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A new source of cytoplasmic male sterility in maize induced by the nuclear gene, *iojap*

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Summary. Cytoplasmic male sterility (cms) was found in plants derived from the F_2 progeny of fertile, normal cytoplasm plants of the inbred R181 pollinated with a genetic stock carrying the recessive nuclear gene, *iojap*. The male sterile plants were maintained by backcrossing with the inbred W182BN which maintains all known sources of cytoplasmic male sterility. The new male sterile progeny were found to exhibit stable male sterility under field conditions in two environments. However, they were partially fertile in the hot, dry summer of 1983 at Aurora, NY. It was found that these lines were restored by lines that characteristically restore cms S group cytoplasms. Pollen phenotype studies indicated that the restoration was gametophytic in nature, also characteristic of the cms S group. Agarose gel electrophoresis of undigested mitochondrial DNA (mtDNA) from these steriles indicated that these lines have the S-1 and S-2 episomes characteristic of the cms S group. Restriction endonuclease digest patterns of mtDNA from these sterile lines digested with BamH I indicated that these steriles fit into the CA subgroup of the cms S group. The new source of cms has been designated cms Ij-1.

Key words: *Iojap* – Restriction endonuclease analysis – *Zea mays* – Cytoplasmic male sterility

Introduction

Three major groups of cytoplasmic male sterility (cms) in maize have been identified on the basis of reactions to nuclear pollen fertility restoration genes (Duvick 1965; Beckett 1971; Gracen and Grogan 1974). These groups have been designated cms T (Texas), cms S (USDA), and cms C (Charrua). Over 80 sources of cms have been identified and placed into one of these three groups. The majority of these sources were identified originally through inbreeding or out-crossing in male sterile plants found in segregating populations (Duvick 1965). The only source derived by putting corn germplasm into a related species is the EP cytoplasm which is derived from *Euchlaena perennis* (*Zea perennis*). This cytoplasm does not fit into any of the 3 major types of cms. It is important to note that most of the sources of cms in the 3 major groups were detected in populations or crosses as nuclear restorer genes segregated. None of the sources appeared to arise as mutations in pure lines known to have normal, fertile cytoplasms (Duvick 1965).

A few of the sources originally classified into the 3 major groups of cms were derived from inbred backgrounds (Duvick 1965; Beckett 1971). Cms I came from a line carrying the gene *iojap* (Rhoades 1950) and cms S came from a genetic strain carrying the genes *iojap* and *teopod* (Buchert 1961; Jones et al. 1957). In the case of the cms S, Rhoades (1950) found varying degrees of pollen abortion in homozygous *iojap* plants. Crosses between normal, fertile green plants and homozygous *iojap* plants produced green, fertile progeny when *iojap* was used as a male. The reciprocal cross produced some stable male sterile individuals. Rhoades concluded that the *iojap* gene had induced cytoplasmic mutations in addition to induction of plastid mutations (Rhoades 1943; Walbot and Coe 1979; Thompson et al. 1983). The two events did not appear to be correlated. Duvick (1965) hypothesized that the *iojap* strain used by Rhoades had a sterile cytoplasm and was carrying nuclear restorer genes. The male sterility was observed as the restorer genes segregated in the cross between the *iojap* strain and a non-restoring inbred.

The purpose of this study was to describe and characterize a new source of cms that was identified in a single plant derived from the F_2 generation of a cross between the inbred R181 and a gene stock carrying the recessive allele *iojap*.

Materials and methods

Field studies

In 1979, a homozygous source of the recessive *iojap* gene was crossed as a male onto the corn inbreds SD10, Oh51A, CO107, R181, and W182BN. These inbred lines were chosen because they lack two or more of the major restorer genes for male sterile cytoplasms. The F_1 progeny were selfed and F_2 plants expressing the *iojap* phenotype were selected to be backcrossed as females to the original inbred parent. Any male steriles found were maintained with W182BN pollen due to its earliness and ability to maintain sterility. Male steriles were then observed in Aurora, NY (summers 1983 and 1984) and Homestead, FL (winter 1983–84 and 1984–85) for stability under field conditions. Fertility ratings were based on a 1–5 scale (Beckett 1971).

Pollen restoration phenotype studies

Crosses were made between the *iojap* cms lines and specific restorers for cms C (CO150), cms S (MS64-7), and cms T (RD4515 and NYD410), and the universal restorer inbred NY821LERF. F_1 progeny of these crosses were planted ear-to-row at Aurora, NY in the spring of 1984 and at Homstead, FL in the fall of 1983 and 1984. After the plants had been shedding pollen for several days, tassels were bagged in the morning. Five to six tassels per row were collected in bags in the evening and taken to the laboratory. Pollen was sprinkled onto a dry, glass slide stained with aceto-carmine, stirred with forceps, and a cover slip was applied. Pollen morphology was examined with a light microscope at 100X. Five hundred pollen grains were counted per slide and categorized as normal or aborted.

Isolation of mitochondria and mitochondrial DNA

Mitochondrial DNA's (mtDNA) were isolated from coleoptile and mesocotyl tissues of etiolated seedlings as described by McNay et al. (1983).

Restriction endonuclease fragment analysis

Mitochondrial DNA's were digested with the endonuclease BamH I for 2 h at 37C according to manufacturer's recommendations (Bethesda Research Laboratories, Inc.).

Horizontal slab gel electrophoresis was conducted on 0.8% agarose (IBI) using TPE buffer at room temperature for 18 h. Ethidium bromide (0.4 μg/ml) was added to gel and running buffer. The gels were placed over a 366 nm UV light source and photographed with a red glass filter. Polaroid type 55 film was used for photography of the gels.

Results

The crosses of *iojap* onto the various lines resulted in varying degrees of expression of the striped *iojap* phenotype. Male steriles were found in crosses between *iojap* and two different inbred backgrounds. The male steriles were obtained from crosses between the normal cytoplasm versions of the inbreds R181 and W182BN as females and an *iojap* line as the male. The male sterile derived from the W182BN background was tested and designated cms T. This line will be discussed in a separate paper when more complete characterization is available. The male sterile line from the R181 background was found in a single plant derived from an ijij F_2 plant of the cross (R181-N×ijij) pollinated with W182BN. The inbred W182BN is an early line

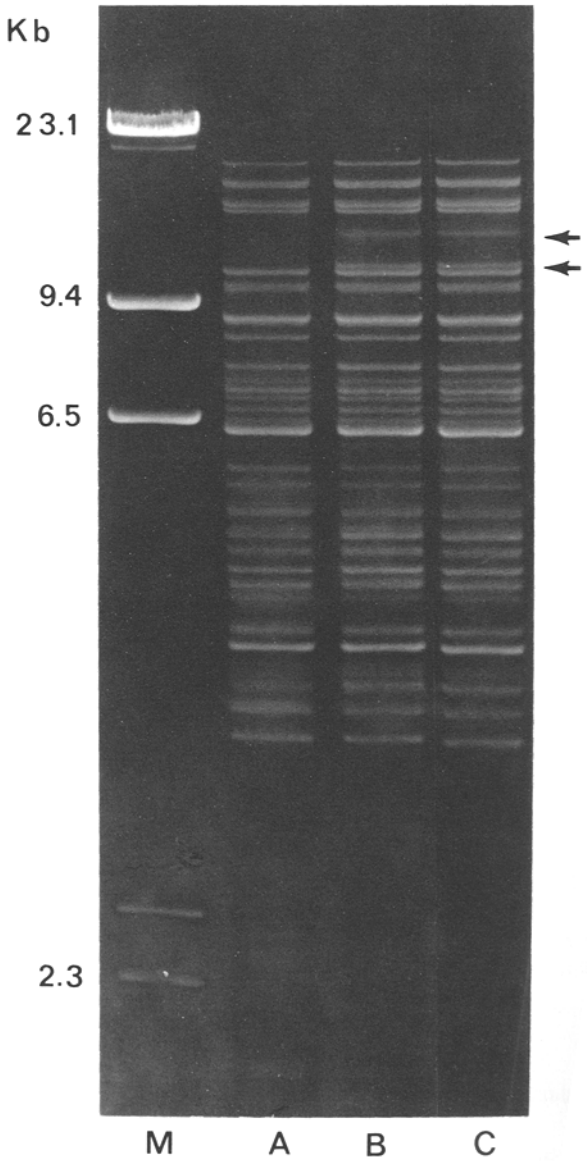

Fig. 1. Agarose gel electrophoretogram of BamH I digested cms S sterile cytoplasm maize mitochondrial DNAs. *M* Molecular weight markers were bacteriophage lambda DNA digested with Hind III (New England Biolabs). *A* W182BN-S, *B* W182BN-CA, *C* W182BN-Ij-1

Table 1. Fertility ratings of several backcross generations of sister line families of a new source of cms

Family	1983[a] Rating	1983–84 Rating	1984 Rating	1984–85 Rating
(R181×ijij)F_2×W182BN-1	1–3	1	1	1
(R181×ijij)F_2×W182BN-2	1–3	1	1	1
(R181×ijij)F_2×W182BN-3	1–3	1	1	1
(R181×ijij)F_2×W182BN-4	1–3	1	1	1
(R181×ijij)F_2×W182BN-5	1	1	1	1
(R181×ijij)F_2×W182BN-6	1–3	1	1	1

[a] The 1983 data involved 3rd backcross to W182BN. The 1983–84 rating involved b.c.-4; 1984 involved b.c.-5 and 1984–85 involved b.c.-6 families

Table 2. Fertility restoration reactions of several sister line families of a new source of cms crossed with different Rf lines

Family	×CO150			×MS64-7			×RD4515			×NYD410	×NY821LERF		
	1983–84[a]	1984[b]	1984–85[c]	1983–84	1984	1984–85	1983–84	1984	1984–85	1984–85	1983–84	1984	1984–85
(R181×ijij)F_2×W182BN-1	1	1	1–3	5	5	5	5	3	3	3	5	5	5
(R181×ijij)F_2×W182BN-2	1	1	–	5	5	5	–	3	3	3	–	–	–
(R181×ijij)F_2×W182BN-3	1	1	1	5	5	5	1–5	1–3	1–3	3	5	–	5
(R181×ijij)F_2×W182BN-4	1	1	1–3	5	5	5	1–5	3	1–3	3	5	5	5
(R181×ijij)F_2×W182BN-5	1	1	1–3	5	5	5	1–3	1–3	1–3	1–3	5	5	5
(R181×ijij)F_2×W182BN-6	1	1	1–3	5	5	5	1–5	3	3	3	5	5	–

[a] Data taken in Homestead, FL 1983–84 season
[b] Data taken in Aurora, NY during 1984 season
[c] Data taken in Homestead, FL during 1984–85 season

that maintains most known cytosteriles. Progeny of the new R181 male sterile line have maintained partial or full male sterility for 4 generations over 2 locations (Table 1).

The results of the crosses between a series of sister line male sterile families derived from the original R181 source of sterility and specific pollen restoration lines are shown in Table 2. The inbred MS64-7, which restores only cms S steriles (Gracen and Grogan 1974), restored the R181 sterile lines to fertility. RD4515 and NYD410, restorers for cms T, partially restored these lines. Restoration varied over seasons and between families. CO150, which carries the restorer genes for cms C, did not restore these steriles except in the 1984–85 season in Florida where these lines exhibited partial restoration.

Standard gel electrophoresis of undigested mtDNA revealed the presence of S-1 and S-2 plasmids characteristic of the cms S group of male steriles. Digestion patterns of BamH I indicated that the sterile from the R181 background is identical to the CA subgroup of cms S (Fig. 1). Two restriction fragments between 23.1 and 9.4 Kb are present in the CA subgroup and cms Ij-1, but not in the S subgroup. As expected, all families derived from the single male sterile plant had identical restriction patterns.

Discussion

A new "mutation" to cytoplasmic male sterility was obtained after crossing a source of the *iojap* gene onto the inbred, R181. This occurrence is similar to that reported by Rhoades in 1950 except that Rhoades was unable to obtain male steriles in crosses using *iojap* as a male (Rhoades 1950). Because of this, Duvick (1965) suggested that Rhoades' *iojap* source was actually a restored cytosterile and that after crossing with putative

non-restorer lines, male sterility was observed as *Rf* genes segregated. This would allow Rhoades' putative *iojap*-induced male sterile to be explained as having been recognized as a result of losing *Rf* genes, rather than as a direct result of mutation to cytosterility. Our recent data show that, at least, one *iojap* source does carry *Rf* genes for cms C and S. Almost all of the known sources of cms in maize have been identified after loss of *Rf* genes, and none have been identified as de novo mutations (Duvick 1965). However, the origin of the R181 cytosterile reported here appears to be via direct cytoplasmic mutation. The R181 inbred lacks all major *Rf* genes and is fully sterile in cms T, C and S backgrounds. Because of this, spontaneous mutations to any of the cms groups should be apparent in this background. No such mutants had been found in the normal cytoplasm version of this line in over 15 years of maintenance in Cornell's breeding nursery. However, after crossing an *iojap* line as a male onto R181 normal cytoplasm as female, a cytoplasmic male sterile plant was derived from the F_2 generation. This cms plant presumably arose via cytoplasmic mutation which occurred after the *iojap* gene was introduced to the R181 background. Some of these cms plants show the *iojap* phenotype while others do not. Since *iojap* was crossed only onto fertile R181 plants and not any cms types, the chances of mixed seed or some type of outcross being responsible for this variant are small. In order to explain both *iojap* phenotype and cms occurring in the same line, we would have to have had a spontaneous mutation to *iojap* occurring in a R181 cms type and simultaneously that mutant type being mixed into the segregating populations of the R181-N × *iojap* cross. The chances of that occurring are low. The more likely explanation is induction of both *iojap* chloroplast and mitochondrial mutations arising in the R181-N × *iojap* cross to give new cms plants with *iojap* phenotypes. The identification of additional cms plants in another non-restoring inbred background that follows a similar pattern to the R181 case, further supports the hypothesis that *iojap* is capable of inducing mutations to cms. The second case of *iojap* induced cms appears to be a cms T type. A full characterization of this mutant is underway and will be published later. Additionally, several recent crosses of *iojap* to other non-restoring backgrounds have given several additional putative cms mutants.

The data on the origin of the new cms source is consistent with a hypothesis that the *iojap* gene or perhaps another unknown but linked nuclear gene is capable of inducing cytoplasmic male sterility as well as a chloroplast mutation. The identification of S_1 and S_2 episomal bands in the new cms source suggests that an alteration in mtDNA, similar to that observed in standard cms S types, has occurred. This suggests that the *iojap* gene is capable of altering the structure of mitochondrial genomes.

A comparison of the new source of cms with standard cms types identifies that the new cms is a cms S type. In addition to the presence of S_1 and S_2 episomal bands, the BamH I digests of mtDNA gives a banding pattern identical to the CA subgroup of cms S. Interestingly, the original cms I and cms S sources, which are both from *iojap* as female lines show a different BamH I banding pattern, but all of these sources fit the cms S type banding pattern when digested with other enzymes.

The expression of male sterility of the new sterile over environments is similar to that of other cms S types. Several versions of the new cms source were ranked as fully sterile over 3 environments but showed some partial fertility in the hot, dry summer 1983 environment. All of the families derived from the new cms S source were fully restored by the inbred MS64-7 which carries the nuclear gene Rf_3 for cms S restoration but lacks *Rf* genes for cms T and C groups. In most environments the line CO150 (cms C *Rf* only) fails to restore the new cms type. However, partial restoration was seen in the Florida 1984–85 generation. The line RD4515 which fully restores cms T only, but which also shows some partial restoration of cms C & S, shows some partial restoration of the new source of cms. The line NYD410 which only restores cms T types also gave partial restoration of the new cms in Florida 1984–85 generation. It is not known whether the partial restoration shown by CO150 and NYD410 is totally due to environmental effects or not. The new cms source maintained by W182BN was fully sterile in the Florida 1984–85 generation. Therefore, basically the new cms follows a cms S pattern of fertility restoration, but some differences in response to CO150 and NYD410 were seen. Further investigation of these effects are in progress. The inbred NY821LERF which has all known *Rf* genes fully restores the new cms source.

The new source of cms follows the gametophytic pattern of pollen abortion typical of the cms S group. This means that fertility restoration follows the pollen grain's phenotype rather than that of the mother plant. In a plant heterozygous for the Rf_3 gene, only the dominant Rf_3 containing pollen grains are viable giving 50% plump, viable pollen grains in an anther (Table 3). Since aborted pollen grains are sometimes hard to detect, usually cms S, *Rfrf* plants give slightly higher than 50% normal appearing pollen. Several lines derived from the new source of cms had from 60–77% viable appearing pollen when crossed with MS64-7 (Rf_3Rf_3 genotype). This is similar to the value reported by Sisco et al. (1985) who found an average of 50–55% normal pollen in the cross W182BN-CA × MS64-7. The inbred NY821LERF has been shown to have 2 inde-

Table 3. Mean percentage normal appearing pollen grains in heterozygous restored plants of several families of the new cms source crossed with various Rf lines

Family	×MS64-7		×RD4515		×NYD410		×NY821LERF	
	1984[a]	1984–85[b]	1984	1984–85	1984	1984–85	1984	1984–85
1	76.93	60.53	54.80 ($n=1$)	–	–	–	82.22	81.32
2	74.97	64.48	61.15 ($n=4$)	52.08 ($n=1$)	–	–	–	–
3	61.40	63.20	–	–	–	–	–	81.30
4	65.02	64.70	57.43 ($n=3$)	–	–	–	91.67	82.05 ($n=4$)
5	62.23	63.36	–	–	–	–	81.97	90.36
6	72.27	72.30	–	61.18	–	–	88.40 ($n=1$)	–

[a] Data taken in Aurora, NY 1984, $n=6$ plants
[b] Data taken in Homestead, FL 1984–85, $n=5$ plants

pendent, dominant *Rf* alleles for cms S pollen restoration (Sisco 1982; Sisco et al. 1985). Therefore, this line should show 75% viable pollen in a RfrfRfrf heterozygote. Our crosses of lines derived from the new cms source with NY821LERF gave from 82 to 92% viable pollen. This is also in agreement with the 73–80% viable pollen reported in crosses of standard cms S types and NY821LERF (Sisco et al. 1985).

The data presented support the hypothesis that a new source of cytoplasmic male sterility was obtained after crossing the *iojap* gene into the R181 background. The exact mechanism of origin of the new cms is unknown, but it appears to have arisen de novo from the R181 × *iojap* F_2 generation. Characterization of fertility restoration reactions and mtDNA restriction digests show similarities between the new cms and members of the cms S group. We, therefore, propose to designate the new cms source as cms Ij-1 and place it in the cms S group.

References

Beckett JB (1971) Classification of male-sterile cytoplasms in maize (*Zea mays* L.). Crop Sci 11:724–727

Buchert JG (1981) The stage of the genome-plasmon interaction in the restoration of fertility to cytoplasmically pollen-sterile maize. Proc Natl Acad Sci USA 47:1426–1440

Duvick DN (1965) Cytoplasmic pollen sterility in corn. Adv Genet 13:1–56

Gracen VE, Grogan CO (1974) Diversity and suitability for hybrid production of different sources of cytoplasmic male sterility in maize. Agron J 66:654–657

Jones DF, Stinson HT Jr, Khoo U (1957) Pollen restoring genes. Conn Agric Exp Stn, New Haven, Bull 610

McNay JW, Pring DR, Lonsdale DM (1983) Polymorphism of mitochondrial DNA 'S' regions among normal cytoplasms of maize. Plant Mol Biol 2:177–187

Rhoades MM (1943) Genic induction of an inherited cytoplasmic difference. Proc Natl Acad Sci USA 29:327–329

Rhoades MM (1950) Gene induced mutation of a heritable cytoplasmic factor producing male sterility in maize. Proc Natl Acad Sci USA 36:634–635

Sisco PH (1982) Differences in fertility restoration and nucleic acids among S-type male sterile cytoplasms of maize (*Zea mays* L.). PhD Thesis, Cornell University

Sisco PH, Gracen VE, Everett HL, Earle ED, Pring DR, McNay JW, Levings CS III (1985) Fertility restoration and mitochondrial nucleic acids distinguish at least five subgroups among cms-S cytoplasms of maize (*Zea mays* L.). Theor Appl Genet 71:5–15

Thompson D, Walbot V, Coe EH Jr (1983) Plastid differentiation in iojap- and chloroplast mutator-affected maize plants. Am J Bot 70:940–950

Walbot V, Coe EH Jr (1979) Nuclear gene iojap conditions a programmed change to ribosome-less plastids in *Zea mays*. Proc Natl Acad Sci USA 76:2760–2764