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FINAL REPORT

Project title: The differentiation of effects of ionization radiation and other mutagenic agents on the functional versus the genetic capacities of cells.

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1. General objectives of the problem.

The general objective has been a comparison of the effects of mutagenic agents, primarily x-rays, on the heritable component of the pollen grain with the effect on its functional capacity.

2a. General summary of work on the project.

It has been possible to demonstrate conclusively that x-rays, at doses up to 8,000 r, produce much more drastic effects on the heritable components of pollen grains than on the functional capacities. Pollen grains germinated in vitro, at doses from 0 r to 8,000 r, showed no differences in growth rates. Application to stigmas of pollen treated with 4,000 r gave close to 100% aborted seeds. Investigations with controlled pollinations, such as a limited pollination with treated pollen followed by a massive pollination with normal pollen, showed that the seed abortion was due to the induction of deleterious hereditary changes introduced by sperm from the treated pollen. A comprehensive study of the embryogeny of the dominant lethal ovules showed that the typical features of the dominant lethality cannot be fully explained by mechanical disturbance of mitosis nor x-rays may have a direct toxic effect on the untreated chromosomes. Further work, with Lilium formosanum was interrupted by serious disease problems and commercial types of L. longiflorum were introduced into the greenhouse for research material. L. longiflorum proved unsatisfactory for several reasons: it is self-sterile; many of the more vigorous commercial clones had chromosome aberrations leading to a high proportion of pollen and ovule abortion without further treatment; and finally, the capsules would not mature unless a fairly high percentage of ovules were developing into viable seeds. Experiments which were made with this material have given uneven results, not suitable for publication, but have provided preliminary information on the "dosage-effect" curves for dominant lethality and for the functional aspects of the pollen grain which have materially helped in planning repeat experiments. These repeat experiments are currently in progress with lines of L. formosanum which have shown vigorous growth and resistance, but not immunity to disease.

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2b. Summary of work during current report period.

1) Studies of direct competition between treated and untreated pollen grains. Statistical analyses of capsules following half-and-half pollinations have shown that there is not only no deleterious effect of x-rays on the germination of grains and growth of tubes following doses of 4,000 and 8,000 r but that the 4,000 r dose probably has, in part, a stimulating effect on the development of the majority of the grains. The preliminary work, with L. longiflorum, has shown that grains treated with 60,000 r may, and those with 100,000 r do suffer a complete handicap in competition with normal grains even though many are still capable of germination and growth in vitro.

2) Investigations of methods for accurately and efficiently estimating pollen grain number. A series of tests were made of a method of packing pollen grains into a small plastic cup and then using this clumped mass of grains for pollinations. Some excellent seed sets were obtained by this method, indicating mechanical injury will not be an important factor, but the L. longiflorum material used precluded an accurate estimate of the percentage of functional grains.

3) Cytological examination of pollen tube mitoses and early embryogeny following different doses of x-rays. Work with the pollen tube mitoses is still in progress, and no reliable information is yet available on characteristic differences, if any, to be found after treatments with different doses. In the embryological studies, Dr. Cave has made several significant observations on development in ovules after pollen treatments. The rate of development in the embryo and endosperm are independent and either may occur at a slower or faster rate than normal. It is of special interest to note that the changes induced by the x-rays may give greater than normal rates of development during the short period prior to the abortion of the tissue.

4) Effects of colchicine treatment of pollen on pollen grain mitoses and embryogeny. After pollen treatments of 0.1% and 1% aqueous colchicine for 15 minutes, pollen tube growth and seed set are accomplished readily following the lower dose only. Examinations of pollen tube mitoses have shown that some mitoses are sufficiently deranged to give either a single, diploid sperm nucleus or two sperm nuclei one of which may lack one or two chromosomes. Embryological studies are now in progress to obtain information on embryo and endosperm development following colchicine pollinations in order to provide material of known types of changes for comparison with the x-ray material.

5) "Killing curves" for the heritable aspects. Rather limited data, obtained from early work with L. formosanum showed evidence for an S-shaped killing curve, as judged from the percentage of dominant lethals, for doses up to and including 4,000 r. Much more extensive work with L. longiflorum, however, indicated a straight-line relationship for the production of dominant lethals at doses up to 4,000 r. It is hoped that work now in progress with L. formosanum will afford sufficient reliable data to define the killing curve for this species. If the curve for L. formosanum actually proves to be different from that for L. longiflorum the results will be of considerable significance.

3. Bibliography:

Spencer W. Brown and Marion S. Cave.
"Induced dominant lethality in Lilium."
Proc. Natl. Acad. Sci. 39(2): 97-102. for February, 1953

Spencer W. Brown and Marion S. Cave.
"The detection and nature of dominant lethals in Lilium. I.
Effects of x-rays on the heritable component and functional ability
of the pollen grain."

Marion S. Cave and Spencer W. Brown.
"The detection and nature of dominant lethals in Lilium. II.
Cytological abnormalities in ovules after pollen irradiation."

Note: The above two papers totaling 34 pages are appearing together in the June 1954 issue of the American Journal of Botany. Reprints will be forwarded as per schedule on receipt.

4. Other information.

- a. Change in direction or emphasis. None.
- b. Personnel changes. Dr. Marion Cave, previously on a Guggenheim Fellowship, returned to the project for several months' work prior to termination. Mr. Daniel Zohary was employed from July 1, 1953, as a Research Assistant on the project.
- c. Number of graduate students on contract. Two. Mr. Donald E. Stone on an hourly basis and Mr. Daniel Zohary as a Research Assistant.
- d. Support from other sources. The Department of Genetics has continued to supply space and materials for growing plants, care of the plants in the greenhouse, and specific technical assistance from Mr. Ernest Jund, the Departmental Technician.
- e. Difficulties encountered. The problem of disease (bulb-rot) described in the previous annual progress report continues to be important. It is hoped, however, that a selection for vigor in L. formosanum will provide suitable resistant, if not immune, plants, for research material.

5. Future plans.

The two collaborators on this project wish to express their sincere appreciation for the material help provided by the contract funds during the contract period. Work currently being undertaken which has not progressed sufficiently far for publication will be continued on the basis of a regular departmental research project. On publication, acknowledgment will be made to the ONR for its support, and reprints of such papers will be provided as per schedule.