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Fertile progeny of a hybridization between soybean [*Glycine max* (L.) Merr.] and *G. tomentella* Hayata

R. C. Shoemaker [1,*], M. S. Heath [2], H. Skorupksa [3], X. Delannay [4], M. Edge [4] and C. A. Newell [4]

[1] USDA-Agricultural Research Service, Departments of Agronomy and Genetics, Iowa State University, Ames, IA 50011, USA
[2] Department of Genetics, Iowa State University, Ames, IA 50011, USA
[3] Department of Agronomy, Iowa State University, Ames, IA 50011, USA
[4] Monsanto Company, 700 Chesterfield Village Parkway, St. Louis, MO 63198, USA

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Summary. A colchicine-doubled F_1 hybrid (2n = 118) of a cross between PI 360841 (*Glycine max*) (2n = 40) × PI 378708 (*G. tomentella*) (2n = 78), propagated by shoot cuttings since January 1984, produced approximately 100 F_2 seed during October 1988. One-fourth of the F_2 plants or their F_3 progeny have been analyzed for chromosome number, pollen viability, pubescence tip morphology, seed coat color, and isoenzyme variation. Without exception, all plants evaluated possessed the chromosome number of the *G. max* parent (2n = 40). Most F_2 plants demonstrated a high level of fertility, although 2 of 24 plants had low pollen viability and had large numbers of fleshy pods. One F_2 plant possessed sharp pubescence tip morphology, whereas all others were blunt-tipped. All evaluated F_2 and F_3 plants expressed the malate dehydrogenase and diaphorase isoenzyme patterns of the *G. max* parent and the endopeptidase isoenzyme pattern of the *G. tomentella* parent. Mobility variants were observed among progeny for the isoenzymes phosphoglucomutase, aconitase, and phosphoglucoisomerase. This study suggests that the *G. tomentella* chromosome complement has been eliminated after genetic exchange and/or modification has taken place between the genomes.

Key words: *Glycine max* – *Glycine tomentella* – Chromosome elimination – Isoenzyme variation

Introduction

The genus *Glycine* Willd. includes two subgenera, *Soja* and *Glycine* (Moench) F. J. Herm. (Newell and Hymowitz 1983). The subgenus *Soja* includes the diploid (2n = 40) cultivated soybean *G. max* (L.) Merr. and the more primitive *G. soja* Sieb. and Zucc. Both species are annual. With the exception of partial sterility in F_1 hybrids between certain combinations of parents, due to chromosomal structural differences, both species intercross freely (Palmer et al. 1987).

The subgenus *Glycine* includes 15 described species (Tindale and Craven 1988). Most species are diploid (2n = 40), with the exceptions of *G. tabacina* (2n = 80) and *G. tomentella* (2n = 80, 78, or 38), and *G. hirticaulis* (2n = 80) (Newell and Hymowitz 1982; Tindale and Craven 1988). All species within the subgenus *Glycine* are perennial.

Members of the subgenus *Glycine* possess many agronomically favorable characteristics not readily found in the subgenus *Soja* (Hood and Allen 1987). These include resistance to various diseases (Kenworthy 1989; Vaughan and Hymowitz 1983; Lim and Hymowitz 1987; Burdon 1988), drought and salt tolerance (Kenworthy 1989; Hymowitz et al. 1987) tolerance to several herbicides (Hart et al. 1988, Kenworthy et al. 1989), and alternative photoperiodism response (Kenworthy et al. 1989). The ability to transfer these characteristics into the cultivated soybean gene pool would be a highly desirable step toward soybean germ plasm enhancement.

Through the use of in vitro embryo-rescue technology, hybrids between soybean and perennial *Glycine* accessions have been obtained (Newell and Hymowitz 1982; Broue et al. 1982; Singh and Hymowitz 1985; Newell et al. 1987). The hybrid plants produced from these crosses were highly sterile. However, extremely low seed set has been reported for colchicine-doubled *G. max* × *G. tomentella* hybrids (Newell et al. 1987; Hymowitz and Singh 1984). In one instance, nine F_2 plants were produced (Newell et al. 1987), and in the

* To whom correspondence should be addressed
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other, one F_2 plant was produced, which then produced four F_3 seed (Hymowitz and Singh 1984). In both instances, the F_2 plants retained the expected chromosome number indicative of their chromosome-doubled hybrid origin. Attempts to backcross these F_2 plants to soybean were not successful.

We report here the production of F_2 and F_3 seed from a colchicine-doubled hybrid of a cross between *G. max* and *G. tomentella*. This report is unique in that progeny of the hybrid demonstrate high levels of fertility, possess a chromosome complement similar to soybean (2n = 40), and exhibit isoenzmye patterns unique to that of the *G. max* parent or to the *G. tomentella* parent or that are altered, suggestive of altered transcription products or altered subunit binding affinities. These data suggest that the *G. tomentella* chromosome complement has been eliminated, but that genetic exchange occurred before elimination.

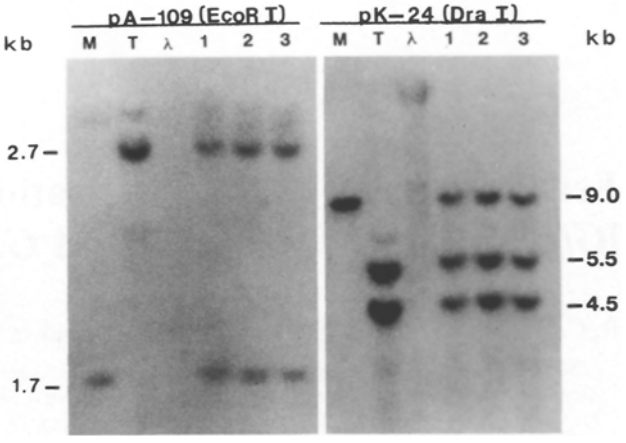

Fig. 1. Autoradiogram of EcoRI- and DraI-digested DNA probed with anonymus probes pA-109 and pK-24. M = DNA from *G. max* (PI 360841), T = DNA from *G. tomentella* (PI 378708). Numbers *1–3* denote DNA preparations from leaf material of F_1 hybrids of a cross between PI 360841 and PI 378708, including the F_1 that produced the fertile progeny. RFLP patterns clearly indicate the hybrid nature of the F_1s

Materials and methods

A hybrid plant of a cross between *G. max* (PI 360841) (2n = 40) and *G. tomentella* (PI 378708) (2n = 78) was produced at Monsanto Co., St. Louis/MO, during January 1984. This F_1 hybrid (2n = 59) was chromosome-doubled with colchicine and was identified as 15x – 2. Several (three) vegetative cuttings of this plant were transferred to Iowa State University, Ames/IA, during October 1987. These plants were maintained in the USDA-ARS greenhouse under varying temperatures and varying day lengths, supplemented with a combination of incandescent and sodium halide lighting. During October 1988, one of these plants gave rise to a branch that produced 41 pods and more than 100 F_2 seed.

Determinations of chromosome numbers were made from root tips of *G. max* and *G. tomentella* parents, and from F_2 and F_3 plants by using the root-tip squash technique of Palmer and Heer (1973). Chromosome counts were made from the 15x – 2 and individual F_2 and F_3 plants from six rooted petioles selected from various positions on each plant. Plants were classified for pollen fertility by staining with a solution of I_2KI. The recombinant DNA clones used as probes in this study were isolated from a PstI genomic library (Keim and Shoemaker 1988). DNA isolation, restriction endonuclease digestion, and subsequent hybridizations were carried out as described by Keim et al. (1989a). Isoenzyme analyses of cotyledon tissue were carried out according to the procedure of Cardy and Beversdorf (1984). Isoenzyme analysis of leaf tissue was carried out by using the extraction buffer of Wendel and Parks (1982).

Results

Restriction fragment length polymorphism (RFLP) analyses confirmed the hybrid identity of the F_1 plant (15x – 2) that produced the fertile progeny (Fig. 1). In addition, chromosome determinations from rooted petioles of 15x – 2 confirmed the expected (2n = 118) chromosome number from the colchicine-doubled hybrid of a cross between PI 360841 (*G. max*; 2n = 40) and PI 378708 (*G. tomentella*; 2n = 78). Chromosome determinations from root tips or rooted petioles indicated 40 chromosomes for all F_2 and F_3 plants tested (Table 1). Pollen viability estimates for F_2 and F_3 ranged from 60% to 98% (Table 1). Pubescence tip morphology and seed coat color of F_2 and F_3 plants are shown in Table 1. Several F_2 progeny exhibited abnormal developmental morphologies, including unifoliate abnormal developmental morphologies, including unifoliate leaves opposite trifoliates or difoliates opposite trifoliolates. Most F_2s possessed trifoliates in opposite arrangement. Only F_2 plant no. 12 possessed trifoliates in alternate arrangement. However, with the exception of the alternately arranged trifoliates observed on F_2 plant no. 12, these characteristics were not found heritable.

Isoenzyme analyses revealed several interesting heritable characters among the F_2 and F_3 progeny. With the exception of F_2 plant no. 12 (data not shown), the isoenzyme pattern of malate dehydrogenase (MDH) was similar to that of the *G. max* parent (Fig. 2); the two uppermost bands were absent in F_2 plant no. 12 and its F_3 progeny. However, without exception, the F_2 and F_3 progeny evaluated retained the diaphorase (DIA) isoenzyme patterns unique to the *G. max* parent (Fig. 3). Conversely, the F_2 and F_3 progeny retained the endopeptidase (ENP) isoenzyme pattern unique to the *G. tomentella* parent (Fig. 4). The isoenzyme patterns observed among F_2 and F_3 progeny for the phosphoglucomutase enzyme (PGM) resembled that of the *G. max* parent, with the exception of altered mobilities of two bands (Fig. 5). Altered mobilities also were observed among bands of the aconitase enzyme (ACO) for F_2 and F_3 progeny, with F_2 plant no. 12 exhibiting a pattern unique from all other

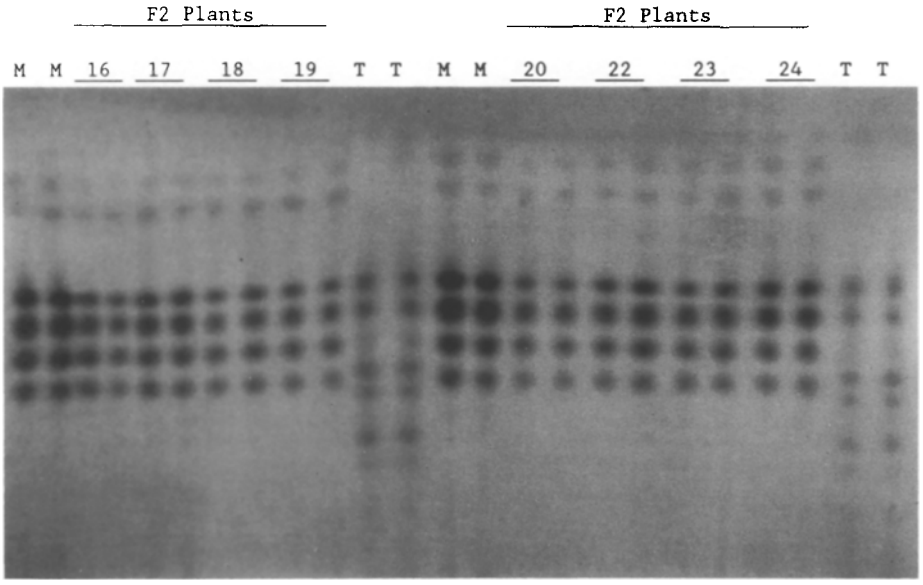

Fig. 2. Malate dehydrogenase isoenzyme patterns for *Glycine max* PI 360841, *Glycine tomatella* PI 378708, and several F_2 progeny of a cross between these two parental types. With the exception of F_2 no. 12, all F_2 and F_3 progeny evaluated possessed the isoenzyme pattern unique to the *G. max* parent. The two uppermost bands were absent in F_2 no. 12 (data not shown). M = *G. max*, T = *G. tomentella*

Table 1. Summation of characteristics observed among *G. max* (PI 360841), *G. tomentella* (PI 378708), an F_1 hybrid from a cross between these parents, and fertile F_2 progeny from this cross

	Chrom. no.	% Viable pollen	Pb[a]	SCC[b]	ACO[c]	ENP[d]	MDH[e]	PGI[f]	PGM[g]	DIA[h]
G. max	40	96	blunt	yellow	M[i]	M	M	M	M	M
G. tomentella	78	91	sharp	black	T[j]	T	T	T	T	T
F_1	118	0.4	sharp	N.D.	H[k]	H	H	H	H	H
F_2s	(22) 40	(14) 91–99 (6) 86–90 (1) 76 (1) 60	(23) blunt (1) sharp	black	(47) Va[1] (1) Vb	(48) T	(47) M (1) V	(48) M	(48) V	(48) M

[a] Pubescence morphology
[b] Seed coat color
[c] Aconitase
[d] Endopeptidase
[e] Malate dehydrogenase
[f] Phosphoglucoisomerase
[g] Phosphoglucomutase
[h] Diaphorase
[i] *G. max* isozyme pattern
[j] *G. tomentella* isozyme pattern
[k] Heterozygous isoenzyme pattern
[l] Variant isoenzyme pattern (different from M or T)
Numbers in parentheses are the numbers of F_2 plants possessing the characteristic listed

F_2 plants (Fig. 6). Mobility variants observed among several, but not all, F_2 plants for phosphoglucoisomerase (PGI) enzyme segregated among F_3 progeny (Fig. 7).

Discussion

The process of chromosome elimination can be devided into two types, developmental elimination or heterokaryon elimination (Orton and Tain 1977). The former is characterized by elimination of specific chromosomes during embryogenesis, and the latter arises after nuclear fusion of somatic cells (protoplasts) or of gametes.

Our data suggest that the *G. tomentella* genomes (2n = 78) were eliminated, thus leaving only the *G. max* chromosome complement (2n = 40). Chromosome elimination has been documented among progeny of crosses between *Fragaria moschata* and *Potentilla fruitcosa* (Macfarlane-Smith and Jones 1985). Preferential elimination of one parental genome has been shown to take place in intergeneric crosses between *Hordeum vulgare* and *Secale cereale* (Fedak 1977) and between *Triticum*

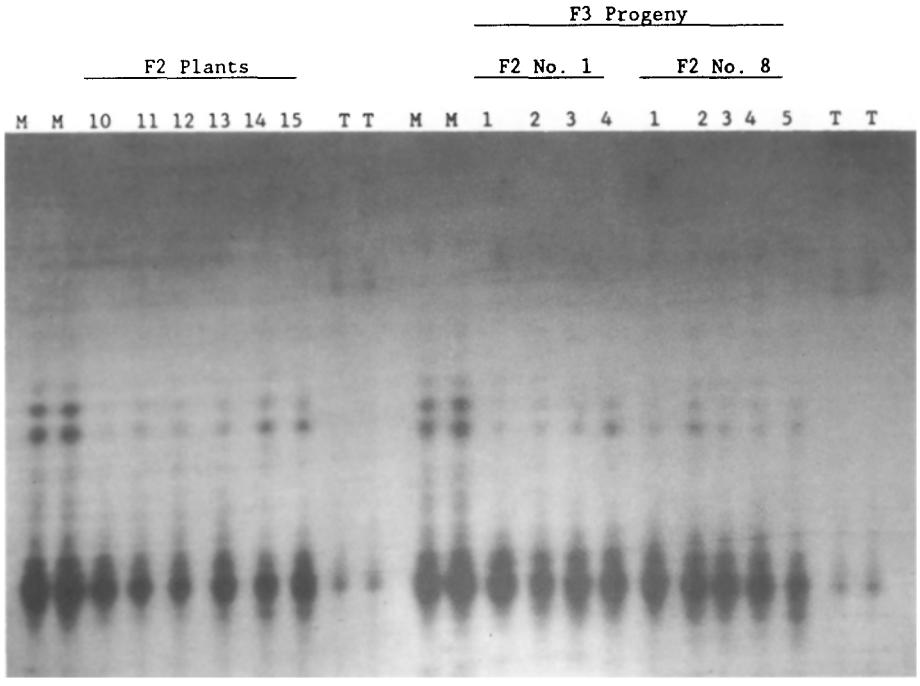

Fig. 3. Diaphorase isoenzyme patterns for *Glycine max* PI 360841, *Glycine tomentella* PI 378708, and several F_2 and F_3 progeny of a cross between these two parental types. Without exception, all F_2 and F_3 progeny evaluated possessed the isoenzyme pattern unique to the *G. max* parent. M = *G. max*, T = *G. tomentella*

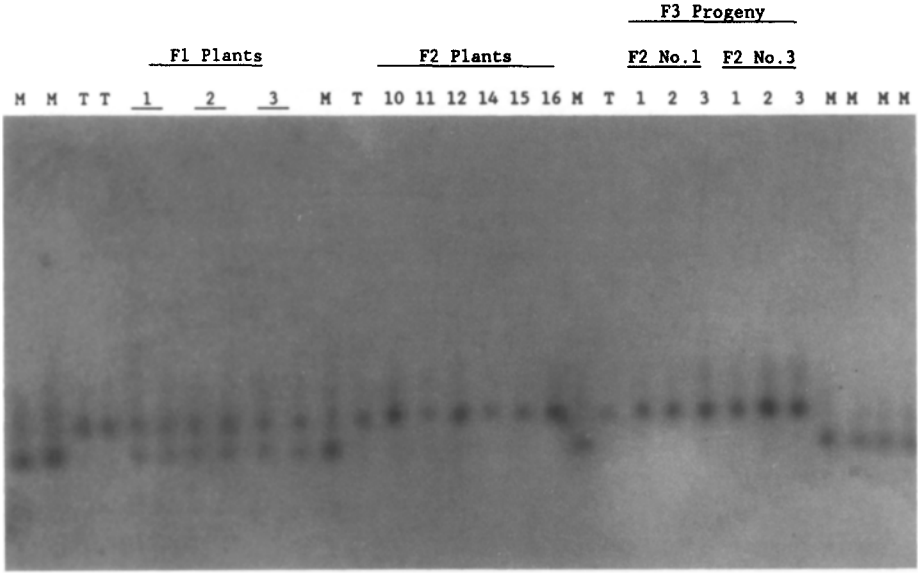

Fig. 4. Endopeptidase isoenzyme patterns for *Glycine max* PI 360841, *Glycine tomatells* PI 378708, F_1 hybrids, and several F_2 and F_3 progeny of a cross between these two parental types. Note the expected hybrid pattern in the F_1 s. Without exception, all F_2 and F_3 progeny evaluated possessed the isoenzyme pattern unique to *G. tomentella*. M = *G. max*, T = *G. tomentella*

aestivum and *Hordeum bulbosum* (Barclay 1975). Preferential elimination of one genome has been reported for interspecific crosses involving the genus *Hordeum* (Thomas and Pickering 1983; Thomas 1988; Subrahmanyam 1982) and the genus *Nicotiana* (Gupta and Gupta 1973).

The exact timing of elimination is unkown. However, because pollen on the F_1 hybrid plant is abnormally highly sterile, it is unlikely that a large number of successful fertilizations occurred, followed by developmental elimination of chromosomes during early embryo development. This scenario relies upon the concurrent occur-

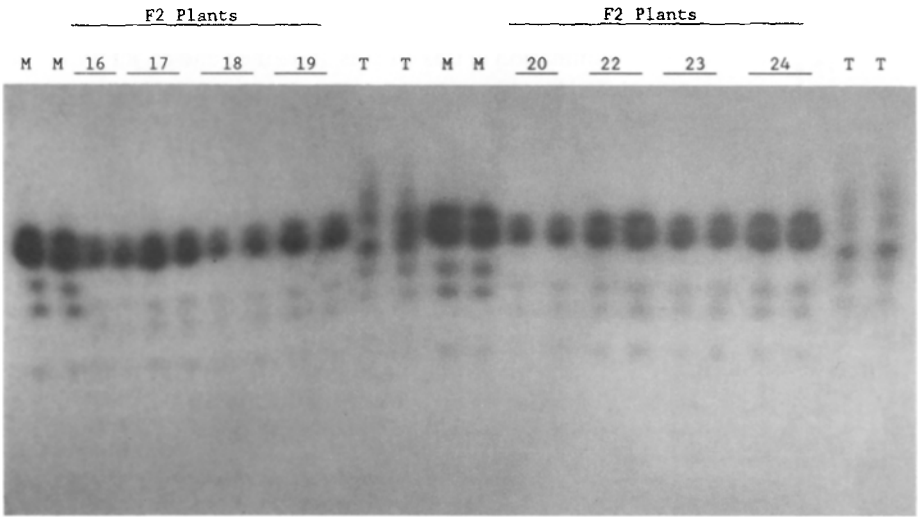

Fig. 5. Phosphoglucomutase isoenzyme patterns for *Glycine max* PI 360841, *Glycine tomentella* PI 378708, and several F_2 progeny of a cross between these two parental types. Without exception, all F_2 and F_3 progeny evaluated possessed isoenzyme patterns similar to that of the *G. max* parent, with the exception of altered mobilities of two bands. M = *G. max*, T = *G. tomentella*

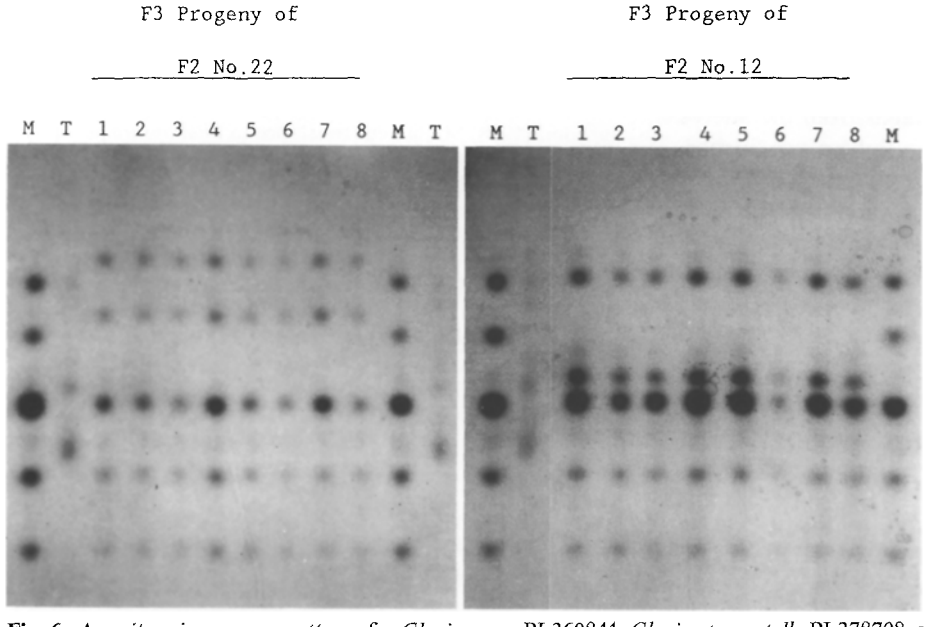

Fig. 6. Aconitase isoenzyme patterns for *Glycine max* PI 360841, *Glycine tomentella* PI 378708, and several F_3 progeny from F_2 no. 22 and F_2 no. 12. All F_2 and F_3 progeny evaluated possessed mobility variants different from either parental type. Most were identical to F_2 no. 22 and resembled the *G. max* pattern, with the exception of mobility shifts for two bands. F_2 no. 12 exhibited a unique pattern, also similar to the *G. max* pattern but with a single band exhibiting a mobility shift. M = *G. max*, T = *G. tomentella*

rence of multiple unlikely events. Similarly, given the Polygonum-type reproductive structure of the soybean, elimination during gametogenesis is also unlikely. More probably, chromosome elimination occurred somatically, thus giving rise to a 2n = 40 sector on the F_1 hybrid. However, chromosome counts from seven rooted petioles collected from the F_1 hybrid after pod harvest could not confirm this. All rooted petioles contained expected chromosome numbers indicative of a colchicine-doubled hybrid (2n = 118).

If the chromosome elimination occurred as a single event in a single meristematic cell ultimately giving rise to a portion of the germ line of the F_1 hybrid, then it could be expected that all F_2s derived from this germ line would be genetically similar. However, this was not observed. Altered isoenzyme mobilities were evident for F_2 plant

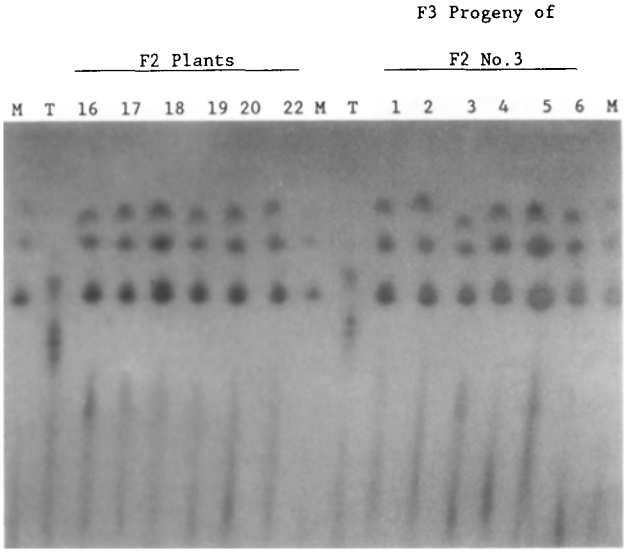

Fig. 7. Phosphoglucoisomerase isoenzyme patterns for *Glycine max* PI 360841, *G. tomentella* PI 378708, several F_2 progeny, and several F_3 progeny from F_2 no. 3. Note that segregation is occurring among F_3 progeny of F_3 no. 3. M = *G. max*, T = *G. tomentella*

no. 12 for ACO, which were dissimilar from the altered mobilities observed for all other F_2s. Pubescence tip morphology also was different for F_2 plant no. 12 than for any other F_2. Neither the ACO variants nor the pubescence tip morphologies segregated among F_3s. Some F_2s gave rise to segregating F_3 progeny for isoenzyme variants for PGI, wherase other F_2s possessing similar mobility variants did not segregate. These data suggest that the chromosome elimination and/or modifications may have occurred sequentially, thus giving rise to multiple products.

Analyses of isoenzymes patterns indicate that portions of the *G. max* genome have been retained, relatively unaltered, thus allowing the *G. max* phenotype to be expressed, e.g., regions coding for MDH and DIA (Fig. 2 and 3) and regions coding for blunt pubescence tip morphology (with the exception of F_2 plant no. 12) (Table 1). Also, it is evident that regions of the *G. tomentella* genome have been retained, relatively unaltered, thus allowing the *G. tomentella* phenotype to be expressed, e.g., regions coding for ENP (Fig. 4), sharp pubescence tip morphology (F_2 plant no. 12), and black seed coat (Table 1). Alterations in isoenzyme mobilities (e.g., PGM, ACO, and PGI) (Figs. 5–7) suggest that genetic recombination has occurred within regions coding for these enzymes or, alternatively, that genetic modifications have occurred, resulting in altered subunit binding affinities and, thus, altered mobilities.

The observation of fertile progeny of a cross between *G. max* subgenus *Soja* and *G. tomentella* subgenus *Glycine* resulting from a putative elimination of *G. tomentella* chromosomes is unique. The mechanism inducing the elimination in this cross is undefined and as yet unrepeated. However, experiments are under way to attempt to induce chromosome elimination in the hybrids. It is evident from these data that progeny of this hybridization have retained characteristics unique to each of the parental types. These progeny also possess characteristics unique from the parental types. It is not known yet if any of the agronomically important traits associated with *G. tomentella* also have been retained. The development of a saturated RFLP map for the soybean (Keim et al. 1989b) will make it possible to locate chromosomal regions containing *G. tomentella* DNA. This will facilitate the sequestering of desirable characters for adaptation to breeding populations.

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