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A CYTOLOGICAL STUDY OF RADIATION INDUCED ALTERATIONS IN CYTOPLASMIC FACTORS CONTROLLING MALE STERILITY IN CORN

Progress Report

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Fertilization Studies

Electron microscopy of thin sections of gymnosperms have been interpreted as demonstrating that the embryo cytoplasm contains In: Biota orientalis only paternal mitochondria and plastids (Chesnoy, 1969); Pinus laricio only maternal mitochondria and plastids (Camefort, 1966); Larix decidua a mixture of maternal and paternal mitochondria and only paternal plastids (Camefort, 1969).

In angiosperms genetic studies indicate that in most species the embryo contains only maternal plastids, the source of mitochondria is unknown. In some species, however, cytoplasmic components of the embryo may have different origins. Biparental transmission of plastids have been demonstrated in Hypercium perforatum, H. acutum, Pelargonium zonale, in many species of Oenothera (Kirk and Tilney-Bassett, 1967), and in Secale cereale (Frost et al, 1970). In the absence of cytological markers the sources of mitochondria of the embryo are also unknown in these cases.

These cytological and genetic investigations suggest that we have much to learn about processes which plants have evolved to maintain cytoplasmic constituents at optimal levels. They also indicate that different plants have developed different solutions to the problem. Cytoplasmic inheritance is based on the behavior of the cytoplasm during fertilization and cell division. Little is known cytologically about this behavior.

At present we are concentrating cytological studies of the fertilization process on reciprocal crosses of normals with a maternally inherited white variegation in tobacco (von Wettstein and Eriksson, 1963; Nilsson-Tillgren and von Wettstein-Knowles, 1970). Here paternal plastids do not participate in zygote development; we are attempting to find out when paternal plastids, and possibly mitochondria, are eliminated.

Cytoplasmic Male Sterility in Corn

According to Buchert (1961) complete pollen abortion is induced by

interactions between the recessive nuclear genes rf_3rf_3 and sterility factors in S-type cytoplasm. Homozygous Rf_3Rf_3 S-cytoplasm plants produce about 100% viable pollen, while heterozygous Rf_3rf_3 plants produce about 50% vialbe pollen. However, this explanation does not account for the differences in reciprocal crosses described by Josephson and Jenkins (1948). The inbred 33-16 (S cytoplasm Rf_3Rf_3) when crossed by MO2RF pollen produces male sterile offspring while the reciprocal cross produces male fertile offspring.

We have observed that male sterile progenies are produced by (33-16 ♀ X MO2RF ♂) ♀ X MO2RF ♂, and about equal proportions of sterile and fertile plants are obtained from (33-16 ♀ X MO2RF ♂) ♀ X 33-16 ♂. Apparently MO2RF contains sterility genes dominant to Rf_3 and expressed only in S-cytoplasm. However, when other inbreds, such as Tx61M or M_p307, are used as pollinators on (33-16 X MO2RF) steriles the offspring are fertile. Here the existance of another type of fertility restorer gene(s) is indicated, genes which are dominant to the sterility inducing genes of MO2RF. Other S-type cytoplasms such as EK (Early King), PS (Pride of Saline) and ML (Moldavian) (Beckett, 1971) are also being studied for their reactions to MO2RF sterility genes.

Modification of Cytoplasmic Sterility Factors

Selection for resistance to Helminthosporium maydis race T infection in T-type cytoplasm corn has been unsuccessful. This failure suggests that sterility factors are either closely linked to cytoplasmic factors controlling susceptibility to H. maydis or that they are pleiotropic for sterility and susceptibility. Recent studies on reactions of different Triticum and Aegilops species to wheat rust (Puccinia recondita) infection indicate that cytoplasmic factors influence nuclear gene expression for resistance (Washington and Maan, 1974).

Selections from gamma irratiated T-type male sterile corn have produced male fertile progenies and progenies containing male sterile and fertile plants. Fertility restoring nuclear genes have been ruled out as controlling fertility

in these populations, since selections from completely fertile progenies outcrossed on T-type male steriles produce only sterile offspring. The irradiation treatment is assumed to have inactivated T-type cytoplasmic sterility factors, and selection is assumed to have produced lines lacking sterility factors. Normal fertile plants derived from gamma irradiated T-type corn are as resistant to H. maydis infection in the field as are plants in normal cytoplasm, and seedling roots of such plants are as little affected by H. maydis race T toxin as are seedling roots containing normal cytoplasm. Whether pleiotropy or linkage between sterility and susceptibility factors is involved here is unknown.

Progenies from irradiated T-type corn which contain sterile and fertile plants are being studied in the field for reaction to H. maydis infection and in the laboratory for reaction to H. maydis toxin.

Ethyl methane sulfonate and ethidium bromide have been applied to corn seed containing normal cytoplasm in attempts to induce sterility factors, and to seed containing T-type cytoplasm in attempts to alter the sterility and susceptibility factors. In the last growing season Fla F44T seed treated with ethidium bromide gave rise to populations which were male sterile with the exception of three fertile plants. These fertile plants were selfed and their offspring will be studied to determine if the alteration in fertility is controlled by genes or cytoplasmic factors and whether changes in reaction to H. maydis infection has occurred. Among Fla F44 normal cytoplasm plants, derived from seed treatment with ethidium bromide, one partially sterile and one sterile plant was obtained. The parially sterile and the sterile plants were crossed by normal F44 pollen. Progeny of these crosses will be studied to determine whether cytoplasmic sterility factors have been induced.

Different Sources of T Cytoplasm

Selection for sterile and partially sterile plants from June varieties

of corn (Golden, Mexican, Honey and White) is continuing. Selection is carried out on the assumption that some selections may possess cytoplasmic sterility factors unassociated with factors controlling susceptibility to H. maydis infection. Originally selections of sterile plants from June varieties gave rise to T-type cytoplasm inbreds (Rogers and Edwardson, 1952) which were used extensively in hybrid corn production. Selection for sterile, blight resistant plants is also continuing in Stinson's unstable T line.

Electron Microscopy of Cytoplasms in Corn

We have examined several male sterile corn cytoplasms in thin sections of apical growing points and young leaves. Normal mitochondria predominate in all cytoplasms examined. The SD, W, PS, RB, ME, R, H, I, VG, and ML sterile cytoplasms contained normal, circular and elongated mitochondria. The T cms cytoplasm contained normal and small circular mitochondria while the L cms contained normal and large ciruclar mitochondria. Fla F44 normal and D cms cytoplasms exhibited only normal mitochondria.

Circular mitochindria occur in (CMS X Fla F44) ♀ X Fla F44 BC5-6 ♂ and in Fla F44 ♀ X (CMS X Fla F44 BC5-6) ♂. The presence of circular mitochondria in progeny of this latter cross and their absence in Fla F44 suggests that abnormal mitochondria were introduced into Fla F44 normal cytoplasm through (CMS X Fla F44 BC5-6) pollen. If corn mitochondria are pollen transmitted then the circular mitochondria may be used as cytological markers to test the proposition that mitochondria are sites of sterility factors. These tests would involve crossing normal cytoplasm maintainers (only normal mitochondria) ♀ by (CMS X restorer) (normal and circular mitochondria) pollen. If circular mitochondria appear in the progeny of this type of cross, pollen transmission of mitochondria would be supported. If male sterility occurs in the S_1-S_n generations of this cross (maintainer) ♀ X (CMS X restorer) ♂ then pollen transmission of sterility factors would be indicated. An occurrence of circular mitochondria in sterile tassels or sectors thereof would associate

mitochondria with sterility factors.

Recently it has been found that in thin sections different normal cytoplasms possess different types of mitochondria. Inbreds Tr, C103, and F44 have only normal mitochondria, while K55, WF9, 38-11, and N6 possess normal and circular mitochondria. Additional inbreds will be examined to determine what types of mitochondria they contain. Maintainer inbreds containing only normal mitochondria will be used as females in crosses by (CMS X restorers) containing normal and abnormal mitochondria. These crosses and their progeny will be examined for abnormal mitochondria and male sterility.

Cytoplasmic Male Sterility in Petunia

The origin of cytoplasmic male sterility in Petunia is unknown. Duvick (1959) states that male sterility occurred in backcross progeny of species crosses when P. hybrida was the male parent in the initial corss, and the recurrent parent in backcrosses. However, the identity of the female parent remains unknown.

Reciprocal crosses between species of Petunia have been made. We have obtained selfs from some of the F_1s of interspecific crosses, and backcrosses by some parental species on the F_1s of interspecific crosses. We will use this material and further backcrosses and selfs to determine whether certain Petunia species contain cytoplasmic sterility factors masked by restorer genes, or whether sterility factors are induced by interactions of hybrid gemones with maternal cytoplasm as Burk (1960) found in interspecific Nicotiana crosses.

After determining which species contain cytoplasmic sterility factors, various graft combinations will be studied to find out whether fertility restorer genes infulence the graft-transmission of sterility and whether certain species contain graft-transmission substances which can induce sterility in normal cytoplasm.

While making interspecific crosses in Petunia we also attempted some

reciprocal intergeneric crosses of Petunia species with NN tobacco. We obtained seed from P. parodii X tobacco and from the reciprocal cross. None of the other intergeneric crosses produced seed. Pogliaga (1952) has reported the production of a 31 chromosome intergeneric hybrid from the cross N. tabacum var. Hybrid 217♀ X P. parodii ♂. He obtained 2 plants which survived to maturity and they were totally sterile.

Our F_1 seedlings resemble the maternal parents. We will obtain chromosome counts on our F_1 plants in order to establish whether the F_1s arose from seed containing a combination of petunia and tobacco chromosomes, or from parthenogenic seed. If we are not dealing with induced parthenogenesis, it should be possible, through treating branches of F_1 plants with colchicine, to produce amphidiploids. Amphidiploids could provide a bridge through which backcrossing with pollen of paternal species would produce combinations of Petunia genomes in tobacco cytoplasm, and tobacco genomes in Petunia cytoplasm. Such combinations would be most interesting in studying cytoplasmically inherited characters.

Non-Mendelian Variegation

Non-Mendelian variegation is of interest to us because: In some aspects it is similar to cytoplasmic male sterility; In some cases abnormal plastids used as cytological markers can provide information about the fertilization process; In species where mutant plastids are transmitted biparentally it should be possible to implicate or exclude plastids as sites of cytoplasmic sterility factors.

At present we are concentrating our studies on variegations in Petunia hybrida, P. axillaris and Nicotiana tabacum. We have determined that a variegation in P. hybrida is non-Mendelian and biparentally transmitted. This varigation usually occurs in small areas of leaves and is difficult to ead (gray-green on green). The variegation in P. axillaris should be easier to work with since it involves large and small yellowish areas of leaves.

Its mode of inheritance is being studied. We have three leaf variegations in tobacco: A non-Mendelian maternally inherited yellowish variegation (Edwardson, 1965); A non-Mendelian maternally inherited white variegation (von Wettstein and Erikson, 1963; Nilsson-Tillgren and von Wettstein Knowles, 1970); A white variegation originating in NN tobacco whose inheritance is being studied. This latter variegation usually covers large areas of the leaves and is easy to read. Reciprocal crosses between NN variegated and NN normal plants have produced some populations containing variegated and normal plants.

The frequencies of variegateds and normals in these populations could be explained by assuming: 1. Biparental transmission of non-Mendelian mutated plastids; 2. Dominant gene(s) controlling variegation which has a high but incomplete penetrance; 3. A seed-and pollen-transmitted virus induces the variegation. The assumption of biparental transmission of non-Mendelian mutant plastids will be acceptable if the dominant gene and virus infection assumptions can be eliminated. If a virus induces the variegation then grafts of variegateds and normals should eventually exhibit variegation on the normal component of the graft. We are in the process of examining these possibilities. Biparentally transmitted plastids in tobacco, and other species, will be used to determine whether plastids are sites of sterility factors.

Graft Transmission of Cytoplasmic Male Sterility

Cytoplasmic male sterility has been transmitted from male sterile stocks to maintainer scions in petunias (Frankel 1956, 1962; Edwardson and Corbett, 1961; Bianchi, 1967), beets (Curtis, 1967, Leonova, 1974), and possibly in sunflowers (Leclercq, 1971). Some of these results and the general absence of information on the loci of sterility factors has led Atanasoff (1964) to propose that virus transmission accounts for all cases of cytoplasmic inheritance. However, there is a considerable amount of experimentation on attempts to

asexually transmit male sterility which does not support Atanasoff's proposal (Edwardson, 1970).

There are a large number of plant viruses whose morphology is unknown, for which no vectors are known, and which are not mechanically transmissable with present methods (Martyn, 1968). These viruses are graft transmissable. The morphology of sterility factors is unknown (however, see attached manuscript ORO-2583-16), there are no known vectors of cytoplasmic sterility factors, and the factors have not been transmitted mechanically. However, there are marked differences in graft transmission of sterility factors and graft transmission of known viruses. Sterility is not expressed in the graft generation (even when the duration of the graft generation is prolonged for years, Frankel, 1962) and not all of the graft combinations produce progeny expressing sterility. Viruses when transmitted from diseased to healthy susceptible graft components induce symptoms in the graft generation. And if a graft union is established the virus is transmitted.

In view of reported failures to transmit sterility factors through some grafts and sussessful transmission in other grafts within the same group of test plants, we are repeating some graft transmission experiments. We have grafted maintainer scions on male sterile stocks in tobacco (90 grafts), Crotalaria mucronata (48 grafts) and sunflower (14 grafts). We have not examined any of the progeny of selfed flowers on maintainer tobacco scions for alterations in pollen fertility. We have examined pollen (using aceto-carmine as a stain) from 315 offspring arising from selfed flowers on maintainer scions of C. mucronata. 310 fertile plants contained vaible appearing pollen, 5 plants were classified as partially sterile. Previously (Edwardson, 1967) had examined pollen from the progeny of 10 grafts in C. mucronata and had observed only viable appearing pollen in all samples. In examining 43 offspring arising from selfed flowers on maintainer sunflower scions we observed 40 fertile and 3 partially sterile plants.

These results do not demonstrate that sterility factors have been transmitted across graft unions. However, completely pollen sterile plants in Petunia were not obtained until the second generation after the graft generation (Edwardson and Corbett, 1961). We plan to use the partially sterile Crotalaria and sunflower plants to produce progenies where selection for increased pollen abortion may lead to establishing complete male sterility. The response of such steriles to maintainers and restorers should indicate whether sterility factors had been graft transmitted.

Cytoplasmic Male Sterility in Vicia faba

Attached to this report is a copy of a manuscript (ORO-2583-16) accepted by Genetics, for publication "Cytoplasmic sterility factors in Vicia faba L." We believe that the cytoplasmic spherical bodies described in previous progress reports and in the attached manuscript are the sterility factors of Vicia faba. Bond et al (1966) reported their failure to graft transmit sterility from cytoplasmic male sterile stocks to normal scions of V. faba. However, this test should be repeated using a large number of grafts. Dr. D. A. Bond (Plant Breeding Inst. Cambridge, England) should be able to carry out the crucial tests involved in detecting pollen abortion in the sexual progeny of grafts. Since we have consistently failed to obtain seed from greenhouse and field plantings we will confine our tests to cytological examinations of apical growing points of normal scions grafted on cytoplasmic male sterile stocks. If the cytoplasmic spherical bodies are transmitted through the graft unions then we should be able to detect them in thin sections.

Blakeslee's Q-Virus

A reprint of our article "Relationships of Datura Quercina and tobacco steak viruses." 1974 Phytopathology 64:1322-1324 is attached to this progress report. In continuing cytological studies of Quercina-infected Datura stramonium ovules, virus aggregates have been observed in cells of

epidermal, nucellar, and integumentary tissues but so far they have not been detected in embryo sacs. Anthers from Quercian-infected plants also contain virus aggregates in epidermal cells. To date our studies of mature anthers from Q-infected plants indicate the "pollen" is retained in the locules. The "pollen" is encased in a thick layer of callose which I assume prevents the "pollen" from shedding (Fig.1). We have not observed deposition of pollen walls in virus infected material.

Pollen abortion in cytoplasmic male sterile Petunia (Frankel et al, 1969) and Sorghum (Warmke and Overman, 1972) has been attributed to abnormalities in deposition and degredation of callose in sterile anthers. In neither of these cases has a virus infection been associated with male sterility. We plan to examine anthers in various stages of development from Q-infected plants in order to trace development of callose layers around pollen mother cells, and to determine the effect of presistent callose on meiosis and microspore formation. In the course of these cytological studies we should be able to learn something about the distribution of virus particle aggregates in anther tissues.

Fig. 1. Cross section of Quercina-infected Datura stramonium anther locule. Note dark staining layer of callose around "pollen". Mag. 645X

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