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PROGRESS REPORT

GENETIC STUDIES ON CYTOPLASMIC MALE STERILITY IN MAIZE

CURRENT CONTRACT

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Introduction

Our research concerns the basic mechanisms of cytoplasmic male sterility (CMS) and fertility restoration in maize. Upon renewal of our DOE contract in April, 1990, we were advised to emphasize the genetic aspects of our studies and to leave the molecular aspects to our collaborators. As a result, the sequence of and emphasis on the different categories presented in our original proposal have shifted.

The molecular determination of CMS is in the DNA of the mitochondria (mtDNA) but specific nuclear restorer-of-fertility (Rf) genes can overrule the male-sterile effect of the cytoplasm. Our approach to the study of the Rf genes is threefold. We are attempting to tag the cms-S Rf genes and the cms-T Rf2 gene with controlling elements (CEs). Since we have identified a number of spontaneous Rf genes for cms-S and have demonstrated that they are themselves transposable, we are also searching for cases in which an Rf gene is inserted into a wild-type gene.

Seven of the independently occurring Rf genes are nonfunctional; they are pseudorestorers. When plants carrying nonfunctional Rf (Rf-nf) genes are crossed as male parents there is no seed set on the ears. Rf-nf genes have been found to be functional in hybrid backgrounds and the genetic nature of this hybrid vigor effect is under study.

Since the newly-arisen Rf genes, as well as the standard Rf3 gene, have been shown to be transposable we are continuing our efforts to characterize these transposition events. Genetic studies will indicate whether Rf elements transpose to a large number of different sites or whether there are preferred sites for insertion.

The other aspect of our research involves the nuclear control over the organization of the mitochondrial genome. We found that the changes in mtDNA organization upon cytoplasmic reversion to fertility were characteristic of the nuclear background in which the reversion event occurred. We have investigated whether these differences are a reflection of differences in the organization of the mtDNA genome before reversion. In order to carry out this study, we converted a number of normal and CMS sources to other nuclear backgrounds. The mtDNA organization of the progenitors and their derivatives, in the absence of reversion to fertility, has been examined.

We are continuing our collaborative arrangements with several other investigators.

Tagging of cms-S and cms-T Restorer Genes

As part of our genetic studies on nuclear restorer-of-fertility (Rf) genes, we are attempting to tag the cms-S Rf genes and the cms-T Rf2 gene with transposable controlling elements

(CES). In the cms-S system we are also searching for cases in which a transposable Rf gene is inserted into a wild-type gene. We began the studies with the cms-S Rf genes under the auspices of the Competitive Grants Section of the USDA Program on Plant Biology and Human Nutrition. The four cases identified in the USDA studies did not yield positive results. We are continuing the cms-S Rf studies under the auspices of DOE and are also proposing to tag the Rf2 gene of cms-T.

Tagging of cms-S restorer genes

In the summer of 1991 we identified five putative cases of insertion of a CE into the RfIV gene of cms-S. Two of the cases involve insertion of the Ds element from the al-m4 stocks and three involve insertion of Mu from the al-Mum2 stock. Since the source of the CE is the al locus in both types of case, the portion of chromosome 3L with which we are dealing can be generalized - RfIV -6.9 - lg2 - 34.9 - al(CE) - 0.25 - sh2. One case of each CE type, along with the appropriate controls, has been planted for analysis in our Hawaii nursery. The others were not sent due to a smaller seed supply. They will be analyzed in Illinois in the summer of 1992. Should we confirm the tagging of RfIV, the molecular analyses will be carried out by one of our collaborators.

Tagging of wild-type genes by transposable restorer genes

We have established in the M825 inbred nuclear background a nonrestoring visible chromosome marker strain for the long arm of chromosome 3 in which RfIV is located. This strain is homozygous rf al sh2 with normal cytoplasm. In the same inbred nuclear background, we have established a restoring train that is homozygous RfIV Al Sh2 in cms-S. We have increased the seed supply of the restoring strain to the point where we can have a small isolation plot to look for cases of insertion of RfIV into Al or Sh2. The cms-S RfIV Al Sh2 strain will be crossed as female parent by the nonrestoring al sh2 strain. Putative cases will be identified as rare al Sh2 or Al sh2 kernels on ears on which the remainder of the kernels are A Sh. Performing these crosses in an inbred nuclear background will assist us in ruling out contaminants. Both the Al gene and the Sh2 gene have been cloned so putative cases can be readily analyzed. This search will be carried out in the summer of 1992. We are handling the genetic component of these experiments; the molecular aspects of this work will be carried out by one of our collaborators.

Tagging of Rf2 in cms-T

Many studies on T male-sterile (cms-T) maize have been published. The mtDNA alteration responsible for the sterility has been identified, as has the protein coded for by this region. Two nuclear genes, Rf1 and Rf2, are required for cms-T.

restoration. The Rf1 gene reduces the amount of the cms-T-specific protein but no function is known for Rf2. A possible approach to the analysis of Rf2 is to tag the gene with a CE and determine its sequence. We have been employing a strain we obtained from Drew Schwartz for these studies. This strain carries an Ac element inserted at the Wx locus which is linked closely to Rf2 on chromosome 9; a Ds element is inserted at the Bz2 locus on chromosome 1. This strain is crossed as male parent onto cms-T R213, an rf2 tester. To date we have examined over 8800 plants from this cross and have identified 90 male-sterile individuals that represent putative insertions of Ac into the Rf2 restorer. The overall frequency of male-sterile plants is 1.0% but the data indicate that most of these result from relatively few premeiotic somatic events, occurring in the Ac-Rf2-carrying male parent. For example, 66 of the 90 male-sterile exceptions among a total of 2219 plants were progeny of 17 crosses involving a single male parent, 89-1833-9, in which a premeiotic mutation led to the production of 3% of pollen grains with the changed rf2 genotype. Two tillers of 89-1833-9 produced no male-sterile progeny among 2044 F1 plants, thus confirming the premeiotic status of the mutational event in plant 89-1833-9. All but one of the remaining 24 male-sterile exceptions can also be explained as the result of a similar premeiotic event in three other pollen parents, so that the frequency of the mutational or insertional event is more realistically given as 1 per 1800, rather than 1 per 100.

Theoretically, the male-sterile plants could result from mutation of, or insertion of Ac into, either Rf1 or Rf2; the premeiotic nature of the change, however, clearly indicates that it is Rf2, closely linked to Ac on chromosome 9, rather than Rf1 on chromosome 3, that has mutated, and this enhances the likelihood that the event is Ac-insertional. The molecular analysis of these male-sterile derivatives is being conducted by Gracia Zabala. She is using the 1.6kb Ac-unique probe to determine whether or not they represent tagged Rf2 alleles.

Genetic Studies on the Pseudorestorer Genes

Exceptional male-fertile plants that arise spontaneously in cms-S maize result from changes at either the nuclear or cytoplasmic level. Among the many spontaneous nuclear revertants that we have identified is a class we now refer to as pseudorestorer. A plant heterozygous for one of these pseudorestorer genes exhibits the phenotype expected of a restorer (Rf) gene. When ears of plants heterozygous for these pseudorestorer genes are crossed by their respective maintainers, the expected genetic behavior is observed. However, when these "fertile" plants are crossed as male parents there is no seed set on the ears. Because this class of "restorer" gene produces nonfunctional pollen, we have given it the symbol Rf-nf. To date, seven independently occurring spontaneous revertants, in four inbred line-cytoplasm combinations, have been identified as

Rf-nf genes. No cases of allelism of the Rf-nf genes have been found. These studies were reported at the Fourth International Workshop on Plant Mitochondria (abstract appended).

In the course of the studies on the allelic relationships between the Rf-nf genes, we found that crossing an Rf-nf plant by an unrelated inbred line yields F1 plants that produce functional pollen. This allowed us to determine, by self pollinating an F1 plant, whether the Rf-nf homozygote is lethal. We have determined that some of the Rf-nf genes are homozygous viable while most are homozygous lethal. The Rf-nf-carrying F1 plants are being successively self pollinated to determine whether the pollen viability of the F1 plants is due to hybrid vigor alone. If these strains retain restorer function as they approach homozygosity, it would suggest the presence of modifier genes that are selectively retained in the inbreeding procedure. Such strains have been continuously self pollinated for two to five generations and the Rf gene continues to function. These experiments are being carried forward.

To further analyze the Rf-nf genes in vigorous backgrounds we are studying them in combination with the inbred line maintainer of each of the other Rf-nf strains and with M825. The F1 plants are being successively crossed as male and as female parents with each of the two inbreds that constitute the F1. Since this procedure establishes four conversion strains for each F1 combination, a lot of field space is being devoted to this project. A subset of these crosses involves comparing the performance of an Rf-nf from an F1 plant when that same F1 plant is crossed both as male and as female. Are we selecting for a transposed or otherwise altered Rf when the F1 plant is used as a pollen parent? If so, the two backcross progenies should differ in their performance as pollen parents. These studies are underway.

Two alternative hypotheses to explain the characteristics of the Rf-nf strain were presented in the original proposal. A protocol has been developed that will differentiate between the two hypotheses. The "helper" experiment involves crossing a cms-S Rf-nf rf plant as female parent with a cms-S Rf rf pollen parent carrying an unlinked functional Rf in the same inbred background. Among the offspring the male-fertile plants carrying both the functional Rf and the Rf-nf genes exhibit 25% aborted pollen. These plants are analyzed to determine if the functional Rf can "help" the Rf-nf to transmit through the pollen. Experiments to date have given no cases in which pollen grains carrying an Rf-nf gene in the presence of a functional Rf gene have functioned. The control experiments involving two functional Rf genes are in progress.

The search for additional Rf genes has yielded several new cases, all of them functional. The search for new Rf-nf genes is continuing.

Transposition of Natural and Spontaneously Occurring Restorer Genes

We reported in the 1989 MGC News Letter (see abstract) Volume 63:120 that a number of restorers of cms-S that occur naturally in certain inbred lines of corn are located at different chromosomal sites. These data, along with data from further studies indicate the following:

- (1) The restorer genes (Rf) of inbred lines R177, LH38, CI21E, Ky21 and probably H95 are not at the same site in chromosome 2 as the standard restorer Rf(CE1) of inbred line CE1 but they are in chromosome 2 as indicated by Rf-wx recombination rates significantly lower than 50%. Moreover, these restorers themselves may be at different chromosomal sites. Rf(CE1) shows a control wx recombination rate of around 10% or lower.
- (2) The restorer genes of inbred lines A634, C123, C103, B14, B14A, Oh45 and Oh43 are also not at the same site as the standard restorer Rf(CE1), nor can they be at the same sites as any of the Rfs mentioned above since they all exhibit Rf-wx recombination rates not significantly different from 50%. They may or may not be in chromosome 2 and they are not necessarily at the same site.
- (3) Contingency tests of Rf-wx recombination rates of different sibling plants carrying the same Rf restorer indicate that at least several of these natural restorers are transposing. We are not doing a detailed study on transposability of these natural restorers since we are carrying out intensive studies of this character using the standard Rf-CE1 restorer (see below).

We are continuing studies on allelism of the natural restorers of cms-S, and to determine whether those restorers mentioned in (2) above are located in chromosome 2 or in another of its nine chromosomes.

The studies on transposability of Rf(CE1) are in their 4th cycle. Evidence for transposition of this natural restorer was abstracted in MGC News Letter 63:121 (see enclosure); these and continuing studies have been most fruitful confirming the transposable nature of this restorer and in identifying certain of its characteristics, as indicated below:

- (1) In the first cycle experiment, 5 of 33 tested Rf(CE1) plants exhibited a higher than normal Rf-wx recombination rate, indicating transposition. In later cycles the rate of transposition is much higher and recombination rates may go from high back to low as well as low to high. There is evidence that

the return from high to low rates reflects loss by recombination of the most distant Rf in a plant that carried two Rfs (see below).

(2) Rf-wx recombination rates to identify transposition events have been determined from the following kind of testcross: (S)rfrf wxwx X (S) (Rf(CE1) N Wx/rf T2-9d wx with kernels scored as Wx or wx on testcross ears. Since it can be argued that the occasionally high level of wx recombinants might be due to a reversion of (S) to (N) cytoplasmic state in the male parent, we made a detailed study of the fertility vs. sterility status, and of the pollen records, among fertile progeny from wx kernels on these ears. Only 42 (4.4%) of 963 plants in this study were male-sterile, indicating that the change from S to N cytoplasmic status, while it does occur, is not sufficient to explain the high rates of Rf-wx recombination that must otherwise be regarded as the result of transposition.

(3) In this same study, the analysis of pollen records of progeny from high vs. low Rf-wx recombination testcrosses supports the transposition interpretation. The ratio of Rf T wx to Rf N Wx progeny should increase correspondingly as Rf-wx recombination rates increase if transposition is involved. We observed a change in this ratio of 60:51 to 54:7 as Rf-wx recombination rates changed from 10% to 50%.

(4) As indicated in item (2) above, heterozygous plants testcrossed as indicated may give high or low Rf-wx recombination. These plants are routinely crossed as female parents by (N) rf T wx plants to produce heterozygotes for test in the next generation. It is expected that Wx progeny from plants that carry Rf in its control untransposed position close to Wx will give only control rates, 10% or less, of male-sterile offspring, while Wx progeny from plants carrying a transposed Rf will give higher rates of male-sterile progeny, the frequency of the latter increasing with the map distance between Wx and the site of the newly-transposed Rf. In such tests of Rf(CE1) heterozygotes, 13 plants with control-rate Rf-wx recombination gave 117 male-fertile and 6 (4.9%) male-sterile offspring. Surprisingly, none of the 7 plants with high-rate Rf-wx recombination gave a high rate of male-sterile offspring; there were 107 fertile and 3 (2.7%) male-sterile offspring. Similar results have been obtained in a study of RfIII and RfI heterozygotes. These data indicate that the transpositions involved here were conservative, or replicative, in nature, that is, a copy of Rf was transposed to a distant site, while the initial Rf remained at the original or nearby site. To our knowledge this is the first evidence of replicative transposition of controlling elements in maize; we plan to investigate this further.

We have accumulated extensive data on the transposition characteristics of the spontaneous restorers RfI, RfIII, RfIV and RfVI. To some extent our studies on the altered characteristics of transposed Rfs have been complicated by the finding noted immediately above, that at least some of the transpositions are

replicative in nature. A great deal of effort recently involves the crossing of transposed Rfs with appropriate gene marker strains, and subsequent testcrosses with such strains, to confirm, by standard procedures, the changed linkage sites of the transposed elements compared with controls. These studies too may be affected by the replicative nature of some transpositions, but we expect that they will nevertheless be successful. Large numbers of testcross progenies will be planted and scored in the greenhouse this winter, and many more testcrosses will be made in the Molokai nursery this winter. The chromosome markers being used in these studies are, for RfI in chromosome 8, virescent-16 and japonica-1, for RfIII and RfVI on chromosome 2, virescent-4 and Chocolate-1 in the long arm, and white-sheath-1, liguleless-1 and glossy-2 in the short arm, and for RfIV in chromosome 3, glossy-6 and liguleless-2 in the long arm, and dwarf-1 in the short arm. The most important of these studies involves RfIV whose control site in the long arm of chromosome 3 is 7 map units distal to liguleless-2.

Nuclear Control over Organization of the Mitochondrial Genome

We have continued our studies on the effect of changes in the nuclear background upon the mtDNA organization. We converted a number of normal and CMS sources to other nuclear backgrounds. The mtDNA organization of the converted material was then compared with the progenitor sources. We found that, in cms-S strains, there is mtDNA reorganization, in the absence of cytoplasmic reversion to fertility, when the nuclear background is changed. The mtDNA organization is under nuclear control. This mtDNA reorganization is not a general phenomenon. It is exhibited only in cms-S and even then not in all cases. It is not detected by all mtDNA gene probes and therefore involves only localized regions of the genome. The mtDNA regions exhibiting reorganization when the nuclear background is changed are the same ones exhibiting reorganization upon cytoplasmic reversion. These regions are those associated with repeats. The molecular aspects of these studies were part of the Ph.D. thesis work of Loida Escote and Carol Leja and were expanded by Gracia Zabala. This work was presented at the Fourth International Workshop on Plant Mitochondria (abstract appended). This work has now concluded and we anticipate publishing the results in the near future.

Collaboration

We are continuing the molecular studies begun in our laboratory in collaboration with Gracia Zabala. Gracia has been supported by our DOE grant in the past but as of August 1, 1991 has had a USDA grant of her own. Gracia has determined that all cytoplasmic revertants to fertility in cms-S, regardless of nuclear background, have rearrangements involving an mtDNA sequence homologous to the unique end of the R1 episome present

in some strains of maize with RU cytoplasm. This sequence is also present in the mtDNA of normal cytoplasm adjacent to one copy of the 5.27 kb repeat. This work was reported at the Fourth International Workshop on Plant Mitochondria (abstract attached). Gracia has analyzed the expression of this R-homologous sequence in the mitochondria of cytoplasmic revertants and their respective sterile progenitors. The transcriptional profile of this R region changes upon reversion in all revertants analyzed. In addition, the Rf3 gene affects expression of the R region. These results were presented at the 1991 Maize Genetics Conference. We now have three results implicating the R sequence of cms-S in the cms-S defect. The R region of the mtDNA undergoes rearrangement in all cytoplasmic revertants analyzed. The transcriptional profile of the R region changes upon reversion in all cytoplasmic revertants analyzed. The Rf3 nuclear restorer gene reduces and alters the transcriptional activity of the R region.

We are collaborating with Kathy Newton at the University of Missouri on the analyses of a number of nonchromosomal stripe (NCS) mutants. NCS mutations are due to mtDNA alterations. In the course of the work in our nurseries we occasionally come across NCS plants. We pollinate these mutants when possible and establish NCS strains. Three publications have resulted from this collaboration and a fourth is pending. We have identified additional NCS mutations and have supplied those to Kathy as well.

We have begun a collaboration with Christiane Fauron at the University of Utah on a comparative study of cms-T and cms-P. Our laboratory has identified an inbred line that restores cms-P but not cms-T. Christiane has determined that the cms-P mtDNA is essentially identical to the cms-T mtDNA. The only difference she detected is an XhoI site found in cms-T that is missing in cms-P. This XhoI site is also absent from normal mtDNA in the homologous region. The cms-P restorer gene has no effect on the mtDNA organization. We will supply Christiane with additional material in order that she might determine possible effects of the cms-P restorer gene on transcription of this region.

Continuation Request and Summary

We propose to continue ongoing research in three of the four major categories described in the PROGRESS REPORT. The studies on the nuclear control over the organization of the mitochondrial genome have, because of their genetic and molecular character, been discontinued in order to comply with the indication that we should not pursue molecular studies in our laboratory. We will, however, continue to supply the special strains we developed to other researchers; since our ideas are involved in the development of such strains we anticipate that their further studies at the molecular level will lead to collaborative publications.

The pathway of future research, including changes in emphasis, have been indicated in the PROGRESS REPORT. The prohibition on molecular studies associated with our previous renewal for the period 1990-1993 has forced us to make difficult choices, as a significant portion of our program, while strongly rooted in genetics studies, was oriented toward ultimate molecular analysis. For example, in the final analysis, evidence for the changed sites of transposed Rfs is best had through molecular analysis, in this case, routine RFLP studies to identify the locations of these elements. We have talked with Chris Chase at the University of Florida, Gainesville, regarding collaboration in such an effort and anticipate sending to her the necessary strains for a preliminary RFLP study after harvest of the Molokai nursery materials.

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