COMPARISON OF POL AND NORMAL CYTOPLASMS IN RECIPROCAL CROSSES OF BRASSICA NAPUS L.

D.D. Chamberlain, C.B. Hill, L.M.J. Sovero

Calgene, Inc., 7664 Moore Road, Memphis, TN 38120

INTRODUCTION

Manually produced F1 hybrids of oil seed rape, Brassica napus L., exhibit significant high parent heterosis for seed yield (Sernyk and Stefansson 1983). A pollination control system such as cytoplasmic male sterility (cms) is required to gain this potential yield advantage in commercially produced F1 hybrid seed.

Cytoplasmic male sterile plants found in Polima, a cultivar of B. napus, from Poland were reported by Fu (1981). This cms which is maintained or partially maintained by most strains of B. napus, was designated as pol cms. One B. napus cultivar, Italy, has been found that will restore the fertility of pol hybrids. The restoration ability of Italy has been attributed to a single dominant nuclear gene, (Rf).

The pol cms has been widely used in university and commercial breeding programs since 1981. Studies of the cytoplasmic effect on characteristics of pol hybrids have been made to determine the practical value of the pol cms system. McVetty et al. (1990) reported that pol hybrids showed significantly lower seed yield and significantly lower oil percent compared to nap hybrids. However, Fu et al. (1990) reported that pol cytoplasm had no deleterious effects on seed yield or on yield components such as siliqua number. Both of these studies were comparisons of A-line (cms) x restorer hybrids with corresponding B-line (maintainer) x restorer hybrids.

A-line x restorer compared to B-line x restorer hybrids will differ some in nuclear genotype depending on the number of backcrosses of the A-line from the B-line, and on the degree of homozygousity of the B-line. Differences in data caused by a slight variation in nuclear genotype cannot be distinguished from differences caused by variation between cytoplasmic genotypes. This experiment has been designed to compare normal cytoplasm and pol cytoplasm crosses that are 100% identical in every way except for their cytoplasm by using equal seed quantities from individually paired reciprocal crosses.

MATERIALS AND METHODS

Three rapeseed cultivars with normal cytoplasm, Bienvenue, Cascade and Santana, were reciprocally crossed with a restorer line having pol cytoplasm. The restorer line was homozygous for the restorer gene (Rf). The restorer line plants and the cultivars were male fertile so that crossing in both directions required hand emasculation and cross-pollination. These hand crosses were named: HC24, HC25 and HC28, respectively, with a suffix N or P to identify the cytoplasm.

Eighteen plants of each of the three cultivars were paired with a restorer line plant, (54 total plant pairs). Each pair was crossed reciprocally. The nuclear genotypes of the progeny produced by the two plants of each pair were identical, while the cytoplasm genotypes differed. The cross-pollinations to Bienvenue, Cascade and Santana plants produced progeny with normal cytoplasm, the crosses to the restorer line plants produced progeny with pol cytoplasm.

To minimize sampling error (since the genotypes of plants from the same cultivar may differ slightly), an equal amount of seed was saved from each of the the two plants that were paired. Excess seed produced by either the normal plant or the pol plant was discarded from the study. Seeds from the 18 normal plants were pooled, as were equal quantities of seeds from the 18 pol restorer plants. Therefore, the nuclear genotypes of the three lots of normal cytoplasm seed should have been identical to that of the three lots of pol cytoplasm seed. At least 100 grams of seed were produced for each of the six crosses.

Except for the cytoplasm, the only difference between the paired crosses was a slight difference in the size of seed produced by the different parent plants. To minimize differences in planting rate, planting packets each containing 900 seeds were prepared. The number 900 was established by weighing 5 grams of the check cultivar Cascade. (5 grams Cascade seed = 900 seeds.). Cascade was chosen as the check cultivar because it was in the same maturity class as the crosses.

The six crosses plus Cascade were planted at five locations in September and October, 1989. Plots four feet wide and twenty-five feet long were planted in four randomized complete blocks at each location. Three of these trial locations survived the winter: Stoneville, Mississippi, Memphis, Tennessee, and Carmi, Illinois.

Plot stands were rated in the fall and in the spring. Winter survival was estimated by dividing spring stand by fall stand. Winter survival notes were taken only at Memphis and Stoneville where winter damage occurred. Fifty percent bloom dates were estimated and plant heights were measured. Full plots were harvested with a plot combine and yields were calculated in pounds per acre based on weight of seed adjusted to 8% moisture. Oil percent was measured on one seed sample from each replication by NMR.

Planned statistical comparisons between normal and pol cytoplasms, and between the different hybrid crosses were made using orthogonal contrast methodology. This analysis was run on SAS computer software.

RESULTS AND DISCUSSION

Table 1 Presents the mean yields for the lines within and over the three locations. Eight of the nine yield comparisons of normal with pol cytoplasm were non significant ($P>0.05$). The one significant comparison occurred at Carmi where HC25P yielded significantly higher than HC25N. These two crosses, which flowered about six days ahead of the others, were damaged by a late spring frost in Carmi while they were in full flower. It is unknown why HC25N was damaged more than HC25P. In Memphis, yields were reduced in both HC24N and HC24P because of low winter survival rates of 40% and 31%, respectively. This damage was a result of temperatures as low as -23 C in December. These same crosses were also most effected by the cold temperatures in Stoneville where their winter survival rates were 60% and 58%, respectively. The Carmi trial was protected by a snow cover in December, but a heavy frost damaged early flowering lines on March 28.

Table 2 presents the means of the percent oil of the crosses within and over the three locations. Analysis indicates no significant difference in percent oil for any of the comparisons.

Table 3 presents normal vs. pol cytoplasm comparisons of the three hybrids individually and collectively, and also comparisons of each hybrid with the other hybrids. Five parameters, seed yield, oil percent, winter survival percentage, fifty percent bloom date, and plant height are compared over locations.

Pol cytoplasm did not differ significantly from normal cytoplasm for seed yield over all crosses over locations. In individual crosses HC25N vs. HC25P was significantly different at one location, Carmi where the pol cytoplasm cross yield higher than the normal cytoplasm cross. However, in comparisons of the various hybrid yields, two of the three were highly significant over locations.

Winter survival of N was significantly higher than P at Memphis where there was more plant death in pol hybrids. All winter survival comparisons between the different hybrids were highly significant at Stoneville and Memphis, as well as in the combined locations. The difference in winter survival between cytoplasms detected at Memphis was minor compared to the highly significant differences observed in survival comparisons of the different hybrids.

Fifty percent bloom date for HC25N differed significantly from HC25P at Memphis and over locations, but not at Carmi. Highly significant differences were detected in bloom dates for two of the three hybrid comparisons over locations.

HC28N was 4.6 cm taller than HC28P, a significant difference across locations. However, no difference was detected when comparing N vs P.

CONCLUSIONS

The few significant cytoplasmic differences observed in this study are evidence of possible cytoplasmic-nuclear interactions which may be expressed under certain environmental conditions. Lack of any consistent trends indicates that chance alone may offer another explanation for the differences detected. Differences in cytoplasmic comparisons were minor compared to those observed between the hybrids. There is no evidence in this study that there are any deleterious effects on hybrids with pol cytoplasm when compared to those with normal cytoplasm.

REFERENCES

FU, T.D. 1981. Production and Research of Rapeseed in the People's Republic of China. Eucarpia Cruciferae Newsletter 6: 6-7.

FU, T.D., YANG, G. and YANG, X. 1990. Studies on "Three Line" Polima Cytoplasmic Male Sterility Developed in Brassica napus L. Plant Breeding 104, 115-120.

McVETTY, P.B.E., EDIE, S.A. and SCARTH, R. 1990. Comparison of the Effect of Nap and Pol Cytoplasms on the Performance of Intercultivar Summer Oilseed Rape Hybrids. Can. J. Plant Science 70: 117-126.

SERNYK, J.L. and STEFANSSON, B.R. 1983. Heterosis in Summer Rape (Brassica napus L.). Can. J. Plant Sci. 63: 407-413.

TABLE 1. YIELDS OF POL AND NORMAL CYTOPLASM RECIPROCAL CROSSES, 3 LOCATIONS.

CROSS	STONEVILLE	DIF. LSD .05 494	MEMPHIS	DIF. LSD .05 303	CARMI	DIF LSD .05 533	3 LOC. AVE.	3 LOC. DIF.
HC24N	2112		1014		2564		1897	
HC24P	2447	335	875	-139	2329	-235	1884	-13
HC25N	2761		1402		1919		2027	
HC25P	2848	87	1285	-117	2616	697*	2250	223
HC28N	2847		1318		2456		2207	
HC28P	2466	-381	1256	-62	2728	272	2150	-57
NORMAL	**2573**		**1245**		**2313**		**2044**	
POLIMA	**2587**	**14**	**1139**	**-106**	**2557**	**244**	**2095**	**51**
CASCADE	1666		726		2067		1486	

TABLE 2. OIL % OF POL AND NORMAL CYTOPLASM RECIPROCAL CROSSES, 3 LOCATIONS.

CROSS	STONEVILLE	DIF.	MEMPHIS	DIF.	CARMI	DIF.	3 LOC. AVE.	3 LOC. DIF.
HC24N	38.4		39.6		37.2		38.4	
HC24P	38.1	-0.3	39.8	0.2	36.6	-0.6	38.2	-0.2
HC25N	38.2		38.4		37.0		37.9	
HC25P	38.2	0.0	38.3	-0.1	37.3	0.3	37.9	0.0
HC28N	38.5		39.2		37.0		38.2	
HC28P	37.9	-0.6	39.5	0.3	36.9	-0.1	38.1	-0.1
NORMAL	**38.4**		**39.1**		**37.1**		**38.2**	
POLIMA	**38.1**	**-0.3**	**39.2**	**0.1**	**36.9**	**-0.2**	**38.1**	**-0.1**
CASCADE	37.2		37.6		37.6		37.5	

TABLE 3. CONTRASTS FOR SEED YIELD, OIL PERCENT, WINTER SURVIVAL PERCENTAGE, 50% BLOOM DATE AND PLANT HEIGHT OVER LOCATIONS.

CONTRAST	SEED YIELD F VALUE	PR > F
HC24N VS HC24P	0.02	0.8840
HC25N VS HC25P	3.18	0.0823
HC28N VS HC28P	0.00	0.9661
N VS P	0.86	0.3591
24 VS 25	7.23	0.0105
24 VS 28	10.65	0.0023
25 VS 28	0.42	0.5208

CONTRAST	OIL PERCENT F VALUE	PR > F
HC24N VS HC24P	0.38	0.5420
HC25N VS HC25P	0.02	0.8980
HC28N VS HC28P	0.06	0.8122
N VS P	0.19	0.6675
24 VS 25	1.23	0.2732
24 VS 28	0.09	0.7651
25 VS 28	0.66	0.4197

CONTRAST	WINTER SURVIVAL F VALUE	PR > F
HC24N VS HC24P	0.05	0.8332
HC25N VS HC25P	0.44	0.5138
HC28N VS HC28P	2.35	0.1355
N VS P	1.93	0.1748
24 VS 25	154.57	0.0001
24 VS 28	43.23	0.0001
25 VS 28	34.31	0.0001

CONTRAST	DAYS TO 50% BLOOM F VALUE	PR > F
HC24N VS HC24P	0.18	0.6744
HC25N VS HC25P	4.77	0.0378
HC28N VS HC28P	0.01	0.9282
N VS P	2.28	0.1429
24 VS 25	186.23	0.0001
24 VS 28	0.01	0.9341
25 VS 28	194.02	0.0001

CONTRAST	PLANT HEIGHT F VALUE	PR > F
HC24N VS HC24P	0.14	0.7057
HC25N VS HC25P	0.26	0.6149
HC28N VS HC28P	5.20	0.0274
N VS P	1.55	0.2202
24 VS 25	2.32	0.1349
24 VS 28	17.72	0.0001
25 VS 28	7.22	0.0101