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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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ABSTRACT

Cytoplasmic male sterile accessions, other than T-type, are being backcrossed to adapted maintainer and restorer inbred corn lines. Fertile selections from gamma-irradiated T-type corn continue to exhibit resistance to infection by race-T of Helminthosporium maydis in field and greenhouse tests. Cytological comparisons of these fertile selections and T-sterile, maintainer and restorer lines are continuing. Dominant male sterility and its suppressio[illegible] cytoplasm corn is being investigated. Induction of cytoplasmic male [illegible]ity in normal cytoplasm corn and suppression of susceptibility to H. maydis infection in T cytoplasm corn is being attempted with chemical mutagens. Consistent differences in cytoplasmic inclusions in sterile and maintainer Vicia faba have been observed. Consistant differences in mitochondria have been observed in cytological comparisons of normal and sterile corn. These abnormal mitochondria and non-Mendelian plastid abnormalities in corn, sorghum, tobacco, and petunia will be used in studying the fertilization process. Investigations of the properties of Datura Q-virus are near completion. Cytological and serological studies indicate the Q-virus is a strain of tobacco streak virus. Graft-transmission of cytoplasmic male sterility is being attempted in sunflower.

Cytoplasmic Male Sterility in Corn

Cytoplasmic male sterile accessions obtained from A. L. Hooker, D. N. Duvick and from Russia are being evaluated for male sterility and resistance to Helminthosporium maydis race-T infection. T-cytoplasm selections from Duvick appear to be as susceptible to H. maydis infection as our T-cytoplasm selections. With the exception of T-cytoplasm, the accessions are being backcrossed to adapted maintainer and restorer inbred lines.

S-type cytoplasmic male sterility is gametophytic; Rf_3rf_3 heterozygotes produce about 50% viable pollen, the Rf_3 gene is transmitted in the viable pollen (Buchert, 1961). The inbred 33-16 contains the S-type cytoplasm and is homozygous for the Rf_3 fertility restoring gene (Josephson, 1955; Duvick, 1965). Reciprocal crosses between 33-16 and MO2RF have produced male sterile populations when 33-16 was the female parent and male fertile populations when MO2RF was the female parent (Josephson and Jenkins, 1948; Josephson, 1955; 1971-72 progress reports). Progeny of crosses (33-16 X MO2RF) X MO2RF are all male sterile while progeny of crosses (33-16 X MO2RF) X 33-16 contain approximately equal members of fertile and male sterile plants. These results indicate that in S cytoplasm certain MC2RF genes act as dominant sterility inducers. The genetics of sterility induction and fertility restoration in S cytoplasm appears to be more complex than the current explanations for cytoplasm-gene interactions involving the rf_3 locus. Male sterility is induced in S cytoplasm by homozygous recessive genes, fertility is restored in the presence of homozygous or heterozygous restorer genes, however, sterility can also be induced by genes derived from MO2RF which are dominant to restorers derived from 33-16.

When (33-16 X MO2RF) steriles were crossed by Tx61M, Mp307, or Tx173D pollen, completely fertile populations were produced, indicating the existence of a different type of restorer gene. The restorers in 33-16 are recessive to the sterility inducing genes from MO2RF, but the restorers in Tx61M,Mp307, and Tx173D are dominant to the MO2RF sterility inducer genes. Other inbreds also contain restorers which are dominant to the dominant sterility inducer genes of MO2RF: Only fertile progenies were obtained in crosses of A132, Mp462, and Tx341 pollen on (33-16X MO2RF)XMO2RF)steriles. Elimination of the MO2RF dominant sterility inducing genes in pollen is suggested by the populations derived from the following sib and self pollinations:

Crosses and self pollinations	Fertile	Partially sterile	Sterile
(33-16 X MO2RF)sterileXTx61M)fertile sibs	1112	0	1
(33-16 X MO2RF)sterileXMp307)fertile sibs	1146	6	18
(33-16 X MO2RF)sterileXTx61M)fertile selfs	1010	4	7
(33-16 X MO2RF)sterileXMp307)fertile selfs	1143	20	37

Studies on cytology and genetics of fertility restoration in S cytoplasm corn are continuing.

Modification of Cytoplasmic Sterility Factors: Irradiation

Some fertile selections from gamma irradiated T cytoplasm corn produce progenies containing only male fertile plants whose reactions to race-T H. maydis infection are indistinguishable from corn with normal cytoplasm. Some male fertile selections from irradiated T cytoplasm corn continue to segregate fertile and sterile plants. Symptoms induced by H. maydis in the field vary from severe to slight in these segregating populations.

Progenies of male sterile plants in these populations are being studied for reactions to H. maydis infections with the objective of obtaining male sterile blight resistant plants.

Different Sources of T cytoplasm

Our selections of inbreds containing T cytoplasm and Duvick's T cytoplasm selections have been uniformly susceptible to race T H. maydis infection.

The T cytoplasm used in commercial hybrid corn production was originally obtained from selections of male sterile plants in June varieties (Rogers and Edwardson, 1952). June varieties are being reexamined for sterility on the assumption that they may possess cytoplasmic sterility factors unassociated with cytoplasmic factors controlling susceptibility to H. maydis infection. The Golden-, Honey-, Mexican-, and White-June varieties have produced, in addition to fertiles, some male sterile and partially sterile plants in Florida. Genetic studies indicate these varieties contain normal and T cytoplasms, restorer genes for T and S cytoplasms, and maintainer genes for T cytoplasm. June variety steriles and their progenies have been as susceptible to H. maydis infection as T sterile inbreds. Male sterile selections and their progenies derived from June varieties will be studied for differences in reaction to H. maydis infection.

A selection of T cytoplasm designated as unstable by H. T. Stinson (A158J) produced uniformly male sterile populations up to the 1972 growing season. Stinson's sterile J cytoplasm is not the same as the J cytoplasm obtained from Hooker. In 1972 some fertile plants (6.5%) occurred in predominantly male sterile populations. In 1973 A158J populations contained 3.9% fertile and 4.8% partially sterile plants.

Progeny of fertile, sterile, and partially sterile selections from these segregating A158J populations are being studied to determine whether cytoplasmic T sterility factors have been lost and whether the loss is accompanied by changes in response to H. maydis infection. The information obtained from fertile segregants of gamma irradiated T cytoplasm corn is interpreted to indicate a simultaneous loss of male sterility and blight susceptibility factors.

Chemical Mutagens

Inactivation of cytoplasmic sterility factors in T cytoplasm corn inbreds and induction of cytoplasmic sterility in inbreds containing normal cytoplasm is being attempted through seed treatments with NMG (N-methyl-N-nitro-N-nitrosoguanidine), NA (nalidixic acid), EB (ethidium bromide), and EMS (ethyl methane sulfonate). Since alterations in, or induction of cytoplasmic sterility factors might not be involved in the tassels of treated plants, but might be involved in the ears or sectors of the ears, the treated, S_1, and BC_1 generations are being examined for changes in fertility. In 1972 one partially male sterile and five tasselless plants were observed in inbred Fla F44 (normal cytoplasm, maintainer genes for T sterility) treated with EMS. These plants were crossed by pollen from untreated Fla F44. The offspring of these crosses were normal. Plants with unaltered phenotypes in all lines (Fla F6, Fla F6T, Fla F44, Fla F44T) were selfed, or crossed by untreated lines. In 1973 four male sterile plants occurred in the progeny of the cross (EB treated Fla F44 X untreated Fla F44). These plants were crossed by untreated Fla F44 and their offspring will be observed for transmission of sterility.

Cytology

That virus infections might explain some cases of cytoplasmically inherited pollen sterility was suggested by Kohler in 1928 (Michaelis, 1964). Graft-transmission of male sterility (Bianchi, 1963; Curtis, 1967; Edwardson and Corbett, 1961; Frankel, 1956, 1962; Leclercq, 1971) indicates the presence of infective sterility agents in petunia, sugar beet and sunflower. However, the nature of these agents has not been demonstrated.

Light and electron microscopy has been used in our cytological studies of monocot and dicot tissues. Cytoplasmic male sterile lines and their normal counterparts in several species have been compared for consistent differences in cytoplasmic components. Tissues of several species infected with mycoplasm, rickettsia, and viruses of different types have also been studied. With the exception of the cytoplasmic inclusions in Vicia faba we have not observed viruses or other pathogens in sterile or normal tissues which could not be accounted for on the basis of natural or controlled infections.

Light microscopy has been very useful in selecting virus infected tissues for electron microscope studies (among others Christie, 1967; Edwardson, Purcifull and Christie, 1968; Edwardson, Zettler, Christie and Evans, 1972). However, light microscopy has not been effective in selecting tissues infected with small spherical virus particles, cytoplasmic particels in Vicia faba and Tropaeolum majus, or tissues containing cytoplasmic sterility factors for electron microscope studies.

Cytoplasmic particles in Vicia faba

In previous reports we have described the large cytoplasmic spherical particles in cytoplasmic male sterile Vicia faba. When male sterile and maintainer seedlings are separated in isolation cages the particles occur

only in male sterile tissues. In 1973 we grafted maintainer scions on male sterile stocks (10 surviving grafts) and obtained 2 seed from one of the maintainer scions. Whether scarification of stigmatic surfaces (Toynbee-Clarke, 1971) had anything to do with obtaining these seed is unknown, in any case we had not previously been able to produce any mature seed. These seed have produced plants which have been examined cytologically; we have observed no spherical bodies. Samples of pollen from the 10 graft combinations and from untreated maintainer plants germinated on artifical medium (Pfahler, 1967). Pollen from the plants derived from selfing the maintainer scion will be applied to this medium and compared with pollen from normal plants to determine whether the graft offspring exhibit reduced pollen fertility. A reduction in pollen viability in the graft offspring and the absence of the spherical particles in further cytological studies would indicate that the particles are not related to sterility.

Tropaeolum majus contains cytoplasmic particles similar in appearance to those in male sterile Vicia faba. In the varieties we have examined the T. majus particles occur in male fertile plants.

Electron microscopy of cytoplasms in corn

In last year's report various types of abnormal appearing mitochondria in corn apical meristems were described. These elongated and circular mitochondria occurred along with normal appearing mitochondria in ten different sterile cytoplasms. Normal cytoplasms in lines thus far examined contain neither circular nor elongated mitochondria. The morphology of abnormal appearing mitochondria is apparently unaltered in the presence of fertility restoring genes. The fertility restored versions of these cytoplasms have been used as pollinators in crosses on an inbred carrying normal cytoplasm. Samples from progeny of these crosses will be

examined cytologically. If abnormal mitochondria are found in this material paternal transmission of mitochondria and non-involvement of mitochondria with sterility factors would be indicated. The absence of abnormal mitochondria would indicate that mitochondria are not paternally transmitted or are inactivated in early zygote development. This assumption would be permissible in cases where the paternal (sterile type) and maternal (normal type) cytoplasms were associated with closely similar nuclear genes, in order to rule out nuclear gene influence on mitochondrial shape. Several of the sterile type cytoplasms have been backcrossed 4-5 times by restorer inbreds. Therefore, in comparing the progeny of (normal cytoplasm-normal mitochondria, RfRf) X (CMS cytoplasm-abnormal mitochondria, RfRf) pollen the influence of nuclear genes on mitochondrial shape is expected to be equalized.

The cytoplasm during fertilization

I do not know of any information on the participation of paternal mitochondria or lack of it in formation of angiosperm zygotes. Genetic studies indicate that in most cases paternal plastids do not contribute to the formation of the zygote. Wagner's (1942) studies on non-Mendelian plastid variegation in rye may be an exception in the Gramineae.

The abnormal mitochondria, discussed in last year's report and in the preceeding section, should be useful in cytological studies of the fate of paternal mitochondria during fertilization and zygote formation.

We are propagating non-Mendelian plastid abnormalities in iojap corn (Rhoades, 1943), sorghum (Karper, 1934), petunia (Edwardson, unpublished), and tobacco (Edwardson, 1965). Our cytological studies of this material involve: 1) Determining whether the abnormal mature chloroplasts develope from abnormal appearing proplastids (Shumway and Weier, 1967 reported young

plastids from white areas if iojap corn leaves contained prominent fibrils and lacked ribosomes, however, their study did not include proplastids); 2) Determining whether abnormal appearing proplastids occur in young zygotes, generative cells and egg cells. Detection of abnormal proplastids in apical growing points and young leaves should be a realtively easy task. However, sectoring of normal and abnormal proplastids is a major problem when germinating pollen, egg cells and young zygotes are examined. Aside from technical difficulties in collection, fixation, and embedding germination pollen, egg cells and young zygotes, these tissues may contain only normal plastids although they are obtained from variegated plants; or the germinating pollen and egg cells may contain a mixture or normal and abnormal proplastids which would make it difficult to interpret the fate of paternal plastids in young zygotes. However, with some luck, I think this problem can be avoided. Some striped plants have been obtained in greenhouse plantings of sorghum and in field plantings of corn. We have not observed striping in sorghum panicles, or corn tassels. The stripes occurring on corn ear shoots have been in our judgement too narrow to be useful. However, Karper (1934) states that in some variegated sorghum plants the entire panicle contains only chlorophyll deficient tissue and the progeny from these panicles are chlorophyll deficient. Rhoades (1943) states that progenies of (striped X normal corn) occassionally consist of only white seedlings, which he assumes were derived from ears containing only abnormal plastids. We will increase the size of corn and sorghum field plantings in order to increase the probability of obtaining some plants in which inflorescences or large sectors of inflorescences, possess abnormal plastids. These inflorescences will be reciprocally crossed with normals to obtain material for cytological studies of the fate of paternal plastids.

Progenies of variegated tobacco contain variegated and normal plants (Edwardson, 1965). We are tissue culturing portions of stem and petiole from yellow-green sectors of variegated plants. Callus has been obtained and plantlets have differentiated from the callus. When the plantlets reach sufficient size they will be used for scions in grafts on normal tobacco. Viable grafts should produce some completely yellow-green inflorescences which will be used in reciprocal crosses with normals to provide material for cytological studies of the fertilization process. We will use the same procedures with petunia tissues exhibiting non-Mendelian plastid inheritance.

Blakeslee's Q-virus

Datura stramonium plants infected with Blakeslee's (1921a,b) Q-virus have exhibited many attributes of cytoplasmic male sterility: Pollen abortion, female fertility, non-transmission of sterility through pollen (under our conditions), high rate of seed transmission (95-99% in several lines), not mechanically transmitted (prior to our studies) graft transmission, and no known vectors. In previous reports we have described mechanical transmission, increased seed transmission, and cytology of Q-virus particles in thin sections. Fulton's (1967, 1971) and Brunt's (1968) descriptions indicate that tobacco streak virus exhibts many characteristics of the Q-virus. Tobacco streak virus in thin sections of tobacco and D. stramonium (Fig. 1-5) is similar to the Q-virus in tobacco and D. stramonium (previous progress reports). Costa and Carvalho (1961) have suggested searching for tobacco streak strains which could be used to induce male sterility in tomato.

To study the serological relationship of these two viruses (D. E. Purcifull and J. R. Edwardson), a culture of tobacco streak virus

(Strain HF) and its corresponding antiserum were obtained from the American Type Culture Collection. Immunodiffusion tests with crude extracts from virus infected tobacco showed that antigens of the two viruses gave reactions of identity (Fig. 6). On the basis of their close serological relationship as well as their similar biological properties, it is concluded that the Q-virus is a strain of tobacco streak virus.

Graft Transmission of Sterility

Cytoplasmic male sterility has been described in sunflower by Leclercq (1969). Preliminary tests suggest that sterility in sunflower is graft-transmitted (Leclercq, 1971). We have reciprocally grafted male sterile and maintainer lines of sunflower; no alterations in fertility have been observed in the graft generation. Progeny of the grafts will be examined for occurrence of male sterility. Cytological comparisons of sterile, maintainer, and restorer sunflower leaf and growing tip tissues have thus far shown no consistent differences in cytoplasmic constituents.

During the period Feburary 28, 1973-December 1, 1973, I have devoted approximately half of my time to this project. I plan to devote about half of my time to this project during the remainder of the current term, December 2, 1973-February 28, 1974.

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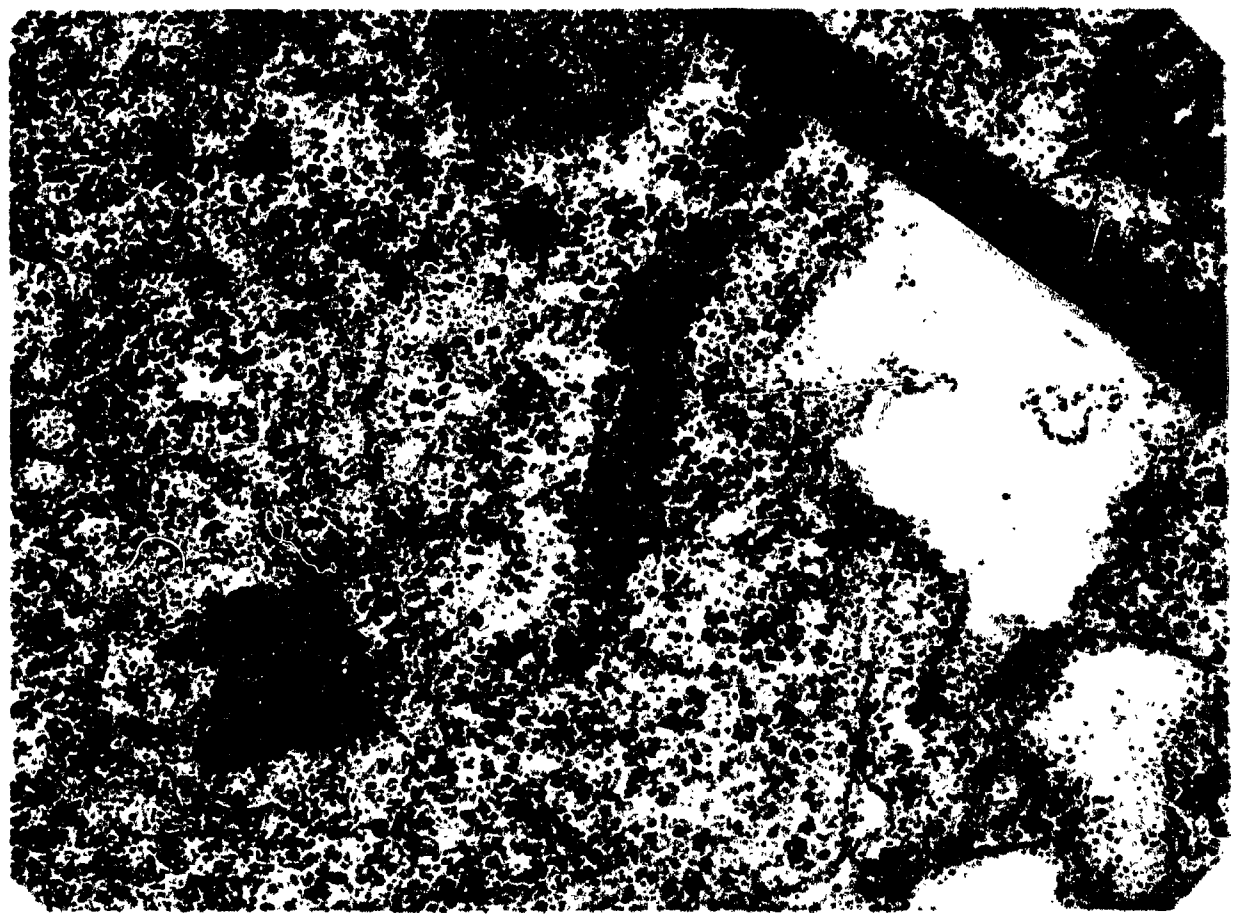

Figure 1. Tobacco streak virus particles aggregated in the cytoplasm of an epidermal cell of tobacco growing tip.

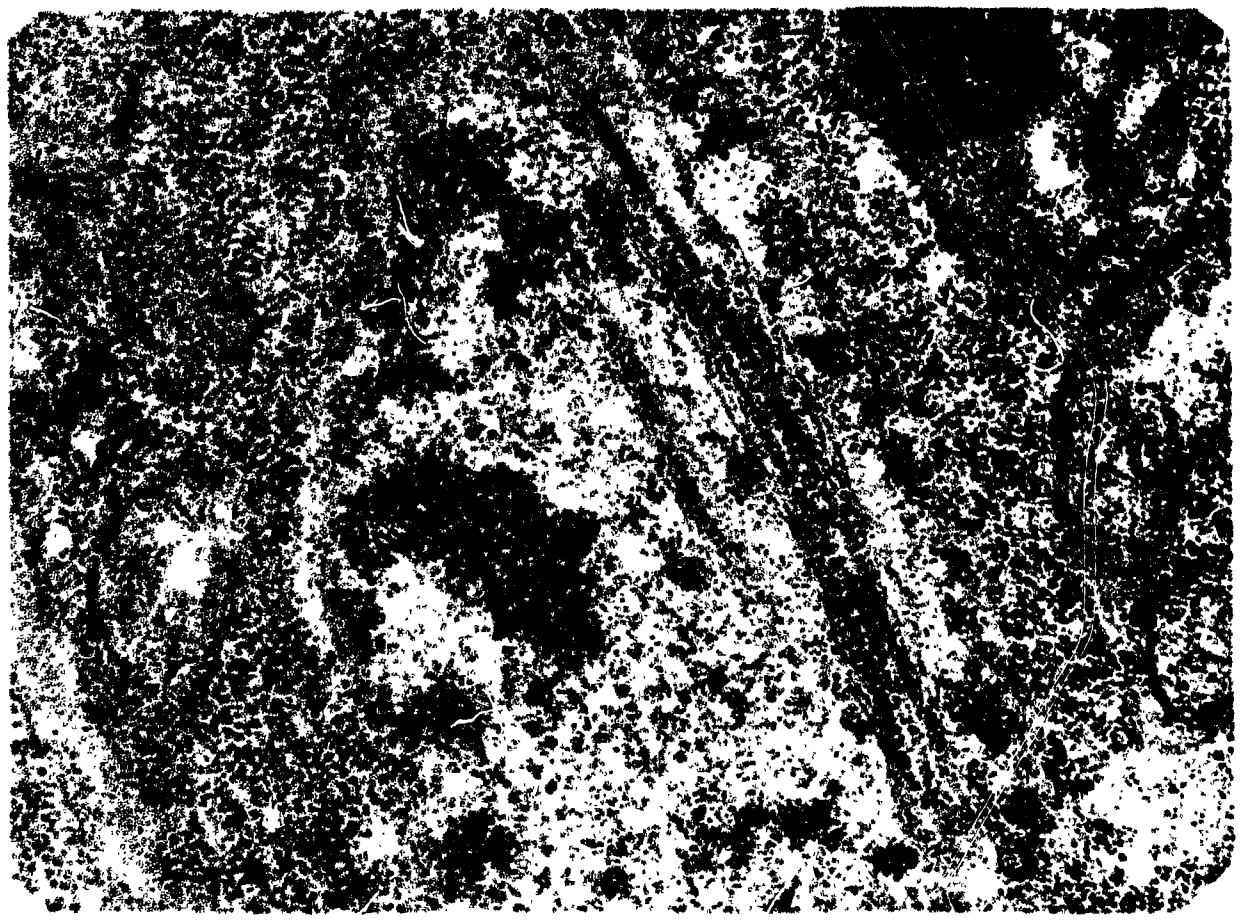

Figure 2. Tobacco streak virus particles aggregated in nucleus of young tobacco leaf epidermal cell.

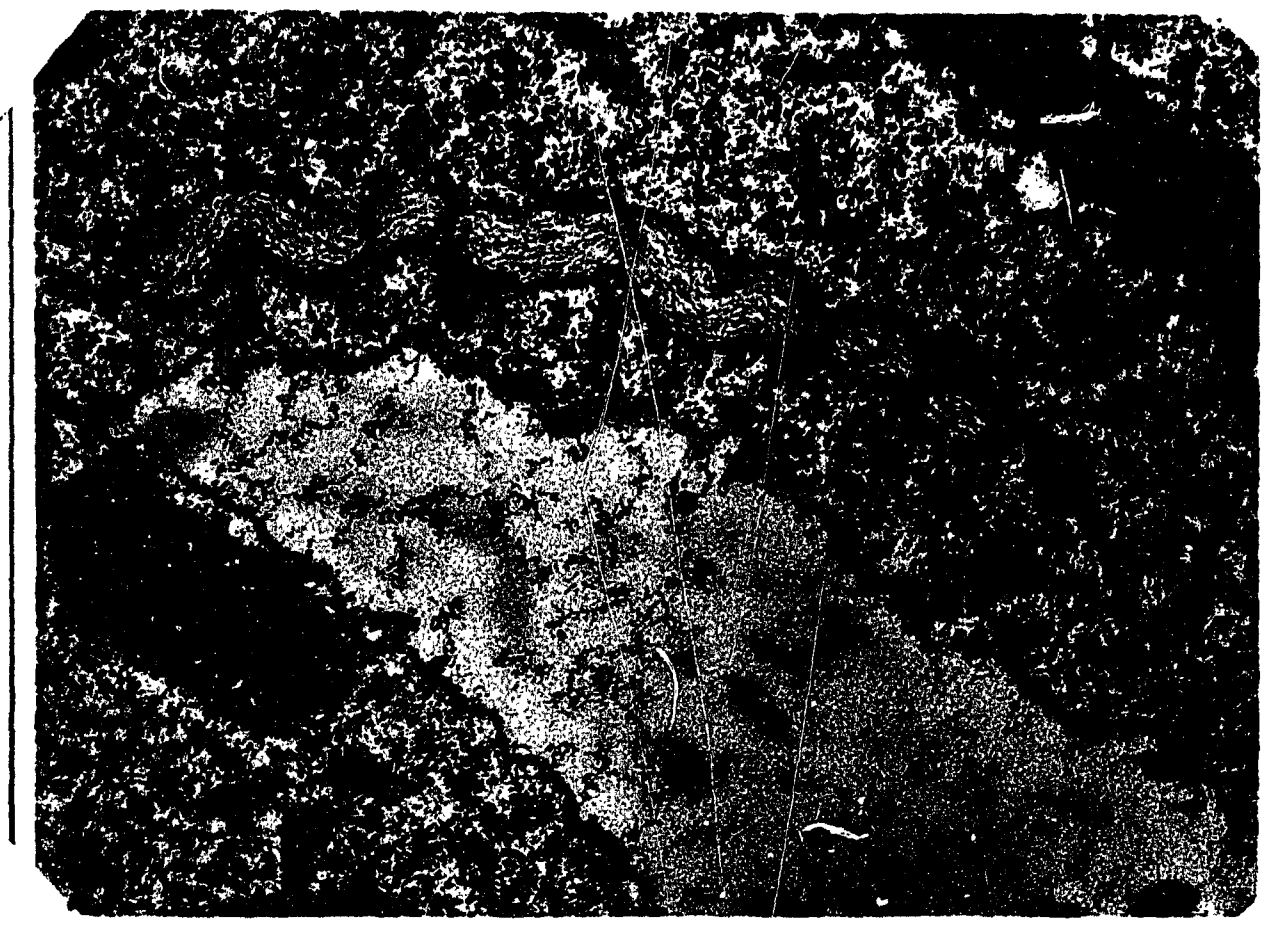

Figure 3. Fibrous inclusions in the cytoplasm of young tobacco leaf epidermal cell infected with tobacco streak virus.

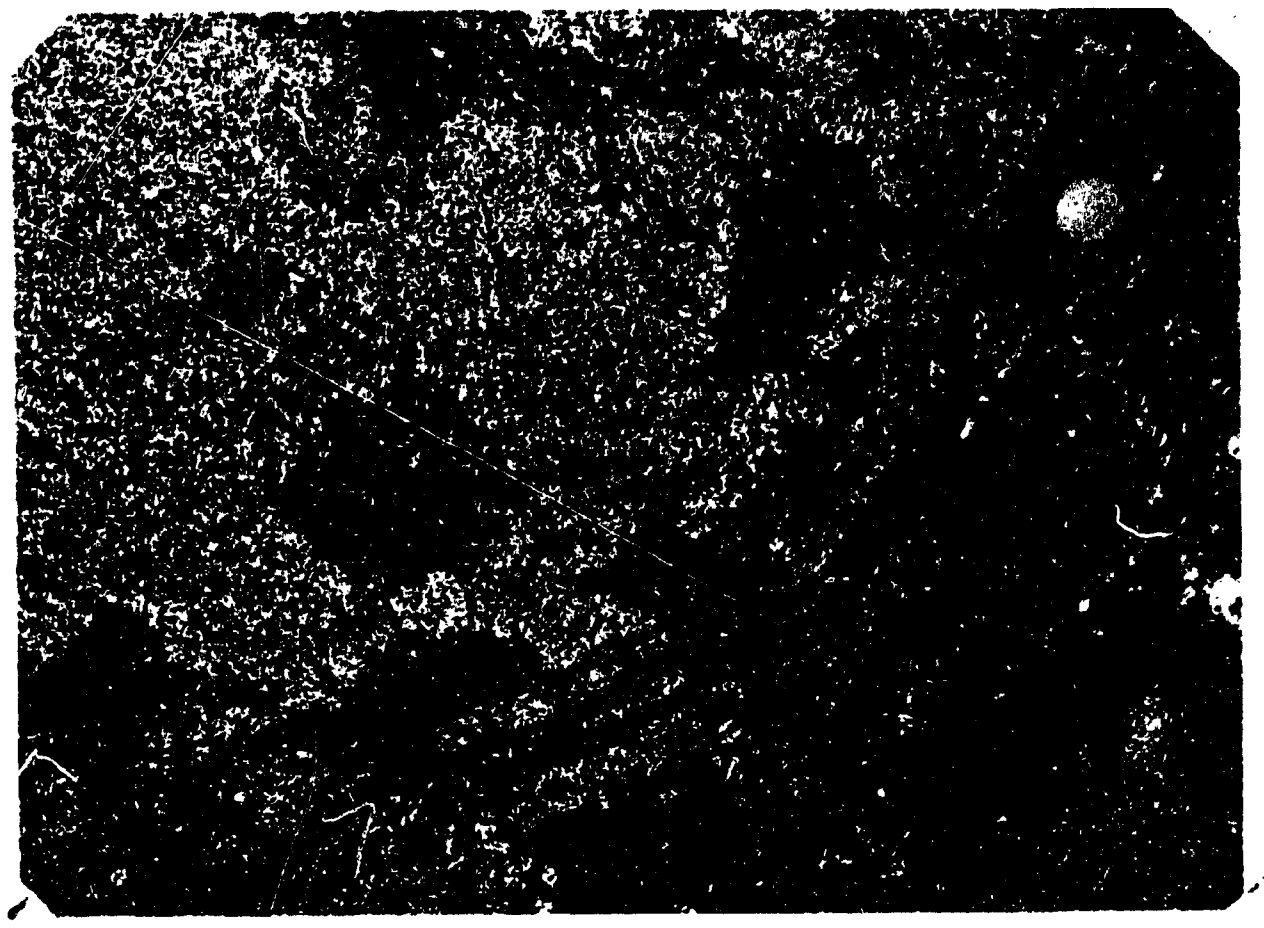

Figure 4. Tobacco streak virus particles aggregated in the cytoplasm of Datura stramonium epidermal cell in the growing point.

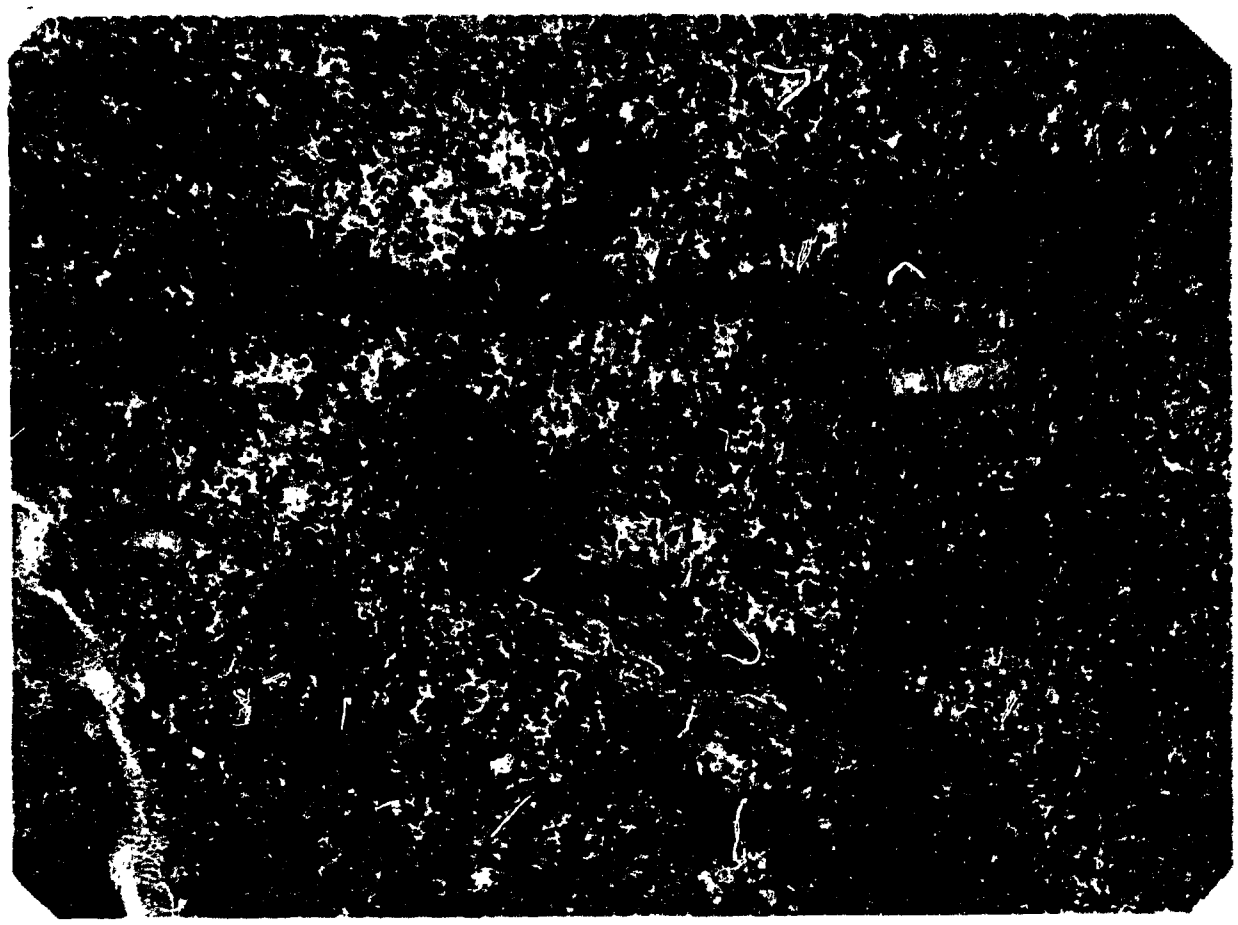

Figure 5. Tobacco streak virus particles associated with fibers in cytoplasm of Datura stramonium epidermal cell in a young leaf.

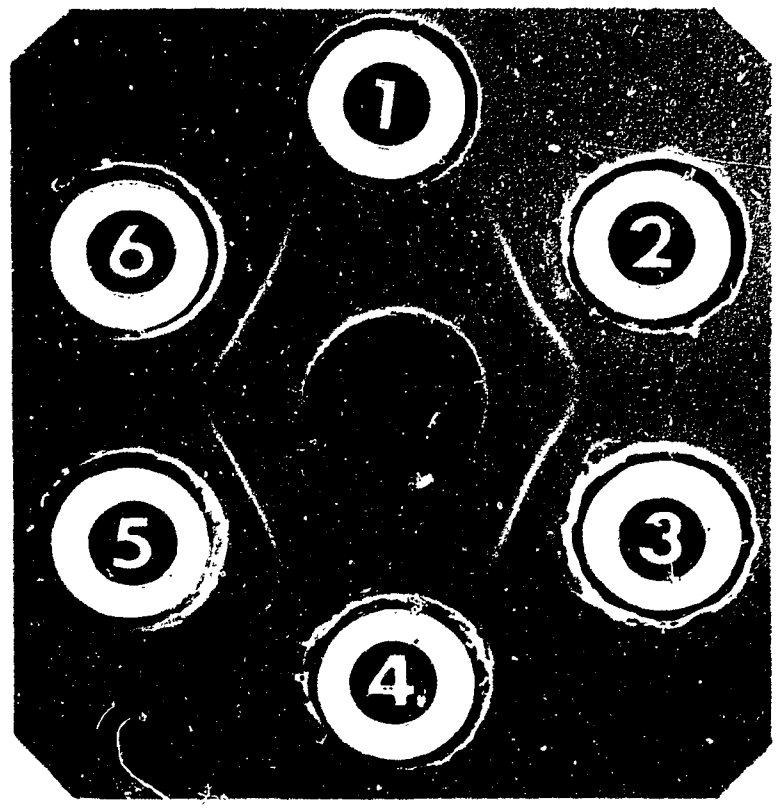

Figure 6. Immunodiffusion test in agar gel, depicting close serological relationship between tobacco streak (TSV) and Datura Q (DQV) virus antigens. The peripheral wells contained crude sap extracted from: 1 and 4, healthy tobacco; 2 and 5, TSV-infected tobacco; 3 and 6, DQV-infected tobacco. The center well contained TSV-antiserum.