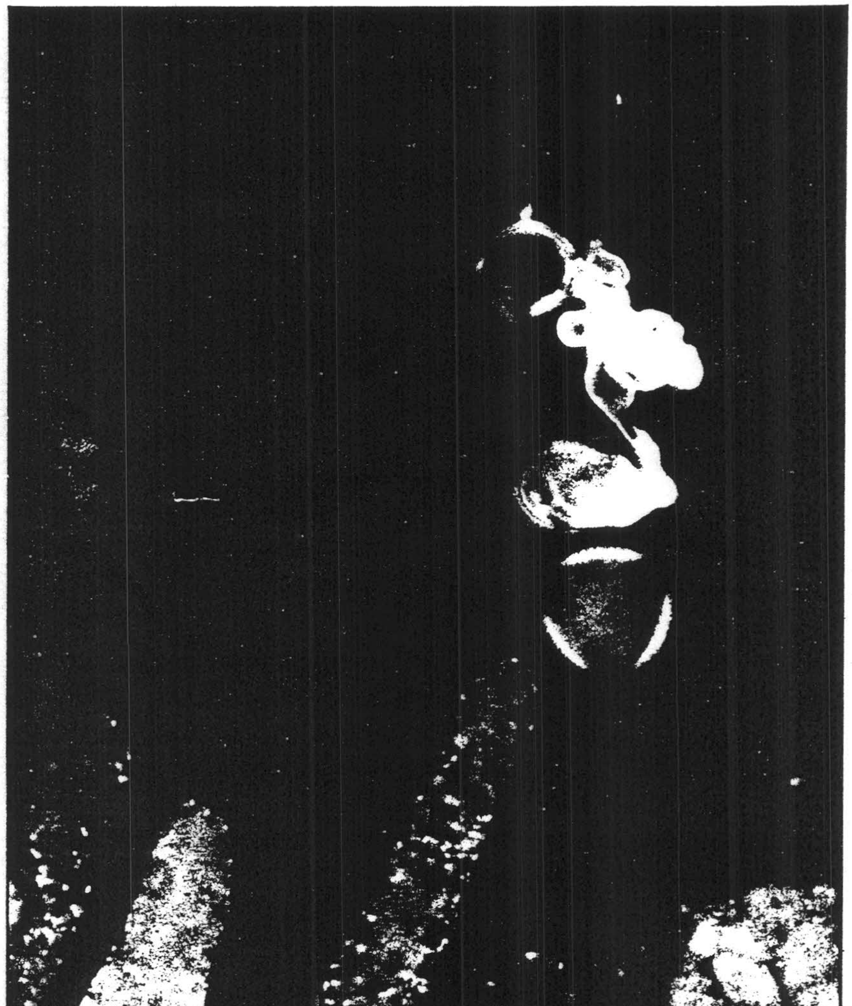
1985 PLANT CELL No. 17

INCOMPATIBILITY NEWSLETTER

the newsletter of self-incompatibility systems, pollen-stigma interactions and other aspects of reproductive and somatic cell biology.



Self Incompatibility and the Site of Pollen Tube Arrest in Brassica Oleracea

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INCOMPATIBILITY PHENOMENA IN AMPHIDIPLOID SPECIES OF BRASSICA

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(submitted to M. Nasrallah in partial fulfillment of Bio. 438 - Independent Research in Biology)

ABSTRACT

Brassica nigra and *B. oleracea* are the parent species of the amphidiploid *B. carinata*; *B. oleracea* and *B. campestris* are the parent species of the amphidiploid *B. napus*. *B. nigra* and *B. campestris* are the parent species of the amphidiploid *B. juncea*. DNA from young leaves was prepared, cut and separated by size through the use of agarose gel electrophoresis. A "Southern Transfer" liberated the DNA from the gel and bound it to nitrocellulose paper. Hybridization with a ^{32}P-labeled "S" locus probe revealed sequences homologous to the probe in the species triangle. To investigate the extent of expression of the self-incompatibility genes of the amphidiploids in relation to each other, to maturity of the bud and to their parent species, open flowers and buds along the inflorescences of the amphidiploids were self-pollinated. The stigmas of the developmental sequences of flowers and buds were then excised into fixative, softened in sodium hydroxide and stained with decolorized aniline blue. Pollen tube growth was then monitored by epifluorescence microscopy under U.V. light. Based on observed coiling of pollen tubes it was shown that a partial self-incompatibility reaction is manifest one day prior to anthesis in 7 out of 9 plants examined from a base population (CrGC #5) of *B. napus* and 8 out of 10 plants examined from a base population (CrCG #4) of *B. juncea*.

INTRODUCTION

Self-incompatibility is the inability of a plant to fertilize itself. Incompatibility in Brassicaceae involves the production of glycoproteins in stigma cells which either inhibit or prevent pollen tube growth (Nasrallah et al. 1985). Each pollen grain interacts with a papilla or finger-like projection of the stigma. It has been shown that if the same allele is present at the "S" locus in the plant receiving the pollen as in the plant donating the pollen, then fertilization cannot occur. In nature incompatibility functions to promote outbreeding and as a result, variability is insured among natural populations.

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In Brassicaceae a species triangle exists whereby in nature, B. nigra (2n=16), B. oleracea (2n=18), and B. campestris (2n=20) crossed in all possible combinations to produce the amphidiploids B. carinata (2n=34), B. napus (2n=38), and B. juncea (2n=36). The term amphidiploid describes a species which has been derived from the crossing of two different species with subsequent doubling of the genomes. Spontaneous chromosome doubling is a necessary phenomenon for the formation of an amphidiploid. When the doubling occurs the species goes from the N=N state with n chromosomes from each parent to the 2n+2n state. After chromosome doubling occurs there is a pairing partner for each chromosome and meiosis can occur normally.

MATERIALS AND METHODS

Plant Stock

Rapid cycling B. oleracea, B. nigra, B. campestris, B. carinata, B. napus and B. juncea seeds of unknown genetic uniformity were obtained from the University of Wisconsin, Madison, WI courtesy of Professor Paul H. Williams.

Assays for Self-incompatibility

Self-incompatibility in relation to bud development was tested by applying pollen to the stigmas of the increasingly less mature buds along an inflorescence. Stigmas were excised into fixative, softened in sodium hydroxide and stained with decolorized aniline blue. Pollen-tube growth was monitored by fluorescence microscopy (Kho and Baer, 1968).

Probing the Amphidiploid Genomes

DNA from young leaves was isolated through the use of a DNA Mini-prep which involved among other steps, precipitation with ethyl alcohol and centrifugation. After cutting with Bam HI and Pst I the total genomic DNA was separated by size through the use of agarose gel electrophoresis. Migration of DNA fragments was induced by passing 125 volts through the .9% gel. For future reference, a polaroid picture was taken with ultra-violet light passing up through the gel. The DNA was denatured, or made to be single stranded, and a Southern Transfer liberated the DNA from the gel and bound it to nitrocellulose paper. The total genomic DNA, bound to the nitrocellulose sheet, was subjected to a ^{32}P-labeled "S" locus probe provided by Dr. M. E. Nasrallah. The pairing of homologous DNA was possible because both the probe and the genomic DNA were single stranded. Bands which were homologous to the probe were revealed through exposure of the nitrocellulose sheet to Kodak X-Omat X-ray film (Eastman-Kodak, Rochester, N.Y., USA) at -70^{o}C.

RESULTS

The Presence, in the Amphidiploids of Sequences Homologous to the Self-Incompatibility Probe

The analysis of several negatives, each showing the extent of the homology between the amphidiploid DNA and the probe, showed that sequences homologous to the "S" locus exist in the amphidiploids as well as in the parent species.

The Change in Self-Compatibility During Bud Development

The immature buds of B. oleracea, B. nigra and B. campestris (the parent species) are self-compatible; the largest buds and the flowers are self-compatible. B. oleracea shows two sharply delineated zones along developing inflorescences: a self-compatible zone exhibiting maximal pollen tube growth, a self-incompatible zone with no pollen tubes (Nasrallah et al. 1985). B. nigra and B. campestris most likely exhibit the same abrupt shift from self-compatibility to self-incompatibility.

Most plants of the rapid cycling B. juncea strain exhibited partial pollen tubes when the most mature buds or the flowers were pollinated. The pollen tube ceased growing at the surface of, or after penetrating, a papilla. As in all six species, pollinated immature buds produced complete pollen tubes and are self-compatible.

B. carinata, the most self-fertile of the amphidiploids, for the most part is self-compatible. Full pollen tube growth can be observed at all stages of bud development along an inflorescence.

The most immature buds of B. napus, are self-compatible. As bud maturity increases to the level characteristic of mid-inflorescence, the buds gradually become self-incompatible. As the bud develops from mid-inflorescence state to a flower the trend reverses; by anthesis full self-compatibility is restored.

DISCUSSION

In this report, it is shown that self-incompatibility sequences are present in the amphidiploid's genomes but they are not entirely functional. Due to the necessary chromosomal doubling that occurs during the formation of an amphidiploid, there are four alleles present at the "S" locus instead of two. Competition resulting in a weakening of the self-incompatibility reaction cannot be ruled out. B. napus shows a progressively increasing tendency towards self-incompatibility until the mid-inflorescence state of bud maturity is reached. As the bud develops from mid-inflorescence state to a flower the trend reverses; by anthesis full self-compatibility is restored. This phenomenon may be caused by a suppressor gene which becomes activated at a certain level of maturity and depresses the level of incompatibility protein in the stigmas, thus causing self-compatibility.

Special thanks to Dr. M. Nasrallah, in whose laboratory this work was done and to James Costa for helpful discussions and Delia Baroni and Donna Curtin for assistance in photography.

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ORGANIZATION OF THE NEWSLETTER

Research Notes

(not exceeding 1000 words)

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Guidelines to follow in the preparation of Research Notes:

The manuscript should be typewritten, doubled spaced on 8½ x 11" paper. It is recommended that manuscripts not exceed 4 typewritten pages. Deviations from this limit could result in publication delays. The number of illustrations (tables and/or figures) should be kept to a maximum of 2. Photographs are not allowed. Tables should be typed, double-spaced on a separate sheet. Each should have a title. For drawings and graphs, use India Ink and heavy white paper or blue-lined coordinate paper. Figure legends should be typed on the page displaying the figure.

Manuscripts and all correspondence should be addressed to:

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ACKNOWLEDGEMENTS: We wish to acknowledge the contributions of the former editors and organizing committees of the Plant Cell Incompatibility Newsletter. We thank them for their dedicated and successful tenure from 1972 to 1982.

M. E. Nasrallah and R. C. [illegible]

The Incompatibility Newsletter was founded in 1972 by the Association EURATOM-ITAL