alc* pericarp contained less than 5% of the anti-PG precipitable protein in extracts from equal weights of Rutgers pericarp. Isozyme gels also show that *alc* lines lack PG2 (Fig. 2). The reduced PG activity and lack of PG2 probably account for the reduced rate of fruit softening characteristic of *alc* fruit (Mutschler 1984 b). The reduced PG activity and the lack of PG2 in fruit of *alc* lines is very similar to the effects of *Nr* (Tucker et al. 1980). In contrast, lines homozygous for either of the other ripening mutants, *rin* and *nor*, lack both PG1 and PG2, and lack detectable PG activity (Hobson 1980; Tucker and Grierson 1982; Grierson and Tucker 1983; Tigchelaar 1978). The PG activity of Alcobaca-red (33.2×10^{-3} μmole galacturonic acid min^{-1} gm fresh wt^{-1}) is similar to Rutgers. Alcobaca-red also shows normal levels of anti-PG precipitable protein and the production of PG2 (Fig. 2).

Effects on specific mRNAs

The poly A + mRNA content in the Rutgers-*alc* samples is similar to that in Rutgers samples; the ratio of the radioactivity detected from the ^{32}P-labeled dT15 bound by Rutgers-*alc*/Rutgers was 1.2, 1.2, 1.1 and 0.7 at the 30 d, MG, MG + 5 and ripe stages, respectively. Differences in content of specific messages cannot be explained as an overall non-specific decrease in mRNA synthesis in *alc* fruit.

Slot blots made using poly A + mRNA from Rutgers and Rutgers-*alc* showed increases in levels of mRNA homologous to pTOM 6, 13, 36, 38 and 92 during ripening of normal fruit pericarp (Fig. 3, Table 3). The rise in

message detected by pTOM 6 is similar to that reported previously for PG clones (DellaPenna et al. 1986; Grierson et al. 1985b; Maunders et al. 1987). The patterns demonstrated by the clones used were of messages which either first appear at MG or are present at very low levels before MG, and rise during ripening reaching a maximum presence either at MG + 5 (pTOM 6, 38, and 92) or at the full ripe (pTOM 13 and 36) stage. The stages at which initiation of accumulation and maximum message level occur were not affected. The main effects of *alc* on the specific messages was to decrease their levels of accumulation (Table 3). This effect was detected at the MG + 5 stage for all 5 clones used. For must of the clones, message was not detected at the 30 d stage, and the message levels were so low at the MG stage, that it was difficult to say with confidence whether *alc* has an effect at this stage. Slot blot hybridizations using the Alcobaca and Alcobaca-red gave very similar results to those using Rutgers and Rutgers-*alc*.

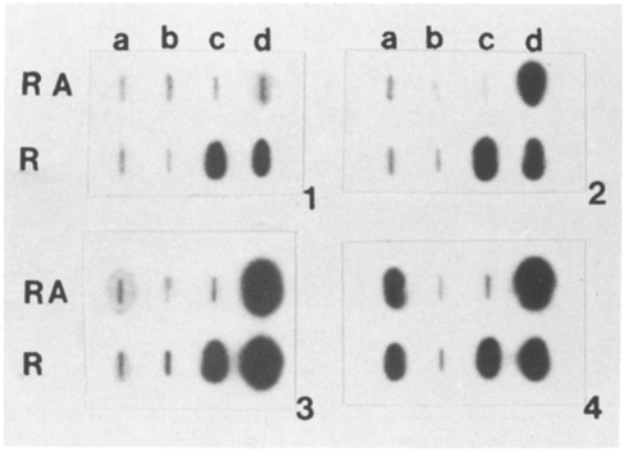

Fig. 3. Slot blots made with 0.5 μg per slot of poly-A + mRNA isolated from Rutgers and Rutgers-*alc* pericarp picked at **a** the 30 d, **b** mature green, **c** mature green + 5 d and **d** ripe stages; the blots were probed with nicktranslated insert from pTOM 6, pTOM 92, pTOM 36, or pTOM 13 (blots **1–4**, respectively)

Since the major measurable effects of *alc* were at the MG + 5 and ripe stages, and since more than one homologous message might be present for some of the clones, further work was done using Northern transfers of poly A + mRNA gels. The level of mRNA homologous to the cloned fragments at the MG + 5 stage is reduced in Rutgers-*alc* compared to Rutgers for the 15 ripening-related or ripening-specific cDNA clones tested (Table 4). The degree of reduction observed for the 15 clones varies depending on the clone used; pTOM 5 shows the least reduction (to about 66% of normal), and pTOM 6 shows

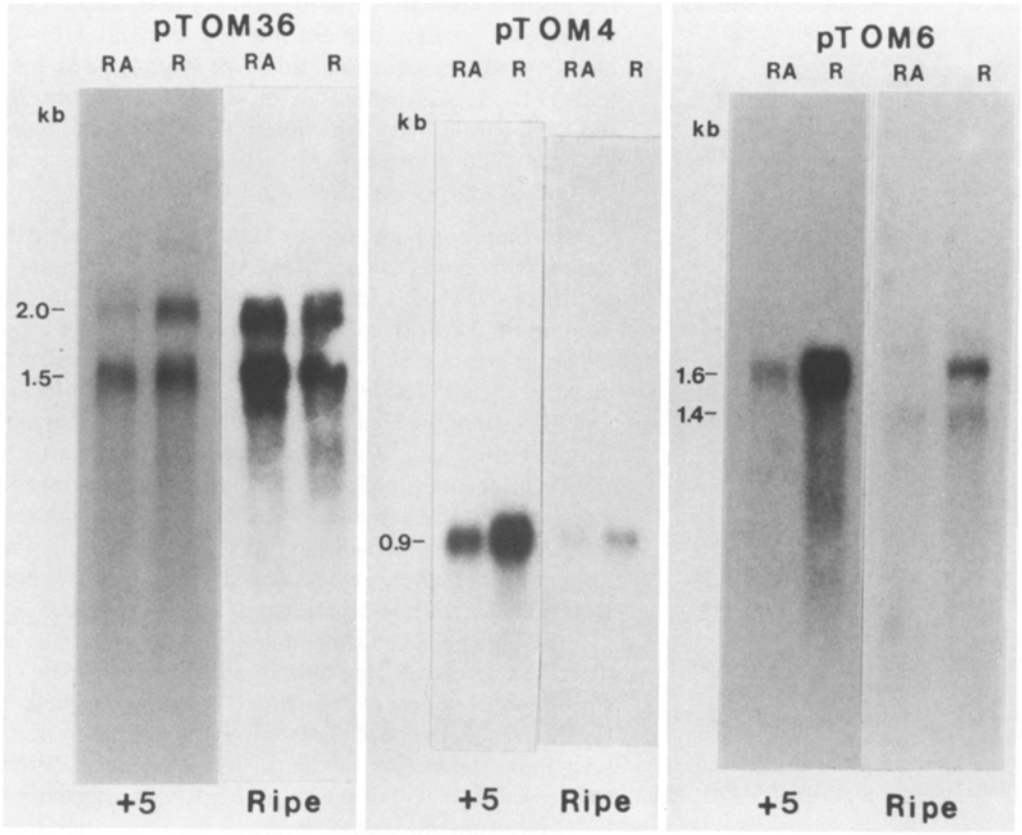

Fig. 4. Northern transfers of poly A + mRNA obtained from Rutgers and Rutger-*alc* pericarp at the mature green + 5 d and ripe stages, and probed with inserts isolated from pTOM 36, 4, and 6

Table 3. Label bound to slot blots of poly A+mRNA from Rutgers, Rutgers-*alc*, Alcobaca, and Alcobaca-red pericarp

Clone	Stage of fruit	Level of bound label[a]		Ratio[b] of Rutgers-*alc*	Level of bound label[c]		Ratio[b] of Alcobaca
		Rutgers-*alc*	Rutgers	Rutgers	Alcobaca	Alcobaca-red	Alcobaca-red
pTOM6	MG	1.1	1.0	1.08	1.3	1.0	1.32
	+5 on	1.4	10.7	0.13	6.5	91.4	0.07
	ripe	2.7	6.2	0.43	1.2	44.2	0.03
pTOM13	MG	0.8	1.0	0.84	NA[d]	NA	NA
	+5 on	1.2	12.6	0.09	NA	NA	NA
	ripe	27.0	13.9	1.94	NA	NA	NA
pTOM36	MG	1.0	1.0	1.03	NA	NA	NA
	+5 on	1.4	14.8	0.09	NA	NA	NA
	ripe	52.9	39.5	1.34	NA	NA	NA
pTOM38	MG	0.5	1.0	0.50	0.3	1.0	0.30
	+5 on	0.8	3.0	0.28	1.0	1.5	0.65
	ripe	2.8	3.1	0.89	0.2	0.7	0.22
pTOM92	MG	NA	NA	NA	2.0	1.0	2.01
	+5 on	NA	NA	NA	15.0	22.6	0.71
	ripe	NA	NA	NA	2.9	12.5	0.23

[a] Levels of label bound presented as proportion of label bound on Rutger at MG stage
[b] Ratio of label bound on Rutgers-*alc* vs. Rutgers and Alcobaca vs Alcobaca-red
[c] Levels of label bound presented as proportion of label bound on Alcobaca-red at MG stage
[d] NA = not available

Table 4. Effects of *alc* on mRNA levels measured by scanning northern transfers of Rutgers and Rutgers-*alc* poly A+mRNA

Class[a]	Clone	mRNA Size[b] (kb)	Signal ratio[c]	
			MG+5 on	ripe
A	pTOM 5	2.0	0.45	NA[d]
	25	0.8	0.35	NA
	31	1.3	0.38	NA
	41	1.4	0.68	NA
	88	1.8	0.21	NA
	92	1.6	0.66	NA
	96	2.5	0.16	NA
	114	0.9	0.50	NA
B	pTOM 13	1.4	0.46	0.81
	36	a) 2.0	0.13	1.17
		b) 1.5	0.50	1.87
	38	1.4	0.18	0.97
C	pTOM 4	0.9	0.31	0.46
	6	a) 1.6	0.10	0.04
		b) 1.4	ND[e]	0.12
	111	0.9	0.15	0.24
D	pTOM 99	1.4	1.2	NA

[a] Class A, B, and C have reduced message level in MG+5 fruit in the *alc* line; ripe fruit message levels were not reduced in class B clones, and were reduced in class C clones; class D clones showed no affects on message levels in MG+5 fruit
[b] For clones homologous to more than 1 size mRNA, the different size bands are labeled alphabetically in order of decreasing size
[c] Ratio of signal: Rutgers-*alc*/*Rutgers*
[d] NA = not available
[e] ND = not detected in either Rutgers or Rutgers-*alc* pericarp

the greatest reduction (to 10% of normal). Three of 6 clones with severely reduced message levels at MG+5 also showed this reduction at the ripe stage (pTOM 4, 6, and 111). The reduction in message homologous to pTOM 6 parallels the reduction in PG activity and anti-PG precipitable protein. Message level of one clone (pTOM 99) shows no effect of *alc* (Table 4).

Northerns prepared using pTOM 36 and 6 resulted in figures with more than one RNA species with homology to the probe. The 2 species homologous to pTOM 36 account for 34% (a) and 66% (b), and 12% (a) and 88% (b) of the area for MG+5 Rutgers-*alc* and Rutgers fruit, respectively, and for 48% (a) and 52% (b), and 36% (b) and 64% (b) of the area for ripe Rutgers-*alc* and Rutgers fruit, respectively. The two species homologous to pTOM 6 account for 65% (a) and 35% (b) of the area for ripe Rutgers pericarp and 39% (a) and 61% (b) of the area for ripe Rutgers-*alc* pericarp, however only the larger (1.6 kb) species was detected in message isolated from mature green +5 pericarp of either genotype.

The data collected show that *alc* has an effect on the detectable levels of some but not all ripening-specific or ripening-related genes. The data do not indicate whether the differences in specific mRNA levels are due to transcriptional differences or due to increased degradation (decreased stability) of the specific mRNAs affected.

Data from all assays indicate that the alteration which created Alcobaca-red is located very near or at *alc*, resulted in a change to normal or near normal phenotype

Table 5. Location and minimum number of loci homologous to some ripening-specific clones

Clone	Minimum no. loci	Chromosome	Linkage[a]
pTOM 4	1	7	pTOM 13
pTOM 6	1	10	
pTOM 92	1	8	Aps-2
pTOM 13	2	7 and 12	Got-2
pTOM 36	2	5 and 8	pTOM 92

[a] Aps = acid phosphatase; Got = glutamic oxaloacetic transaminase

in all traits affected by *alc*, and is most likely a reversion at the *alc* locus.

Mapping loci homologous to ripening-related clones

The results of RFLP mapping 5 of the ripening-related clones show that there are between 1 or 2 regions of chromosome containing homologous sequences to these probes (Table 5). In those cases in which more than 1 locus is indicated, the data do not show which of the loci codes for the cloned sequence, what degree of homology exists among the loci, or whether all of the loci are expressed. The estimates of loci numbers generally agree with the results of cloning and sequencing studies which suggest the existence of one PG locus (Bird et al. in preparation) and 3 pTOM 13 loci (Holdsworth et al. in preparation).

Homology to the cDNA clone of PG (pTOM 6) is limited to 1 region on the short arm of chromosome 10. This is the chromosome arm that contains the *alc* and *nor* loci. The linkage data available do not necessarily imply that either of the 2 loci codes for PG; further mapping tests are underway to determine the relationship and proximity of *alc*, *nor*, and the regions homologous to the PG cDNA clone.

The regions homologous to the other 4 clones are not located on chromosome 10. Since the levels of mRNA homologous to these clones are moderately (pTOM 13, 36, 92) to strongly (pTOM 4) affected by *alc*, the linkage study suggests that the effect of the *alc* locus on these messages is transactive. The linkage data also suggests that some clustering of ripening related genes may exist (the regions homologous to pTOM 4 and 13, or to pTOM 36 and 92), but further information is required to determine whether this holds true.

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