INTERGENERIC HYBRIDS DESIGNED TO TRANSFER RESTORATION OF MALE FERTILITY FROM RADISH (*Raphanus sativus*) TO RAPE (*Brassica napus*) WITH THE OGU MALE STERILE CYTOPLASM AND THE EFFECT OF ELEVATED TEMPERATURES ON SEED PRODUCTION FROM INTERGENERIC CROSSES

by

MICHAEL JAMES HURST

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ABSTRACT

Michael J. Hurst, M. Sc., The University of Manitoba, Sept. 1985. Intergeneric hybrids designed to transfer restoration of male fertility from the radish (Raphanus sativus) to rape (Brassica napus) with the ogu male sterile cytoplasm and the effect of elevated temperatures on seed production from intergeneric crosses.

Restorer gene(s) for the ogu male sterile cytoplasm in B. napus L. (n=19) are not available. Restorer gene(s) appear to be present in an oilseed radish, R. sativus L. (n=9), obtained from seed stocks at the Department of Plant Science, University of Manitoba. Brassica napus (cv Karat), with the ogu male sterility inducing cytoplasm (ogu cms Karat) was crossed with this radish to transfer restorer gene(s). Crosses and backcrosses (BC) were made with the recurrent parent Karat to recover the B. napus genome while maintaining the restorer gene(s) from the radish in the ogu cytoplasm. Pollen was observed in the F1 and BC1 generation but not in later BC generations. A BC3 generation was produced but female fertility in these plants was reduced to an extent that BC4 seeds were not produced.

Cuttings from the F1, BC1, BC2 and BC3 generation were transplanted to blank rows left in a dense stand of Karat in a field plot located at the University of Manitoba. This was done in an effort to obtain more seed than was available from manual pollinations. Hybrids between B. napus and R. sativus were treated with colchicine to produce doubled

hybrids. The doubled hybrids were crossed with Karat. Embryos were cultured to facilitate production of hybrids. However, none of these procedures resulted in the development of a restorer. The presence of the male sterile radish cytoplasm in the hybrids may have had a detrimental effect on the expression of male fertility and also on female fertility. The white flower color gene from the radish may have also effected female fertility when it was present in the genome. Another possibility is that the number of pollinations made to produce F1, BC1, BC2 and BC3 plants may not have been large enough to obtain a restorer if restoration depends on complex gene and/or chromosome combinations. The lack of restorer development indicated that the inheritance of fertility restoration for the ogu male sterile cytoplasm was probably not simple.

The chromosome pairing was examined in pollen mother cells (pmc's) from the cross ogu cms B. napus X R. sativus. At diakinesis of meiosis I an average of 12.75 univalents, 7.3 bivalents and 0.65 trivalents were present. The bivalent associations probably arose from homoeologous chromosome pairing between the A and C genomes of napus.

Seven other R. sativus cultivars were examined as potential sources of restoration for the ogu male sterile cytoplasm. However, no pollen was observed in any F1 plant involving the seven cultivars.

High temperature treatment of the female parent before flowering significantly increased the seed production (the number of seeds per floret pollinated) in intergeneric crosses. Three different female parents were tested; ogu cms Karat and the two B. napus cultivars Regent

and Karat. These were crossed with R. sativus and compared with control groups of untreated female parents. There were genotypic differences between the female parents. Pods formed and developed quickly when B. napus (cv Regent) was heat treated and crossed with R. sativus. Pod formation and development occurred more slowly when ogu cms Karat and B. napus (cv Karat) were used. Regent appeared to be more cross compatible with R. sativus than the other two female parents in both treated and control groups.

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INTRODUCTION

Rapeseed has become a major crop in Western Canada in the last three decades. Improvements in the quality of rapeseed resulted in a new commodity called canola rapeseed. Canada is the world's largest exporter of rapeseed and canola rapeseed. Interest in the development of hybrid cultivars has been stimulated by reports that cytoplasmic male sterility (cms) could be used to produce the hybrid cultivars with higher yields due to heterosis (Shiga and Baba, 1971, 1973; Thompson, 1972). Sernyk and Stefansson (1982) reported heterosis in F1 hybrids. Seed yields of the best F1 hybrids exceeded those from conventional summer rape cultivars by 43%.

Numerous pollination control systems have been suggested for producing hybrid rape. Some of the more common methods include sporophytic self-incompatibility (Hinata and Nishio, 1980), male gametocides (Van Der Mer and Van Dam, 1979), monogenic male sterility induced by gamma irradiation (Takagi, 1970), genic male sterility (Shiga, 1980) and cytoplasmic male sterility (Shiga and Baba, 1971, 1973; Thompson, 1972). The most economically feasible pollination control system that can be used on a large scale under field conditions appears to be cms.

Four cms systems are being evaluated for their potential use in hybrid rapeseed production in Brassica napus L. (n=19) at the University of Manitoba. These include the nap, mur, pol and ogu cms systems. This

study concentrated on the ogu cms system. A restorer has not been developed for this cms system in B. napus. Restorer gene(s) have been reported in certain European oilseed radish varieties, Raphanus sativus L. (n=9), (Bonnet, 1975). Therefore, to introduce restorer gene(s) into B. napus, an intergeneric hybrid must be produced. Producing intergeneric hybrids can be very difficult. A study by Dolstra (1982b) illustrates the difficulties encountered. He produced Brassioraphanus (n=19) hybrids (R. sativus X B. campestris) and BC progenies from these hybrids. The F1 hybrids were relatively easy to produce but the BC generations were much more difficult and only a few plants were produced.

For this study, a radish cultivar with the capability for restoration was obtained from seed stocks at the Department of Plant Science, University of Manitoba. Crosses were made to transfer restorer gene(s) from this radish to B. napus (cv Karat). During the process of introducing restorer genes into B. napus several related experiments were undertaken. Chromosome pairing in pollen mother cells (pmc's) of F1 hybrids was examined. Several potential sources of restoration for the ogu male sterile cytoplasm from R. sativus were examined. The effect of a univalent and bivalent chromosome from the radish (controlling flower color) on seed production (the number of seeds per floret pollinated) was evaluated. Finally, the use of elevated temperatures to improve seed production in intergeneric hybrids was explored.

LITERATURE REVIEW

CYTOPLASMIC MALE STERILITY

Cytoplasmic male sterility is a genetic system controlling pollen sterility based on an interaction between factors in the cytoplasm and genes in the nucleus. There is evidence suggesting these factors are gene(s) located on mitochondrial chromosomes (Pring et al, 1977). This sterility factor can be overcome by the presence of nuclear genes usually referred to as male fertility restorers. These gene(s) are usually dominant in their mode of action.

A pollination control system is implemented by the development of cms and maintainer forms of the female parent of a hybrid and a restorer form of the male parent of that hybrid. The cms female parent is maintained by crossing it with it's male fertile, maintainer form. The male fertile, restorer, male parent is maintained by self pollination. The F1 hybrid seed is then produced by interplanting rows of cms female parent with rows of the male fertile, restorer, male parent. Pollen from the male is transferred to the female parent on which the F1 hybrid seed is produced. Plants of commercial F1 seed are male fertile due to the dominant effect of the nuclear, male fertility restorer gene(s) contributed by the male parent. Cms has been identified and used for hybrid seed production in a number of crop species. Some of these include sunflowers (Whelan, 1980), rice (Shinjyo and Omura, 1966;

Shinjyo, 1972), onion (Jones and Clarke, 1943), sorghum (Stephens and Holland, 1954) and corn (Rhoades, 1933; Duvick, 1965). Cytoplasmic male sterility in oilseed rape (B. napus) was first reported by Shiga and Baba (1971, 1973) and by Thompson (1972).

THE OGURA SYSTEM

Ogura (1968) identified male sterility in an unidentified variety of Japanese radish which was growing in Japan. This system was later classified by Shiga (1980) as the ogu cms system. The sterility of the male sterile radish was induced by the interaction between homozygous recessive genes (ms ms) and a sterility inducing cytoplasm(S). This cms radish possessed morphological abnormalities such as small flower buds, style deformities and abnormal flowering habits. All Japanese radish varieties tested were shown to be homozygous recessive (ms ms) and carry no fertility restorer genes.

Bonnet (1975, 1977) introduced the male sterile radish from Japan to France and found European radish varieties which carried restorer genes for the ogu male sterile radish. Through backcrossing to European varieties, superior agronomic types were developed and in 1977 an F1 hybrid radish variety was registered. Bannerot (1974) introduced the cabbage (B. oleracea, n=9) nucleus into the cms Japanese cytoplasm by a series of four backcrosses using B. oleracea as the recurrent parent.

Bannerot (1977) later produced ogu cms B. napus by repeated backcrossing of cms B. oleracea with B. napus. He also crossed ogu cms B. napus with a wide range of different B. napus accessions. Restorer

genes were not identified in any accessions, therefore all B. napus cultivars are maintainers for the ogu male sterile cytoplasm.

Bartkowiak-Broka et al (1979) studied the morphology of this cms system in B. napus. Petal width was almost normal in the ogu cms lines and atrophy of the stamens and complete absence of pollen grains were always seen. Female fertility of the ogu cms lines was normal as long as sufficient pollen was provided for pollination.

There are several undesirable characteristics associated with this cms system in B. napus. Rouselle (1979) has reported that all ogu male sterile rape lines exhibit severe yellowing of the young leaves when plants are grown at temperatures below 12°C. Curled pods are a common occurrence and this is also an undesirable characteristic. The nectaries may not be fully developed and nectar production may be limited. Insect pollinators may not be attracted and reduced seed set often results.

The transfer of restorer gene(s) for the ogu male sterile cytoplasm from the radish into the B. napus genome has not been accomplished. Heyn (1979) attempted to introgress male fertility restorer gene(s), from the radish, into the B. napus genome using Raphanobrassica (n=18) hybrids (B. oleracea X R. sativus). He suggested that male fertility restoration was controlled by two loci with complementary dominant gene action. He also suggested that one of the male fertility restorer genes was linked to the gene for white flower color in radish. Apparently, the presence of dominant white flower color in any progeny could be used as a marker for the presence of restorer gene(s). Rouselle (1979) also

attempted to develop a restorer in the same manner. In both studies positive results were indicated but only BC1 data was given.

CYTOLOGICAL INVESTIGATIONS

A cytohistological study conducted by Bartkowiak-Broda et al (1979) indicated meiosis and microspore production occurred normally in the ogu cms B. napus lines examined. Then microspore degeneration occurred, probably due to breakdown of the tapetum which occurred after the tetrad stage of meiosis.

Introgression of Raphanus genes may depend on pairing between Raphanus and Brassica chromosomes (Dolstra, 1982b). Pairing-and-exchange between R (radish) and A (campestris, n=10) chromosomes is low. Dolstra (1982a) produced AR hybrids. In 67% of pmc's examined, no chromosome associations occurred and from all cells examined, the mean number of bivalents seen per cell was 0.47. Pairing and exchange between R (radish) and C (oleracea) chromosomes may be higher. Observations of up to nine bivalents in CR hybrids have been reported (Harberd and McArthur, 1980; Namai, 1976, 1980; McNaughton, 1973). Apparently, the R genome is more capable of pairing with the C genome than with the A genome. However, in an ACR hybrid, homoeologous pairing between the A and C genomes would most likely occur before pairing of R with either the A or C genome of napus. Namai (1976, 1978) reported multivalent associations between A and C chromosomes in AAC hybrids whereas in AAR hybrids, minimal multivalent associations were reported. From another study, the average chromosome association per cell in AACR hybrids was 14.48 univalents, 10.59 bivalents, 0.75

trivalents and 0.02 quadrivalents (Dolstra, 1982b). He reported that as the number of multivalents in a cell increased, the number of bivalents decreased. This was interpreted as the pairing of one of the C chromosomes (or less likely an R chromosome) with a pair of A chromosomes.

THE EFFECT OF HIGH TEMPERATURE ON SEED PRODUCTION IN THE FEMALE PARENT INVOLVED IN AN INTERGENERIC CROSS

Treatment of the female parent with high temperature has often been used to break down the self-incompatibility (SI) reaction which is controlled by oppositional S allele systems (Townsend, 1968). Examples include certain members of the clover family such as red clover, Trifolium pratense (Leffel, 1963; Kendall and Taylor, 1969) and diploid alsike clover, T. Hybridum (Townsend, 1968). Elevated temperature to overcome SI has also be used on several radish varieties (El Murabaa, 1957).

High temperature treatments have been used in an effort to overcome breeding barriers associated with interspecific and intraspecific crosses. Anderson and Taylor (1974) evaluated the effect of temperature on hybridization of red clover with another clover species, T. medium and also the effect on diploid-tetraploid crosses of red clover (T. pratense). Buds on plants were isolated in a chamber maintained at 40°C for two or three days. After treatment the plants were transferred to greenhouses maintained at 25°C and pollinated immediately. Plants maintained at 25°C in these greenhouses were pollinated throughout the experiment. Seeds were harvested 30 days later, grown and examined

cytologically to determine whether hybridization or self pollination had occurred. Seeds produced on the female parents which were heat treated were the result of self pollination and not hybridization. Therefore, the temperature treatment did not facilitate interspecific or intraspecific hybridization.

El Murabaa (1957) evaluated the effect of high temperature on seed production in crosses between compatible clones and in self pollination of incompatible clones of two cultivars of R. sativus. One plant of each clone was removed to a growth chamber at 17°C and another one at 26°C a few days before flowering. At anthesis, flowers were emasculated and pollinated. Pollen for crossing was gathered from one plant belonging to the other clone; that for self pollination was taken from the same flower. Clones remained in their respective growth chambers until all flowers had been pollinated. Then they were transferred to cabinets maintained at 20°C until the seeds were harvested. High temperature treatment had a detrimental effect on the seed production from crosses between compatible clones. There was a slight increase in the seed production from self pollination of incompatible clones which were heat treated.

MATERIALS AND METHODS

Plants used in this study were grown from seed stocks from the Department of Plant Science, University of Manitoba. Plants were given a 16 hr. daylength period, watered daily and maintained at a temperature averaging 20°C. The experiments were initiated in January 1983 and continued until April 1985. All harvested seeds were germinated on petri plates. The seedlings were transplanted into flats. At the three leaf stage, the plants were transplanted into pots containing a soil mixture of two parts black earth, one part peat moss and one part sand.

RESTORER DEVELOPMENT

Brassica napus (cv Karat) with the *ogu* male sterility inducing cytoplasm (*ogu* cms karat) was crossed with *R. sativus*. F1 seeds were harvested, grown and crossed with Karat (the recurrent male parent). Each BC generation was grown and crossed with Karat to recover the recurrent parent's genome while maintaining the restorer gene(s) from the radish genome in the *ogu* cytoplasm. The number of florets pollinated, the number of pods and seeds harvested, the number of plants produced and the flower color were observed and recorded.

The F1, BC1 and BC2 generations were treated with colchicine to produce doubled hybrids. Cuttings from each generation were submerged in a solution containing 5% colchicine and 1.5% dimethyl sulfoxide (DMSO) for a period of 5 hours. Air was bubbled through the solution

with an aquarium air pump. The treated cuttings were transferred to pots containing vermiculite. After the root system was well established, these plants were transplanted to pots containing soil. When the doubled hybrids flowered they were pollinated with pollen from Karat. The number of florets pollinated and the number of seeds harvested were recorded.

Embryo culture was used to develop embryos produced from the F1, BC1, BC2 and BC3 generation. Harberd's (1969) procedure for culturing Brassica embryos was used. About 12-15 days after pollination, ovules were sterilized, slit open and transferred to a modified Murashige and Skoog's liquefied medium formulated from ingredients outlined in Gamborg and Wetter (1975). An elevated sucrose (8%) and protein level (400 ppm casein hydrolysate) were used in the solution. The suspension was continuously agitated to wash out the ovary. On the twentieth day after pollination the developing embryos were transferred to an MS solid medium. When the plants were well established they were transplanted to pots containing soil.

In the spring of 1984 cuttings from F1, BC1, BC2 and BC3 plants were taken. These shoots were placed vertically in pots containing a mixture of perlite and vermiculite. This mixture was packed firmly around the stems and the pots were placed into plastic bags containing water. The bags were sealed and transferred to growth cabinets for periods of one to three weeks. After roots were produced the young plants were transferred to pots containing soil and placed outside into small portable greenhouses for a period of one month to facilitate hardening. Two weeks before flowering these plants were transplanted to blank rows

left in a dense stand of Karat in a field plot located at the University of Manitoba. Furadan was used as an insecticide for flea beetle control. The number of cuttings transplanted to the field, the number of seeds harvested and the number of plants produced were recorded.

CYTOLOGICAL INVESTIGATIONS

Pollen mother cells were collected from the F1 generation and examined under a Zeiss light microscope. Flower buds 1.4 to 1.6 mm long were removed from the plants between 8 and 9:30 in the morning and fixed in a mixture containing three parts ethanol and one part glacial acetic acid. For examination, an individual anther was removed from the bud and squashed on a slide using 1% aceto carmine solution. Five F1 plants were examined and the number of cells examined per plant and average chromosome associations per plant were recorded.

Pollen grains were stained with an I2-KI solution and examined under the microscope. This stain is starch specific and the intensity of stain is an indication of pollen grain viability. One hundred pollen grains, representing a random sample, were examined. Pollen stainability was calculated and expressed as the percentage of stained pollen grains.

POTENTIAL SOURCES OF RESTORATION FOR THE OGURA MALE STERILE CYTOPLASM

Approximately 25 seeds from each of the following *R. sativus* cultivars were grown and crossed with *ogu* cms Karat; Raonla, Red Boy, French Breakfast, Celery Belle, White Icicle, Long Black Spanish and a cultivar from Iraq. Seeds were harvested and the *R. sativus* parent used

in the cross was recorded. The anther and pollen development of the F1 plants was observed and recorded.

THE EFFECT ON SEED PRODUCTION OF A UNIVALENT AND BIVALENT CHROMOSOME FROM THE RADISH, CONTROLLING FLOWER COLOR

Two white flowering B. napus strains were used as pollen parents in the cross. Chromosomes were counted to determine whether the radish chromosome occurred as a univalent or bivalent. Ogu cms Karat was used as the female parent. The number of florets pollinated, the number of pods harvested and the number of seeds harvested were recorded. This experiment was replicated twice.

THE EFFECT OF HIGH TEMPERATURE ON SEED PRODUCTION IN THE FEMALE PARENT INVOLVED IN AN INTERGENERIC CROSS

The following R. sativus cultivars were used as male parents; Raonla, Red Boy, French Breakfast, Celery Belle, White Icicle, Long Black Spanish and a cultivar from Iraq. The female parents were ogu cms Karat and the two B. napus cultivars Regent and Karat. One or two days before flowering the female parents were removed from the greenhouse and placed in growth cabinets maintained at 30°C for 16 hours of daylength, 26°C for 8 hours of darkness. After the female parents had been in the cabinets for seven consecutive days they were transferred to a growth chamber maintained at 20°C for 16 hours of daylength, 15°C for 8 hours of darkness. Pollen parents remained in this chamber throughout the experiment and all florets were pollinated in this chamber. The B. napus cultivars Karat and Regent were emasculated before pollination to prevent self pollination from occurring. Crossing continued for up to

one month after the female parents were removed from the high temperature cabinets. A control group of untreated female parents were also kept in the growth chamber and pollinated in the same manner. The number of florets pollinated, the number of pods harvested and the number of seeds harvested were recorded from treated and control groups. For the cross ogu cms Karat X R. sativus, the treated group was replicated five times, the control group three times. For the crosses Regent X R. sativus and Karat X R. sativus, two replications were used.

ANALYSIS OF DATA

A two tailed students t test was used to determine whether pollen from the white flowered B. napus strains would have an effect on seed production in ogu cms Karat. One tailed student's t tests were used to determine whether heat treatment of the female parent would increase the seed production in the three intergeneric crosses. Bartlett's test for homogeneity of variance was used to determine which formula should be used to calculate the t values. The t tests were used as suggested by Stelle and Torrie (1980).

RESULTS

The summary of the characteristics recorded from the F1 and BC progenies of crosses involving ogu cms Karat and R. sativus are presented in Table 1. Forty four white flower colored F1 plants were produced. The leaves from all F1 plants were mildly chlorotic. Anther development varied between and within F1 plants but generally was good. Pollen was produced but most F1 pollen grains were shrivelled and sterile.

Eighteen BC1 plants were produced from 3677 pollinations made onto F1 plants. Thus, 204 pollinations were required to produce one BC1 plant. Seventeen of the BC1 plants were white flower colored and one was yellow. The leaves from all BC1 plants were mildly chlorotic. Sporadic anther and pollen development was observed in one white flowered BC1 plant but the rest were completely male sterile. Pollen stainability from the BC1 plant with pollen development was approximately 30%.

Six BC2 plants were produced from 3378 crosses made onto white flowered BC1 plants. Thus, 563 pollinations were required to produce one BC2 plant. The BC1 plant with yellow flowers produced no pollen but when it was pollinated with pollen from Karat seed set was normal. All BC2 plants produced white flowers and the leaves did not appear to be chlorotic. Pollen development was absent in this generation.

Eight BC3 plants were produced from 1050 pollinations made onto BC2 plants. Thus, 131 pollinations were required to produce one BC3 plant. All BC3 plants produced white flowers and the leaves did not appear to be chlorotic. Pollen development was again absent in this generation.

The majority of buds on the BC3 plants aborted before they could be pollinated. However, 952 pollinations were made onto the 8 BC3 plants but no BC4 seeds were produced.

A number of doubled hybrids were produced and crossed with Karat (Table 2). Twenty three F1, 21 BC1 and 19 BC2 cuttings were successfully doubled. These plants were male sterile and seeds were not produced from any of the crosses made onto them.

BC1, BC2 and BC3 embryos were grown in vitro in an attempt to develop plants in this manner. One BC1 embryo developed but it died after a short period of time.

F1, BC1, BC2 and BC3 plants were transplanted to blank rows left in a dense stand of Karat in a field plot. The characteristics recorded from this trial are outlined in Table 3. Seed set on the white flowered plants was low (figure 1 a, b and c). Twelve seeds from F1 plants, seven from BC1 plants and two from BC2 plants were harvested. However, these seeds did not produce any plants. Insect pollinators visited the white flowered plants on a regular basis. Cuttings from the yellow flowered BC1 plant were also transplanted to the field plot. Insect pollinators also visited these plants regularly and the seed set on these plants was good (figure 1 d).

The chromosome pairing was examined in pmc's of five plants from the cross ogu cms Karat X R. sativus. At diakinesis of meiosis I an average of 12.75 univalents, 7.3 bivalents and 0.65 trivalents were present (Table 4).

Seven R. sativus cultivars were examined as potential sources of restoration for the ogu male sterile cytoplasm (Table 5). Moderate to good anther development was observed in F1 plants from crosses involving Raonla or White Icicle. However, no pollen was observed in any F1 plants involving the seven R. sativus cultivars.

The effect on seed production of a univalent and bivalent chromosome from the radish, controlling flower color, was evaluated (Table 6). Bartlett's test for homogeneity of variance was not significant at the 5% level. The t value was then calculated assuming independant sample size and equal variance estimates. The calculated t value was 0.92. The t value at the 5% level of significance for 2 df was 4.30. Therefore, the t test was not significant. Thus, pollen from the B. napus lines with the radish chromosome in the univalent or bivalent state did not affect seed production in ogu cms Karat.

The effect of elevated temperatures on seed production in intergeneric crosses was evaluated (Tables 7, 8 and 9). Bartlett's test for homogeneity of variance was not significant at the 5% level in all three crosses. The t values were then calculated assuming independant sample size and equal variance estimates. The calculated t value for the cross ogu cms Karat X R. sativus was 3.40. The t value at the 5% level of significance for 6 df was 1.94. The calculated t value for the

cross B. napus (cv Karat) X R. Sativus was 9.99. The t value at the 5% level of significance for 2 df was 2.92. The calculated t value for the cross B. napus (cv Regent) X R. sativus was 12.46. The t value at the 5% level of significance for 2 df was 2.92. Therefore, the t tests were significant in all three crosses. Thus, in these intergeneric crosses, the seed production in the treated parents was significantly higher than it was in control parents (Figure 2 a, b and c).

In this study high temperature treatments consistently improved seed production in intergeneric crosses, however, genotypic differences did exist. Pods formed and developed quickly when B. napus (cv Regent) was heat treated and crossed with R. sativus. Pod formation and development occurred more slowly when ogu cms Karat and B. napus (cv Karat) were used. Regent appeared to be more cross compatible with R. sativus than the other two female parents in both treated and control groups.

TABLE 1

Characteristics of F1 and BC progenies from crosses involving ogu cms *B. napus* as the recurrent parent and *R. sativus* (cv oilseed radish) as the donor parent

	Generation			
	F1	BC1	BC2	BC3
no. of florets pollinated	3677	3378 (onto white)	1050	952
no. of pods harvested	267	232	116	0
no. of seeds harvested	287	121	147	0
flower color	white	17 white 1 yellow	white	white
average no. of seeds per pod	1.074	0.524	1.27	0
average no. of seeds per floret pollinated	0.078	0.036	0.140	0
no. of plants produced in next generation	18	6	8	0
no. of pollinations needed to produce 1 plant in next generation	204	563	131	

TABLE 2

Effect of pollen from *B. napus* (cv Karat) on F1 and BC progenies from crosses involving *ogu* cms *B. napus* (cv Karat) and *R. sativus* (cv oilseed radish) after chromosome doubling with colchicine

	Generation		
	F1	BC1	BC2
no. of doubled hybrids produced	23	21	19
no. of florets pollinated	189	169	173
no. of seeds produced	0	0	0

TABLE 3

Effect of interplanting vegetatively propagated F1 and BC progenies from crosses involving ogu cms *B. napus* (cv Karat) and *R. sativus* (cv oilseed radish) in a stand of *B. napus* (cv Karat) under field conditions

	Generation			
	F1	BC1	BC2	BC3
no. of cuttings transplanted	58	34	31	25
no. of seeds harvested	12	7	2	0
no. of plants produced	0	0	0	0

TABLE 4

Chromosome pairing in F1 plants from the cross ogu cms *B. napus* (cv Karat) and *R. sativus* (cv oilseed radish)

F1 plant	no. of cells examined	average chromosome association		
		I	II	III
1	100	11.60	8.10	0.66
2	100	13.44	6.80	0.62
3	100	13.16	6.30	0.87
4	100	12.56	7.90	0.55
5	100	12.99	7.40	0.55
	overall average	12.75	7.30	0.65

TABLE 5

Anther and pollen development of F1 plants from crosses involving ogu cms *B. napus* (cv Karat) and cultivars of *R. sativus*

Cultivar	no. of F1's examined	anther development	pollen development
From Iraq	118	poor	none
Raonla	130	mod-good	none
Red Boy	109	moderate	none
French Breakfast	100	poor	none
Celery Belle	111	poor	none
White Icicle	186	mod-good	none
Long Black Spanish	86	moderate	none

poor 0 to 0.5 stigmaal length
moderate 0.5 to 1 stigmaal length
good 1 to 1.5 stigmaal length

TABLE 6

Seed production from crosses involving ogu cms B. napus (cv Karat) and pollen from white flowered B. napus (cv Regent) with a univalent or bivalent chromosome from R. sativus

Rep no.	no. of florets pollinated	no. of pods harvested	no. of seeds harvested	no. of seeds per pod	no. of seeds per floret pollinated
			ogu cms Karat X univalent strain		
1	123	65	964	14.83	7.84
2	475	318	4775	15.01	10.05
total	598	383	5739	14.92	8.95[1]
			ogu cms Karat X bivalent strain		
1	106	93	981	10.55	9.25
2	563	408	6581	16.13	11.69
total	669	501	7562	13.34	10.47[1]

[1]these values not significantly different at 5% level

TABLE 7

Effect of elevated temperature (30°C for 16 hr. day/26°C for 8 hr. night, for 7 days) on the seed production of ogu cms B. napus (cv Karat) pollinated with pollen from R. sativus

Rep no.	no. of florets pollinated	no. of pods harvested	no. of seeds harvested	no. of seeds per pod	no. of seeds per floret pollinated
			Treated group		
1	100	73	67	0.92	0.67
2	656	155	282	1.82	0.43
3	641	241	314	1.30	0.49
4	299	134	198	1.48	0.66
5	688	255	241	0.95	0.55
total	2384	858	1102	1.29	0.56[1]
			Control group		
1	243	67	3	0.045	0.012
2	184	51	11	0.22	0.06
3	431	180	10	0.555	0.023
total	858	298	24	0.27	0.032[1]

[1]these values significantly different at 5% level

TABLE 8

Effect of elevated temperature (30°C for 16 hr. day/26°C for 8 hr. night, for 7 days) on the seed production of *B. napus* (cv Karat) pollinated with pollen from *R. sativus*

Rep no.	no. of florets pollinated	no. of pods harvested	no. of seeds harvested	no. of seeds per pod	no. of seeds per floret pollinated
			Treated group		
1	61	13	51	3.92	0.836
2	260	68	249	3.66	0.958
total	321	81	300	3.79	0.90[1]
			Control group		
1	60	2	14	7.0	0.233
2	325	29	89	3.07	0.274
total	385	31	103	5.04	0.25[1]

[1]these values significantly different at 5% level

TABLE 9

Effect of elevated temperature (30°C for 16 hr. day/26°C for 8 hr. night, for 7 days) on the seed production of B. napus (cv Regent) pollinated with pollen from R. sativus

Rep no.	no. of florets pollinated	no. of pods harvested	no. of seeds harvested	no. of seeds per pod	no. of seeds per floret pollinated
			Treated group		
1	27	22	30	1.36	1.04
2	243	101	278	2.75	1.15
total	270	123	308	2.06	1.10[1]
			Control group		
1	267	56	109	1.95	0.41
2	310	112	120	1.07	0.39
total	577	168	229	1.51	0.40[1]

[1]these values significantly different at 5% level

Figure 1. F1 and BC generations from crosses involving ogu cms B. napus and R. sativus (oilseed radish).

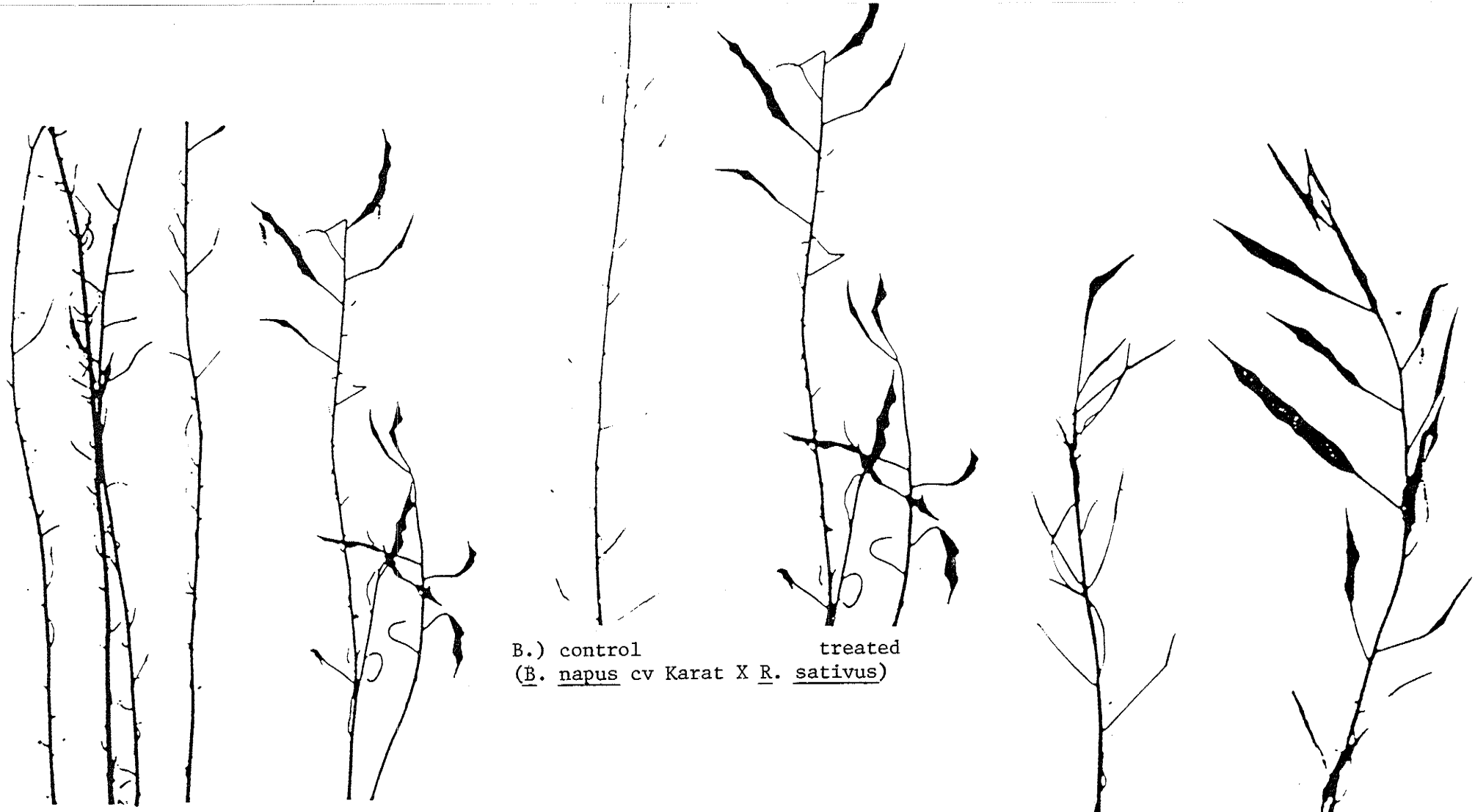

Figure 2. Branches of heat treated and control plants from the three intergeneric crosses.

DISCUSSION

RESTORER DEVELOPMENT

The possibility of developing a restorer for the ogu male sterile cytoplasm was investigated. Ogu male sterile Karat was crossed with an R. sativus cultivar known to possess fertility restorer gene(s). Crosses and backcrosses were made with the recurrent parent Karat to recover the B. napus genome while maintaining the restorer gene(s) from the radish in the ogu cytoplasm. The F1 and first three BC generations were produced, however, BC4 seeds were not obtained by pollinating the BC3 plants.

There may be a number of explanations for the difficulties encountered in developing a restorer for the ogu male sterile cytoplasm. The lack of homologous chromosomes in the F1 hybrids could have exerted a detrimental effect on female fertility. Doubled F1 hybrids were successfully produced (2n=56, AACCRR). These plants were male sterile and seeds were not produced from any crosses made onto them. Therefore, female fertility was low when the chromosomes were present in the univalent state and also when homologous chromosome pairing occurred. These results differ from results obtained by Heyn (1979). He crossed a hexaploid form of Raphanobrassica (2n=56, AACCRR) onto ogu cms B. napus to transfer restorer gene(s) from radish to B. napus. The Raphanobrassica was white flowered and self fertile. The Raphanobrassica

and the doubled F1 hybrids produced in this study both contained fertility restoration gene(s) but the doubled F1 hybrids were male sterile. The Raphanobrassica used by Heyn (1979) had a male fertile cytoplasm and the doubled F1 hybrids used in this study had a male sterile cytoplasm. Therefore, the presence of the male sterile radish cytoplasm in the hybrids may have had a detrimental effect on the expression of male fertility and also on female fertility.

The white flower color gene may have had a detrimental effect on female fertility. The seed production on white flowered BC1 plants was low. However, the seed production on the yellow flowered BC1 plant was almost normal. Possibly, a detrimental interaction could have taken place between the white flower color gene from the radish and the B. napus chromosomes in the male sterile radish cytoplasm. This interaction would effect female fertility whenever the white flower color gene was present in the genome. When this gene was eliminated from the genome, as it was in the yellow flowered BC1 plant, female fertility increased. Kato and Tokumasu (1976) reported similar results in their experiments. They found yellow flowered Brassicoraphanus (n=19) plants (B. japonica X R. sativus) which had unusually good female fertility. These plants were produced from white flowered F1's. They assumed that the yellow flower color gene from B. napus and the white flower color gene from R. sativus were located at corresponding loci of only partially homologous chromosomes. They also assumed that the gene for flower color was closely linked to a gene which controlled the development of embryos (or endosperm) and this gene promoted the development of embryos when it was in the homozygous condition. Therefore, the embryo having only the

yellow flower color gene would develop more easily into viable seed than the embryo having both the white and yellow flower color gene. In the present study, embryos from white flowered hybrids were extracted and placed on culture media to develop. However, no plants were obtained from cultured embryos. Kato and Tokumasu (1976) also suggested that the sterility of the white flowered Brassicoraphanus plants could have been caused by a discordance between the cytoplasm of Brassica and the white flower color gene from Raphanus. The F1 hybrids produced in this study were in the male sterile cytoplasm from Raphanus, therefore, the same explanation does not appear to be applicable.

Sernyk and Stefansson (1982) produced homozygous (2n=40) and heterozygous (2n=39) white flowered rapeseed strains. The white flower color chromosome was present in the bivalent or univalent condition, respectively. Vigor and female fertility in these two strains were very similar to normal yellow flowered rapeseed. Using these strains, similar results were observed from a test which compared the effect on seed production of a univalent and bivalent chromosome from the radish. Pollen from the B. napus strains with the radish chromosome in the univalent or bivalent state did not effect seed production when crossed with ogu cms Karat. Apparently, the white flower color gene from the radish did not effect seed production in these two experiments. However, the two experiments involved intraspecific crosses while the development of the restorer for the ogu male sterile cytoplasm and the Brassicoraphanus plants produced by Kato and Tokumasu (1976) involved an intergeneric cross. Also, there was only one radish chromosome present in the genomes of the plants used in the two experiments while all

radish chromosomes were present in the genomes of plants used to produce the Brassicoraphanus plants and in the development of the restorer. Therefore, the white flower color (gene or chromosome) by itself does not explain male and female sterility encountered in the intergeneric hybrids.

The number of pollinations made to produce F1, BC1, BC2 and BC3 plants may not have been large enough to obtain a restorer if restoration depends on complex gene and/or chromosome combinations. If the inheritance of fertility restoration was conditioned by a single dominant gene, then theoretically 50% of the BC1's should have been male fertile. In this study one of 18 BC1 plants showed sporadic pollen development, the rest were male sterile. Therefore, the inheritance of fertility restoration for the ogu male sterile cytoplasm was probably not simple.

Other methods may be more useful for developing a restorer for the ogu male sterile cytoplasm. Protoplast fusion, which has received considerable attention recently, has been used successfully to create a non chlorotic ogu male sterile B. napus genotype (Pelletier, 1983). The flowers of this plant type also have normal nectary development. The favorable recombinations were achieved by using protoplast fusion to combine mitochondria from ogu male sterile B. napus with chloroplasts from triazine resistant B. napus plants.

CYTOLOGICAL INVESTIGATIONS

The chromosome pairing was examined in pmc's from the cross ogu cms Karat X R. sativus. At diakinesis of meiosis I an average of 12.75 univalents, 7.3 bivalents and 0.65 trivalents were present. The introgression of Raphanus genes may depend on pairing between Raphanus and Brassica chromosomes (Dolstra, 1982b). Allosyndetic pairing between R and A or C chromosomes would probably be low when they are present in the same genome because of homoeologous pairing between A and C chromosomes of B. napus. Thus, introgression of Raphanus characters into B. napus chromosomes may be difficult.

POTENTIAL SOURCES OF RESTORATION FOR THE OGURA MALE STERILE CYTOPLASM

Seven other R. sativus cultivars were examined as potential sources of restoration for the ogu male sterile cytoplasm. However, no pollen was observed in any F1 plants involving the seven cultivars. Thus, these strains do not appear to carry restorers for the ogu male sterile cytoplasm and other cultivars would be needed as sources of restoration.

THE EFFECT OF HIGH TEMPERATURE ON SEED PRODUCTION IN THE FEMALE PARENT INVOLVED IN AN INTERGENERIC CROSS

The effect of elevated temperatures on seed production in intergeneric crosses was evaluated. Three different B. napus parents were used as female parents in the crosses. The female parents were subjected to heat treatment and crossed with R. sativus. Control groups of untreated female parents were used for comparison. Seed production on the heat treated plants was significantly greater than that on the control groups.

These results are different from results obtained by researchers using other crops. Anderson and Taylor (1974) reported that high temperature treatment did not facilitate interspecific (T. pratense X T. medium) or intraspecific (diploid X tetraploid T. pratense) hybridization. However, heat treatment of the female parent has been used to break down the SI reaction in certain clover species such as red clover, T. pratense (Leffel, 1963; Kendall and Taylor, 1969; and Townsend, 1968), and in R. sativus (El Murabaa, 1957). In the present study high temperature treatments consistently improved seed production in intergeneric crosses between B. napus and R. sativus. However, genotypic differences were also apparent. Heat treatments probably do not have the same effect on all interspecific, intraspecific or intergeneric crosses and it appears to be necessary to determine the conditions which give the best results for difficult crosses.

SUMMARY

The possibility of developing a restorer for the ogu male sterile cytoplasm in B. napus was investigated. Crosses were made between ogu male sterile B. napus (cv Karat) and an R. sativus cultivar known to possess fertility restoration gene(s). Crosses and backcrosses were made with the recurrent parent Karat to recover the B. napus genome while maintaining the restorer gene(s) from the radish in the ogu cytoplasm. The F1 and first three BC generations were produced, however, BC4 seeds were not obtained by pollinating the BC3 plants.

Cuttings from the F1, BC1, BC2 and BC3 generation were transplanted to blank rows left in a dense stand of Karat in a field plot located at the University of Manitoba. This was done in an effort to obtain more seed than was available from manual pollinations. Hybrids between B. napus and R. sativus were treated with colchicine to produce doubled hybrids. The doubled hybrids were crossed with Karat. Embryos were cultured to facilitate production of hybrids. However, none of these procedures resulted in the development of a restorer. The presence of the male sterile radish cytoplasm in the hybrids may have had a detrimental effect on the expression of male fertility and also on female fertility. The white flower color gene from the radish may have also effected female fertility when it was present in the genome. Another possibility is that the number of pollinations made to produce F1, BC1, BC2 and BC3 plants may not have been large enough to obtain a

restorer if restoration depends on complex gene and/or chromosome combinations. The lack of restorer development indicated that the inheritance of fertility restoration for the ogu male sterile cytoplasm was probably not simple.

The chromosome pairing was examined in pmc's from the cross ogu cms B. napus X R. sativus. At diakinesis of meiosis I an average of 12.75 univalents, 7.3 bivalents and 0.65 trivalents were present. The bivalent associations probably arose from homoeologous chromosome pairing between the A and C genomes of napus.

Seven other R. sativus cultivars were examined as potential sources of restoration for the ogu male sterile cytoplasm. However, no pollen was observed in any F1 plants involving the seven cultivars.

High temperature treatment of the female parent before flowering significantly increased seed production in intergeneric crosses. Three different female parents were tested; ogu cms Karat and the two B. napus cultivars Regent and Karat. These were crossed with R. sativus and compared with control groups of untreated female parents. There were genotypic differences between the female parents. Pods formed and developed quickly when B. napus (cv Regent) was heat treated and crossed with R. sativus. Pod formation and development occurred more slowly when ogu cms Karat and B. napus (cv Karat) were used. Regent appeared to be more cross compatible with R. sativus than the other two female parents in both treated and control groups.

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