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Characterization of a *Lycopersicon esculentum* x *Solanum lycopersicoides* somatic hybrid lacking a glutamate oxaloacetate transaminase isozyme

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Abstract. Morphological, cytological, isozyme and chloroplast DNA analyses were used to determine possible mechanism(s) for the loss of glutamate oxaloacetate transaminase-4 (GOT-4) isozyme activity in a somatic hybrid. Plant 204-1, derived by cell fusion between tomato (*Lycopersicon esculentum*) and *Solanum lycopersicoides*, was characterized for both *Got-4* and acid phosphatase-2 (*Aps-2*), two isozyme loci which are closely linked (recombination 2.5 cM). This hybrid was determined to be chimeric for both *Got-4* and *Aps-2*. The *S. lycopersicoides* plant used to provide cells for the fusion was determined to be heterozygous for both *Got-4* and *Aps-2*. Only one *S. lycopersicoides* allelic form of *Aps-2* and *Got-4* was found in plant 204-1. This observation indicated that either the alternative copy of the *S. lycopersicoides* chromosome region encoding *Got-4* and *Aps-2* is deleted or the entire chromosome is absent. Plant 204-1 was cytologically determined to be aneuploid with approximately 62 chromosomes. Sixty-two somatic hybrids of separate callus origin were analysed for GOT-4 and a high proportion (27%) lacked the *S. lycopersicoides* form of *Got-4*. The loss of this allele and the linked *Aps* allele most likely occurred in the suspension culture of *S. lycopersicoides* used to provide cells for fusion.

Introduction

Genetic improvement of crop species depends upon both identifying and utilizing desirable genetic variation. In some cultivated crop species there is limited variation or absence of the trait(s) of interest. One method of introducing new variation is through cell fusion to create somatic hybrid plants. Such plants may be either symmetric or asymmetric hybrids with regard to the balance of parental chromosome numbers [5]. Thus, traits of one parent may be present in some hybrids while absent in others. Identifying the genetic variation resulting from cell fusion is important in gaining an understanding of the processes responsible for such variation and ultimately determining the viability and utility of the resulting plants in breeding.

In our laboratory, somatic hybrid plants were created between tomato and *S. lycopersicoides* in an attempt to transfer chilling tolerance to tomato [4]. Eight plants of separate callus origin examined to date have been verified by cytological, biochemical and morphological studies as intergeneric hybrids. Two hybrid plants, 67 and 204, expressed only the tomato form of glutamate oxaloacetate transaminase-4 (GOT-4) [4]. Plant 67 has not formed roots to date and hence is maintained only in vitro.

In this study, plant 204 has been investigated in further detail to determine the possible mechanism(s) of loss of the *S. lycopersicoides* Got-4 isozyme activity.

Materials and methods

Somatic hybrid plants between *Lycopersicon esculentum* 'Sub Arctic Maxi' and *Solanum lycopersicoides* (LA 1990) were created and verified as described [4]. Shoot 204 was selected from callus 204. This shoot was rooted in vitro and a terminal shoot tip cutting was excised and maintained in vitro on rooting medium as shoot 204. At the time the first tip cutting was excised, the rooted basal portion of the shoot, identified as shoot 204-1, was transferred to soil. Terminal shoot tip cuttings were transferred in vitro at monthly intervals. Three months after 204-1 was placed into planting medium, the process was repeated for 204-2 and then for 204-3. Plants 204-4 to 204-22 are rooted tip cuttings taken from several branches of 204-1 after transfer to the greenhouse.

All biochemical assays were conducted on greenhouse-grown plants as previously described [4]. Extracts enriched for chloroplasts were prepared and GOT isozymes were analysed as previously described in [4] except that the GOT stain was modified [1]. Acid phosphatase (APS) isozymes were studied by electrophoresis in a 9% polyacrylamide gel with SDS omitted [7]. Gels were stained for Aps using β-naphthyl acid phosphate as the substrate [13]. Tomato isozyme marker lines LA 1810 (*Aps-1*^{n}), LA 1811 (*Aps-1*^{1}), LA 1813 (*Aps-2*^{n}) and LA 1815 (*Aps-2*^{2}) with identified APS alleles were supplied by C.M. Rick. These genotype markers were used to verify the location of APS bands on the gels. Intensity of APS bands was determined by scanning the gels at 540 nm on a Gilford 250 spectrophotometer. Peak area for the *S. lycopersicoides* forms of APS-2 were expressed relative to the peak area for the tomato forms of APS-2.

Chloroplast DNA was extracted as described by Palmer [10] and digested with Pst-1 according to supplier's instructions (Bethesda Research Laboratories). Samples from tomato, *S. lycopersicoides*, and both forms of hybrid

204 were separated on a 0.7% agarose gel using 0.089 M Tris, 0.089 M borate, 0.002 EDTA buffer [8], stained with ethidium bromide and visualized with ultraviolet light.

Pollen viability was determined by aniline blue-lactophenol staining. Two hundred pollen grains from each flower were scored and eight flowers were examined from each GOT form of hybrid 204.

Chromosome counts were made from cuttings rooted in planting medium. Roots tips were pretreated with p-dichlorobenzene, fixed in Farmer's solution, hydrolysed in 1 N HCl for 20 minutes and prepared with Feulgen's stain and mounted in acetocarmine.

Result and discussion

Hybrid plant 204-1 was determined to be chimeric for GOT-4 activity (Fig. 1). Ten samples of leaves were collected from the four major branches of 204-1 approximately six months after transfer to the greenhouse. These

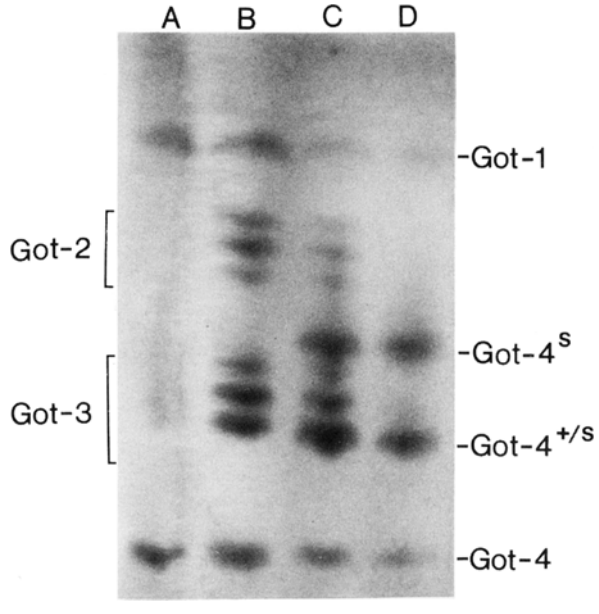

Fig. 1. GOT isozyme patterns of somatic hybrid 204. Lane B and C are leaf extracts; A and D are chloroplast extracts. Lane A and B are from 204-4 which lacks the *S. lycopersicoides* form of GOT-4 (*Got-4*^{s}) and Lane C and D are from 204-3 which has both the active *Got-4*^{s} and the tomato form of GOT-4 (*Got-4*$^{+}$). The *S. lycopersicoides* homodimer of GOT-3 co-migrates with the GOT-4 heterodimer (GOT-4$^{+/s}$).

lateral branches of the single main stem arose during growth in the greenhouse. Five samples from two major branches expressed both the tomato (GOT-4^+) [3] and *S. lycopersicoides* (GOT-4^s) forms of GOT-4. All four samples collected from another major branch expressed only GOT-4^+. A single sample collected from the fourth branch expressed GOT-4^+ at a normal level and GOT-4^s at a low level. When this branch was resampled after two months, no GOT-4^s activity was noted. All samples of plants 204-2 and 204-3 expressed both GOT-4^+ and GOT-4^s. These plants were derived from tip cuttings of the same shoot that gave rise to 204-1. Cuttings were collected from identified portions of chimeric plant 204-1. After two months growth in the greenhouse all 18 rooted cuttings expressed the isozyme patterns expected based on their positions on the original plant. The lack of GOT-4^s activity in hybrid 204 was observed only in 204-1 and in plants derived from cuttings of 204-1. No case was observed in this study where a plant lacking GOT-4^s activity regained this activity.

Although there was a detectable difference in GOT-4 isozymes (Fig. 1), the two forms of hybrid 204 are otherwise similar. Both forms of hybrid 204 have flowered and set fruit. Pollen viability did not differ statistically as flowers from branches expressing both GOT-4^+ and Got-4^s had 45% viable pollen and 55% viable pollen for flowers from branches expressing only GOT-4^+. Both forms of the plant averaged 1.5 developed ovules per fruit with normal appearing embryos. Similarly, both forms of hybrid 204 had identical zymograms for phosphoglucoisomerase-1 (PGI-1), phosphoglucomutase-2 (PGM-2), 6-phosphogluconate dehydrogenase-2 (6-PGDH-2), shikimic acid dehydrogenase-1 (SKDH-1), GOT-2 and GOT-3.

These six isozyme markers represent genes localized to tomato chromosomes 1, 4, 7 and 12 [11, 14, 15, 16]. GOT-4 has been mapped to tomato chromosome 8 [16]. Although APS isozymes have been described previously [9, 16], neither *Aps-1* nor *Aps-2* had been previously studied in these somatic hybrids. *Aps-1* has been mapped to chromosome 6 [9, 12] and *Aps-2* to chromosome 8 of tomato [16]. Both APS-1 and APS-2 isozyme patterns confirmed the hybridity of the somatic hybrid (Fig 2). *S. lycopersicoides* (LA 1990) is null for APS-1 and 'Sub Arctic Maxi' expressed an active form of APS-1. The somatic hybrid also expressed APS-1 (Fig. 2). The *S. lycopersicoides* forms of APS-2 are slower migrating than the tomato forms. Both tomato and *S. lycopersicoides* forms of APS-2 are present in the hybrids (Fig. 2). There is an additional slower migrating form of APS. The *S. lycopersicoides* form of this isozyme is slower migrating than the tomato form. Three bands are present in the hybrid indicating this isozyme functions as a dimer.

GOT is a dimeric enzyme with distinct chloroplast, mitochondrial, micro-

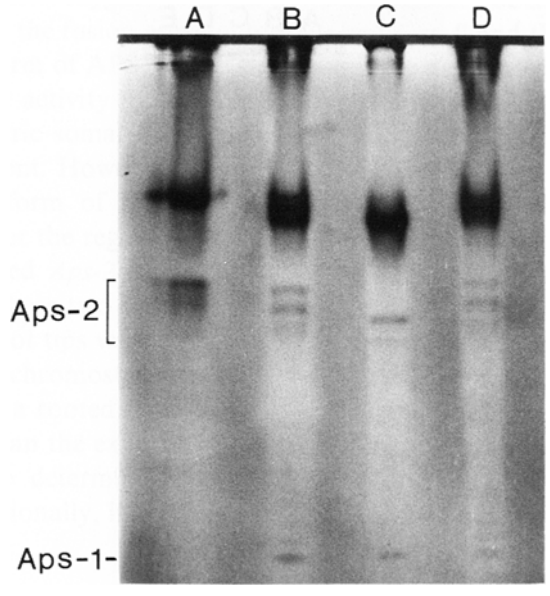

Fig. 2. APS isozyme patterns of tomato and *S. lycopersicoides*. Lane A: *S. lycopersicoides*; B: sexual hybrid between tomato and *S. lycopersicoides*; C: tomato; D: a representative somatic hybrid between tomato and *S. lycopersicoides*.

body and cytosolic forms [6]. *S. lycopersicoides* (LA 1990) and 'Sub Arctic Maxi' differ for 3 of the 4 forms of GOT. In hybrid plants, 10 different forms are present. To clarify the specific form(s) of GOT absent in hybrid 204 the subcellular compartment for GOT-4 was identified. GOT-4 is associated with the chloroplast fraction (Fig. 1). GOT-1 most likely represents microbody contamination of the chloroplast-enriched fraction. GOT-4^s was detected in 204-3 but not in 204-4. The heterodimer between GOT-4^+ and GOT-4^s was present in 204-3 and absent in 204-4 indicating the presence and absence, respectively, of active GOT-4^s subunits.

Thus, Got-4 is an enzyme mapped genetically to chromosome 8 [16] and the activity localized to the chloroplast. The somatic hybrids could have either tomato or *S. lycopersicoides* chloroplasts. Pst-1 digests of chloroplast DNA indicated both forms of hybrid 204 had chloroplast restriction fragment patterns indentical to tomato (Fig. 3). Therefore, the differential GOT-4 activity is not due to a species-specific plastid type.

In order to determine the possible mechanism of the loss of GOT-4^s activity, the isozyme genotype of the *S. lycopersicoides* parent used in fusion was investigated by analysing sexual diploid intergeneric hybrid plants between tomato and *S. lycopersicoides*. The hybridity of ten sexual hybrids

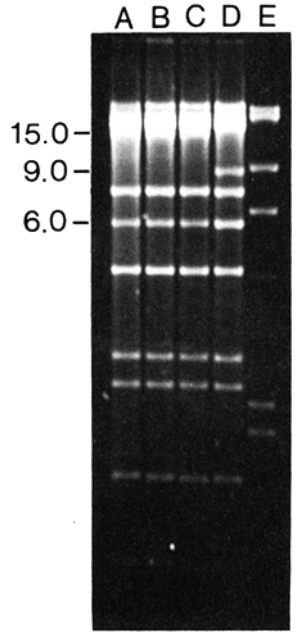

Fig. 3. Pst-1 digest of chloroplast DNA. Lane A: tomato; B: 204-3 which expresses GOT-4^s; C: 204-4 which lacks GOT-4^s activity; D: *S. lycopersicoides*; E: Hind III digest of lamda.

was verified by PGI-1, GOT-2 and GOT-3 isozymes. Seven of these hybrids expressed both GOT-4^s and GOT-4$^+$ and three only GOT-4$^+$. Thus, the *S. lycopersicoides* parent used as the protoplast source in the fusion was heterozygous with an active allele (*Got-4*^{s}) and a null allele for *Got-4* (*Got-4*^{n}). The lack of GOT-4 activity in somatic hybrid 204 could have resulted from a single mutation or deletion of *Got-4*^{s}.

A single mutation event would affect only the activity of GOT-4 while a deletion could affect linked loci. In tomato, *Aps-2* is tightly linked to *Got-4* on chromosome 8 with an estimated recombination rate of 2.5 cM [16]. The genotype of the *S. lycopersicoides* parent was investigated for *Aps-2* by analysing the same sexual diploid hybrids analysed for GOT-4.

In the three sexual hybrids with *Got-4*^{n} the *S. lycopersicoides* form of APS-2 stained less intensely than in the seven sexual hybrids with *Got-4*^{s} (Fig. 4). The intensity of the *S. lycopersicoides* APS-2 bands was expressed relative to the tomato forms of APS-2 of the same sample. Densitometer scans of these gels indicated a 40% reduction in stain intensity for the three hybrids. Therefore, the two *S. lycopersicoides* alleles of *Aps-2* could be distinguished by stain intensity even though they co-migrated on the gel.

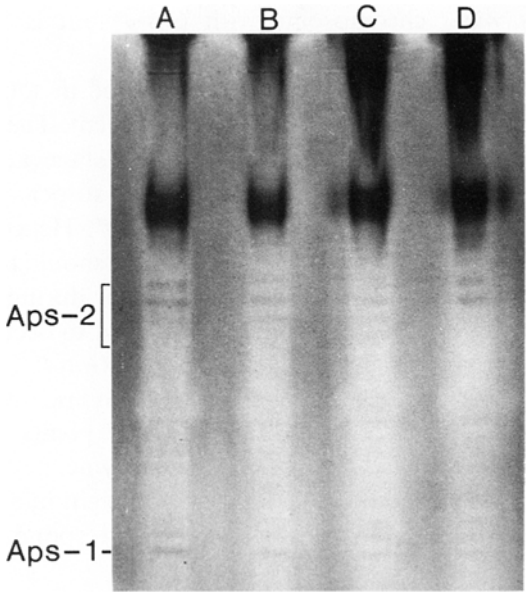

Fig. 4. APS isozymes of hybrids between tomato and *S. lycopersicoides*. Lanes A and B: sexual hybrids; C and D: somatic hybrid 204. A and D are from plants with *Got-4*^{s} and higher activity for *S. lycopersicoides* form of *APS-2*. B and C are from plants without *Got-4*^{s} and have decreased activity for the *S. lycopersicoides* form of Aps-2.

From these analyses it was concluded that the *S. lycopersicoides* partner in the fusion was heterozygous for both *Got-4* and *Aps-2*. The *S.lycopersicoides* partner used in the fusion appears to have had *Got-4*^{s} and the allele for the high-activity form of APS-2 on one chromosome and *Got-4*^{n} and the allele for the reduced activity form of APS-2 on the homolog.

In a symmetric somatic hybrid both *S. lycopersicoides* alleles of *Aps-2* should be present. However, only the reduced activity form of APS-2 was found in the form of the somatic hybrid lacking the *Got-4*^{s} (Fig. 4). Therefore, either the region of the *S. lycopersicoides* chromosome with the *Got-4* and linked *Aps-2* loci was deleted or one copy of the entire chromosome with these two loci was lost.

Mitosis in root tips was examined to provide evidence for loss of one *S. lycopersicoides* chromosome. Approximately 62 chromosomes were found in plant 204-9, a rooted tip cutting of hybrid 204 not expressing GOT-4^{s}. This is more than the expected tetraploid number ($2n = 4x = 48$). It was not possible to determine cytologically the absence of a specific chromosome. Additionally, it is possible that this hybrid has multiple copies of

the *S. lycopersicoides* chromosome with *Got-4*^{n} but lacks the homolog carrying *Got-4*^{s}.

The loss of the *Got-4*^{s} and *Aps-2* alleles detected in somatic hybrid 204 could have occurred at several stages of development. The expected consequences are likewise dependent on the specific stage at which the loss occurred. For example, if the loss occurred in the suspension culture, after several subcultures, many cells would lack *Got-4*^{s}. Hence, many hybrids resulting from a fusion with this suspension culture should lack *Got-4*^{s}. If the active allele was lost as a consequence of fusion or during development of the callus and shoot, the loss(es) would be expected to occur independently in each hybrid and only a few plants would lack *Got-4*^{s}.

To distinguish between these alternatives, 62 hybrid plants of separate callus origin were analysed for GOT. All these plants were previously verified as hybrids by GOT-2 and GOT-3 isozyme patterns. Seventeen hybrid plants (27%) lacked *Got-4*^{s}. Additionally, eight hybrid plants (13%) had intense stain for this isozyme which would correspond to an additional copy of the *Got-4*^{s} allele.

Three somatic hybrids lacking *Got-4*^{s} and three hybrids with a high level of activity were selected for analysis for APS. APS-2 stain activity was consistent with the loss and gain, respectively, of the high-activity *Aps-2* allele.

Since plants with extra copies of *Got-4*^{s} were detected and a high proportion of the somatic hybrids lacked *Got-4*^{s}, the gain or loss of *Got-4*^{s} and *Aps-2* most likely occurred in the *S. lycopersicoides* suspension culture prior to fusion.

An alternative explanation for the loss could be a somatic instability resulting from the fusion process. In this case, the loss would occur subsequent to the fusion, but in a high proportion of the somatic hybrid plants. Hybrid plant 204-1 is chimeric for *Got-4*. If the loss of one allele of *Got-4* occurred in the *S. lycopersicoides* suspension culture prior to the fusion there were at least two cell types initiating the callus which produced plant 204-1 and plant 204-1 did not develop from a single hybrid cell. These cell types sorted out rapidly in the developing plant as evidenced by the analysis of plants 204-2 to 204-22.

Aneuploidy and polyploidy of plant cells in culture has been demonstrated cytologically for many species including tomato [2]. In this hybrid, more chromosomes than the expected ($2n = 4x = 48$) were observed cytologically, but a portion or one entire *S. lycopersicoides* chromosome was lacking. This variant plant was detected only because one of the parents was heterozygous for a marker gene. This level of variability may be present for other markers on other chromosomes, but would be more difficult to detect.

Although plant 204-1 was created by cell fusion and culture, this process did not create a new form of GOT but only allowed existing variation to be detected. Determination of the possible mechanisms accounting for the loss of GOT-4 activity was only made possible through the use of the detailed genetic map of tomato including linked isozyme loci on chromosome 8.

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