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Originals

Conditional lethality involving a cytoplasmic mutant and chlorophyll-deficient malate dehydrogenase mutants in soybean*

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Summary. Conditional lethality in soybean, *Glycine max* (L.) Merr., occurred in F_2 plants when cytoplasmic-chlorophyll mutant Genetic Type T275 was the female parent and when either nuclear mutants T253 or T323 plants were the male parents. Mutant T253 [*Mdh1-n* (Urbana) *y20* (Urbana) *k2*] is missing two of three mitochondrial malate dehydrogenase isozymes [*Mdh1-n* (Urbana)] and has yellowish-green leaves [*y20* (Urbana)] and a tan-saddle pattern seed coat (*k2*). Mutant T323 [*Mdh1-n* (Ames 2) *y20* (Ames 2)] also is missing two of three mitochondrial malate dehydrogenase isozymes [*Mdh1-n* (Ames 2)] and has yellowish-green leaves [*y20* (Ames 2)], but has yellow seed coat (*K2*). Mutants T275, T253, and T323 are viable both in the field and glasshouse. The genotypes *cyt-Y2 Mdh1-n* (Urbana) *y20* (Urbana) *k2/Mdh1-n* (Urbana) *y20 (Urbana) k2 and cyt-Y2 Mdh1-n* (Ames 2) *y20* (Ames 2)/*Mdh1-n* (Ames 2) *y20* (Ames 2) are conditional lethals. These genotypes are lethal under field conditions, but plants survive in reduced light under shadecloth in the glasshouse. We do not know if their interaction with *cyt-Y2* is due to *Mdh1-n*, *y20*, or *Mdh1-n y20*. The reciprocal cross (*cyt-Y2* as male parent) gives viable genotypes. These conditional lethal genotypes should be useful for studies on the interaction between organelle and nuclear genomes.

Key words: Soybean – *Glycine max* – Malate dehydrogenase – Nuclear – organelle interaction

Introduction

Both nuclearly and cytoplasmically inherited chlorophyll-deficient mutants are known in soybean [*Glycine max* (L.) Merr.]. More than 36 single-gene recessive nuclear mutants and eight cytoplasmically inherited mutants have been characterized (Palmer and Kilen 1987; Cianzio and Palmer 1992). These mutants are maintained in the Soybean Genetic Type Collection and are identified with T-numbers.

Chlorophyll-deficient mutants are useful as marker genes in genetic studies and in physiology and biochemical research. The interaction of the plastid and mitochondrial genomes with the nuclear genome and subsequent gene expression is of major interest to biologists. In eukaryotes, plastids and mitochondria are the predominant carriers of extra-chromosomal genetic information (Grun 1976).

Palmer (1984) reported that soybean mutant T253 (*y20 k2*), which has chlorophyll-deficient foliage (*y20*) and a tan-saddle-pattern seed coat (*k2*), could not be separated genetically into its two phenotypes. The nuclear mutant phenotypes, chlorophyll deficiency and tan-saddle pattern, cosegregated and were inherited as a single-gene trait. The lack of recombinant genotypes indicated either that the loci were tightly linked or that there was a pleiotropic effect. It was suggested that the mutant phenotypes were the result of a small chromosomal deletion that would preclude recombination between the two loci (Palmer 1984). Soybean mutant T239 (*Y20 k2*), a near-isogenic line of T253, has normal green foliage (*Y20*) and a tan-saddle pattern seed coat (*k2*).

Cytoplasmically inherited mutant T275 (*cyt-Y2*) has yellowish leaves that become greenish yellow as the plant develops (Palmer and Mascia 1980). A condi-

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tionally lethal phenotype was observed in the F_2 when T275 (female parent) was crossed with T253 (male parent) (Palmer and Cianzio 1985). The genotype *cyt-Y2 y20 k2/y20 k2* was lethal under field conditions, but plants survived under reduced light in the glasshouse. The reciprocal cross, T253 × T275, gave the expected 3 *Y20 K2/—*: 1 *y20 k2/y20 k2* ratio in the F_2 in both the field and glasshouse. This interaction is unique to *cyt-Y2* and *y20 k2* because reciprocal crosses with T275 and T239 (*Y20 k2*) gave the expected segregations for plant color and seed-coat color in the F_2 (Palmer and Cianzio 1985). They showed that gametophytic lethality, either pollen or ovule, was not the explanation for failure to find the *y20 k2/y20 k2* genotype among F_2 plants of the cross T275 × T253. In addition, sporophytic lethality at the developing seed stage was not a plausible explanation (Palmer and Cianzio 1985).

Five nuclearly inherited chlorophyll deficient single-gene recessive mutants have been identified in soybeans to be missing two of three mitochondrial malate dehydrogenase (MDH) (E.C.1.1.1.37) bands. Three of these mutants, T323, T324, and T325, were isolated in an experiment designed to generate transposable element-induced mutations (Palmer et al. 1989). The fourth mutant (T317) was identified as a somaclonal variant in the Chinese cv 'Jilin 3' (PI 427.099) (Amberger et al. 1992). The fifth mutant (T253) was tested electrophoretically for malate dehydrogenase, and the banding pattern was identical with the pattern found for T317, T323, T324, and T325. Genetic tests with all five chlorophyll-deficient, MDH-null mutants indicated allelism (Hedges and Palmer 1992). The yellow-green plant phenotype and the MDH-null phenotype were co-inherited. That is, in segregating populations, no crossovers between plant color and the MDH phenotype were observed (Hedges and Palmer 1992).

Plants contain multiple molecular forms of malate dehydrogenase. NAD-dependent malate dehydrogenase is a key enzyme in metabolic pathways in the cytosol, mitochondria, glyoxysomes, and peroxisomes. These various isozymes are encoded in genes of the nucleus and synthesized on cytoplasmic ribosomes.

Our objective in the investigation presented here was to test for conditional lethality of T323 with T275. We tested for malate dehydrogenase in the seed and determined percentage survival of MDH-present versus MDH-null plants. Genetic type T323 is designated *Mdh1-n* (Ames 2) *y20* (Ames 2). As controls we used T239 (*Mdh1 Y20 k2*), T253 [*Mdh1-n* (Urbana) *y20* (Urbana) *k2*], and *cyt-G2* (green leaves, sibling of *cyt-Y2*).

Materials and methods

The genetic stocks used in these experiments are listed in Table 1. Hybrid seeds were obtained by following the procedure described by Walker et al. (1979). Cross-pollinations and progeny testing were done at the Bruner Farm, Ames, Iowa, and at the University of Puerto Rico-Iowa State University Soybean Nursery at Isabela, Puerto Rico. Reciprocal crosses were obtained for all genetic combinations. Control populations, crosses 1–6, and experimental populations, crosses 7–12, are given in Table 2.

Seeds of the parents and the F_2 and F_3 generations were analyzed for malate dehydrogenase by using starch gel electrophoresis (Cardy and Beversdorf 1984). A sample for electrophoretic analysis was taken from the cotyledons of 4-day-old seedlings. The seedlings were transplanted to peat pots. After several days of hardening-off in the glasshouse, the seedlings were transplanted to the field.

About 1000 F_2 seedlings from cross 8 (T275 × T253) and about 1000 seedlings from cross 9 (T275 × T323) were transplanted. Survival was about 97%. Forty F_2 plants from cross 8 and 30 F_2 plants from cross 9 were single-plant threshed. F_3 seeds from these F_2 plants were examined electrophoretically for malate dehydrogenase.

F_2 seeds from the other 10 cross combinations were hand planted directly into the soil in the field. A random sample of 100 F_2 plants from each of the 10 cross combinations was single-plant threshed, and F_3 seeds were tested for malate dehydrogenase. The remaining F_2 plants from the 10 cross combinations were not tested for malate dehydrogenase. They were classified only for plant color and seed-coat color because the data from the random sampling and previous results with T323 (Hedges and Palmer 1992) indicated no recombination between *Mdh1-n* and *y20*.

Backcrosses were made for cross number 8, F_1 × T253, and for cross number 9, F_1 × T323. Hybrid seeds of these two genetic combinations were germinated in a growth chamber, the seedlings then were sampled for malate dehydrogenase and subsequently transplanted to the glasshouse and classified for plant color and seed-coat color.

Table 1. Soybean genetic stocks used to test for conditional lethality

Genetic type	Gene symbol	Phenotype	Reference
T239	*k2*	Tan saddle on seed coat	Palmer 1984
T253[a]	*Mdh1-n* (Urbana) *y20* (Urbana) *k2*	MDH null, yellowish-green leaves (weak plant), tan-saddle on seed coat	Palmer 1984
	cyt-G2	Green leaves (normal sibling of T275)	Palmer and Mascia 1980
T275	*cyt-Y2*	Yellowish leaves, becoming greenish yellow	Palmer and Mascia 1980
T323	*Mdh1-n* (Ames 2) *y20* (Ames 2)	MDH null, yellowish-green leaves (reduced vigor)	Hedges and Palmer 1992

[a] Previous gene symbol for T253 was *y20 k2*

Table 2. Reciprocal cross-pollinations of nuclear and cytoplasmic soybean mutants

Control populations			Experimental populations		
Cross number	Female parent	Male parent	Cross number	Female parent	Male parent
1	*cyt-G2*	T239	7	T275	T239
2	*cty-G2*	T253	8	T275	T253
3	*cyt-G2*	T323	9	T275	T323
4	T239	*cyt-G2*	10	T239	T275
5	T253	*cyt-G2*	11	T253	T275
6	T323	*cyt-G2*	12	T323	T275

Photosynthetic photon flux density (PPFD) was determined both for field-grown plants and for glasshouse-grown plants during July and August, 1991. The average PPFD in the field from 9 different days (three replications per day) was 1585 μE $m^{-2}s^{-1}$, whereas the average PPFD in the glasshouse under the shadecloth from 9 different days (three replications per day) was 92 μE $m^{-2}s^{-1}$. All readings were taken between 1100 and 1300 hours.

Results

In the control populations, the F_2 data were compatible with the expected results. In reciprocal cross-pollinations of *cyt-G2 with* T239, T253, and T323, the genetic markers segregated in a 3:1 ratio (Table 3). In crosses 1 and 4, all F_2 plants had green leaves, MDH was present, and segregation was 3 *K2–*:1*k2 k2*. In crosses 2 and 5, all F_2 plants were either green leaves, yellow seed coat, and MDH present or yellow leaves, tan-saddle seed coat, and MDH null. For crosses 3 and 6, all F_2 plants were yellow seed coat, and segregation was 3 green leaves, MDH present: 1 yellow leaf, MDH null. In crosses 2, 3, 5, and 6, all F_3 seed tested from F_2 plants with yellow leaves were MDH null, as expected. For crosses 2, 3, 5, and 6, all F_3 seed tested from F_2 plants with green leaves gave families that were all MDH present or segregated 3 MDH present:1 MDH null. In these segregating F_2 families, the yellow F_3 seedlings were MDH null. F_2 family segregation was the expected 1 nonsegregating:2 segregating (data not presented).

In the experimental populations (Table 4), the F_2 plants from cross 7 were yellow leaves, MDH present, and, as expected, segregation was 3 *K2–*: 1 *k2 k2*. In the reciprocal cross (number 10, Table 5), all F_2 plants were green leaves, MDH was present, and segregation was 3 *K2–*:1 *k2 k2*.

Cross 8 was *cyt-Y2* × T253 and cross 9 was *cyt-Y2* × T323. All MDH-null F_2 plants died within 14–28 days after germination. A severe yellowing was noticed on the unifoliolates before the first trifoliolates were fully expanded. The F_2 family segregation for MDH in crosses 8 and 9 was the expected 1 nonsegregating:2 segregating (Table 6). Within segregating families, the F_3 seedlings segregated 3 MDH present:1 MDH null.

Table 3. Phenotype and number of F_2 plants from reciprocal cross-pollinations of nuclear and cytoplasmic soybean mutants: control populations

Cross number	Green leaves yellow seed coat MDH present	Green leaves tan-saddle seed coat MDH present	Yellow leaves and viable tan-saddle seed coat MDH null	Yellow leaves and viable yellow seed coat MDH null	χ^2 (3:1)	*P*
1	718	230	–	–	0.28	0.60
2	679	–	220	–	0.13	0.71
3	683	–	–	233	0.09	0.76
4	668	226	–	–	0.05	0.85
5	665	–	218	–	0.02	0.83
6	647	–	–	213	0.06	0.87

Table 4. Phenotype and number of F_2 plants from crosses of cytoplasmic soybean mutant T275 as female parent and nuclear mutants as male parents: experimental populations

Cross number	Viable[a] yellow seed coat MDH present	Viable[a] tan-saddle seed coat MDH present	Lethal[a] MDH null	χ^2 (3:1)	*P*
7	688	225	–	0.06	0.80
8	773	–	219	2.02	0.15
9	769	–	233	1.63	0.20

[a] All plants had yellow leaves

Table 5. Phenotype and number of F_2 plants from crosses of nuclear soybean mutants as female parents and cytoplasmic mutant T275 as male parent: experimental populations

Cross number	Green leaves yellow seed coat MDH present	Green leaves tan-saddle seed coat MDH present	Yellow leaves and viable tan-saddle seed coat MDH null	Yellow leaves and viable yellow seed coat MDH null	χ^2 (3:1)	P
10	683	233	–	–	0.09	0.76
11	691	–	219	–	0.42	0.52
12	681	–	–	217	0.33	0.56

Table 6. Number of F_2 families and F_3 seedlings from soybean cross number 8 (T275 × T253) and cross number 9 (T275 × T323) tested for malate dehydrogenase (MDH)

Cross number	Number of F_2 families				Number of F_3 seedlings in segregating F_2 families			
	True breeding MDH	Segregating MDH	χ^2 (1:2)	P	MDH present	MDH null	χ^2 (3:1)	P
8	12	28	0.20	0.65	209	71	0.02	0.89
9	8	22	0.60	0.44	168	52	0.22	0.64

In cross 11, segregation of green leaves:yellow leaves, and MDH present:MDH null were the expected 3:1. In cross 12, segregation of green leaves:yellow leaves, yellow seed coat:tan-saddle pattern seed coat, and MDH present:MDH null were the expected 3:1. All green plants were MDH present and all yellow plants were MDH null; there were no exceptions.

Backcrosses were made between (T275 × T253) × T253 and between (T275 × T323) × T323. The BC_1F_1 seed from the first cross gave 21MDH present:25 MDH null plants. All 25 MDH-null BC_1F_1 plants were kept in the glasshouse under shadecloth and produced BC_1F_2 seed. The BC_1F_1 seed from the second cross gave 16 MDH present:14 MDH null plants. All 14 MDH-null BC_1F_1 plants were under shadecloth in the glasshouse and produced BC_1F_2 seed. The 1:1 segregation of MDH present : MDH null in both backcrosses fits the expected ratio.

Discussion

Our understanding of nuclear/organelle interactions has been facilitated by the existence of variants in cytoplasmically inherited genes (Grun 1976). In eukaryotes, mitochondria and plastids are the predominant carriers of extrachromosomal genetic information.

Interactions between nuclear and organelle genomes form the basis for the use of cytoplasmic male sterility-restorer systems for the production of hybrid maize (Duvick 1965). Specific rearrangements in the mitochondrial DNA have led to three types of cytoplasmic male sterility (CMS) groups in maize (Hanson 1991). The maize CMS-S group also has chloroplast DNA that can be distinguished from chloroplast DNA of normal maize by restriction endonuclease analysis (Pring and Levings 1977). In Oenothera, disharmony resulting from incompatibility between nuclear and organelle genomes generated a broad range of plant color phenotypes from yellow to almost green (Stubbe 1964). Epigenetic changes induced by nuclear gene mutants, e.g., iojap in maize, occur, causing cytoplasmic chlorophyll-deficient phenotypes (Thompson et al. 1983).

In soybean, five nuclearly inherited chlorophyll-deficient mutants that are missing two of three mitochondrial malate dehydrogenase bands are known (Amberger et al. 1992; Hedges and Palmer 1992). Both phenotypes cosegregate and are inherited as single-gene recessives. Thus, both the chloroplasts and the mitochondria are affected. Eight cytoplasmically inherited chlorophyll mutants are known in soybean (Palmer and Kilen 1987; Cianzio and Palmer 1992).

Palmer and Cianzio (1985) concluded that a nuclear-cytoplasmic effect occurs between *cyt-Y2* (T275) and *y20-k2* (T253), but not between *cyt-Y2* and *Y20-k2* (T239). T253 and T239 are near-isogenic lines in the cv 'Harosoy' (Bernard 1991). The present results show unambiguously that a nuclear-cytoplasmic effect occurs between *cyt-Y2* and *Mdh1-n* (Ames 2) *y20* (Ames 2) (T323). Genetic type T253 [*Mdh1-n* (Urbana) *y20* (Urbana) *k2*] is also missing two of three mitochondrial malate dehydrogenase isozyme bands (Hedges and

Palmer 1992). They were not able genetically to obtain recombination between the *Mdh1* locus and the *Y20* locus. Genetic stocks of *Mdh1 y20 K2* or *Mdh1-n Y20 K2* are not available to test for a nuclear-cytoplasmic effect with *cyt-Y2*. Thus, it has not been resolved whether the nuclear-organelle interaction is due to *y20*, *Mdh1-n*, or *Mdh1-n y20* in combination with *cyt-Y2*.

Hedges and Palmer (1992) were also unable to determine whether the *Mdh1 and Y20* loci are two separate loci that are tightly linked or a single locus with pleiotropic gene action. Gottschalk (1968, 1976) considered four possibilities in his interpretations of pleiotropic gene action. His case 2, 'the effect is not caused by a gene mutation, but by a chromosome mutation, a minute deficiency involving a small group of genes' (Gottschalk 1976), may be the most plausible description to explain these results. Hedges and Palmer (1992) presented evidence that T323, T324, and T325, which were found in a transposon-containing soybean population, may be the products of deletions or of more complex rearrangements.

The ability of the *cyt-Y2 Mdh1-n* (Urbana) *y20* (Urbana) *k2*/*Mdh1-n* (Urbana) *y20* (Urbana) *k2* and *cyt-Y2 Mdh1-n* (Ames 2) *y20* (Ames 2)/*Mdh1-n* (Ames 2) *y20* (Ames 2) plants to survive and produce seed under some environmental conditions (glasshouse) means that seed of the conditional lethal genotypes can be produced. These conditionally lethal genotypes may be useful for studying the interaction between nuclear and organelle genomes.

In addition to *cyt-Y2*, cytoplasmically inherited, chlorophyll-deficient foliage mutants have been recently reported in soybean (Cianzio and Palmer 1992). Shoemaker and Palmer (1984) reported that, in crosses of T278 M (*cyt-Y3*) and T253, in the F_2 generation, the *cyt-Y3 y20 k2/y20 k2* genotype is lethal. Reciprocal cross-pollinations between the remaining five cytoplasmically inherited chlorophyll-deficient soybean mutants and T253 and T323 are planned.

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