Effects of temperature, and age of siliques, on hybrid embryo yield from interspecific crosses between *Brassica napus* and *B. oleracea* var. *alboglabra*

R. Bennett, M. R. Thiagarajah, J. R. King, M. H. Rahman

Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada T6G 2P5.
Email: Habibur.Rahman@afhe.ualberta.ca

Abstract

Reciprocal crosses were made between *Brassica napus* (AACC) and *B. oleracea* var. *alboglabra* (CC) with the objective of broadening genetic diversity in *B. napus*. An ovule culture technique was applied to obtain interspecific hybrid plants, and the efficiency of this technique was investigated between 6 and 16 days after pollination (DAP) under two temperature conditions, 20/15°C and 15/10°C (day/night). Due to interspecific crossability barriers, a low total number of interspecific hybrid embryos/plants were obtained. Data were therefore pooled across plants and a chi-square test applied for statistical analysis. Response to temperature differed depending on which species was the female parent. Using *B. oleracea* var. *alboglabra* as the female parent, a significantly greater number of hybrid embryos were obtained from plants grown at 20/15°C, and between 12-16 DAP. With *B. napus* as the female parent, hybrid embryo numbers were greatest at 15/10°C, and 14-16 DAP. Thus, for this particular cross, the rate of interspecific hybrid production depends greatly on the interaction between the female parent and the growing temperature. The slower growing parent, *B. oleracea* var. *alboglabra,* was more productive in generating interspecific hybrids under higher temperatures.

Key words: Interspecific hybrid, ovule culture, *Brassica napus*, *Brassica oleracea* var. *alboglabra*

Introduction

Genetic diversity in canola (*Brassica napus*, AACC, 2n=38) is narrow (Hasan et al., 2003; Seyis et al., 2003), and this needs to be broadened. The resynthesis of *B. napus* by crossing *Brassica rapa* (AA, 2n=20) and *Brassica oleracea* (CC, 2n=18) has been considered to be an important strategy for broadening the genetic diversity in *B. napus* (Seyis et al., 2003; Engqvist and Becker, 1994). However, in this approach non-canola quality traits are also introduced into the seeds of resynthesized *B. napus* from its parental species, especially from *B. oleracea* where no canola quality (⩽2% erucic acid in oil and ⩽30 μmol glucosinolate/g seed meal) germplasm is available. In this context, development of a canola quality *B. oleracea* is needed for efficient broadening of the genetic diversity in canola. This can be accomplished through introgression of canola quality genes from *B. napus* by crossing and backcrossing between these two species.

The cross between *B. napus* and *B. oleracea* is known to be quite difficult (Downey et al., 1980). It has been used to transfer self-incompatibility traits (Ripley and Beversdorf, 2003) and cabbage aphid resistance (Quazi, 1988) from *B. oleracea* to *B. napus*; as well as triazine resistance (Ayotte et al., 1987) and resistance to *Plasmodiophora brassicae* (Chiang et al., 1977) from *B. napus* to *B. oleracea*. In *Brassica* it often occurs that self-pollinated progeny from the cross between amphidiploid and diploid species is stabilized into the amphidiploid type; while backcrossing of the hybrids with the diploid parent yields diploid type plants in the segregating population (Rahman, 2001; Zaman, 1988). Therefore, it is difficult to introgress a trait from canola *B. napus* into *B. oleracea,* as extensive effort is needed to obtain viable F_1 hybrids; as well as backcrossing the F_1 with *B. oleracea* to obtain BC_1 hybrids. To create an ample amount of offspring to work with, an efficient method of producing F_1 and BC_1 hybrids from these two species is needed. The objective of this study was to determine the optimal temperature and time of rescue of hybrid embryos for the interspecific cross between *B. napus* and *B. oleracea* var. *alboglabra.*

Materials and methods

Two canola quality *Brassica napus* doubled haploid lines, HiQ and A01-104NA, and one highly inbred (F_7) non-canola quality *Brassica oleracea* var. *alboglabra* line were used. Parental plants were grown in two growth chambers set at 20°/15°C and 15°/10°C day/night temperatures with a 16 hr. diurnal period. In each growth chamber the three parents were grown in two replications. There were four plants for each *B. napus* parent and eight *B. oleracea* plants in each replication. Reciprocal crosses were made where individual female plants were pollinated with bulk pollen from 4-8 randomly selected male parents. For all crosses siliques were harvested at two day intervals between 6 and 16 days after pollination (DAP), and were used for rescue of hybrid embryos. Excised siliques were surface sterilized with a 7% calcium hypochlorite solution for 10 minutes, and then rinsed twice with distilled water. The siliques were longitudinally dissected under aseptic conditions, and developing (fertilized) ovules were excised and counted. Developing ovules were determined to be those with a healthy and non-shrunken appearance. A small incision was made on the non-micropylar end of the developing ovules and these were floated on approximately 5 mL liquid medium in a 60×15 mm petrie dish. Liquid medium was the same as that used by Ripley and Beversdorf (2003): Nitsch and Nitsch (1967) medium supplemented with 300 mg L^{-1} casein hydrolysate, 200 mg L^{-1}

glutamine, and a 13% concentration of sucrose. The medium was adjusted to a pH of 6.0, and was filter-sterilized. Cultured ovules were placed on a shaker set at 60 rpm. After 2-3 weeks on the shaker, the number of developing embryos resulting from cultured ovules was recorded.

Comparisons of mean fertilization rates of ovules were done by two sample t-test with unequal variances. For yield of hybrid embryos, chi-square analysis was performed using proc CATMOD in the SAS system (Statistical Analysis System, Inc. 1999), with a null hypothesis that there was no difference in the number of hybrid embryos per pollination between the six DAP (6-16) and two temperatures (20°/15°C and 15°/10°C). Due to the small number of embryos produced, data from the crosses involving HiQ and A01-104NA were pooled as '*B. napus*'. Self-pollination of *B. napus* and *B. oleracea* were used as a control in this experiment.

Results

Self-pollination of *B. napus* yielded (17.0 ± 2.4) fertilized ovules per silique, while self-pollination of *B. oleracea* yielded (9.8 ± 5.7) fertilized ovules per silique. In general, interspecific crosses using *B. napus* as the female parent yielded a lower number of fertilized ovules per silique (3.8 ± 2.3) than crosses using *B. oleracea* as the female (7.4 ± 3.8). No significant difference for number of fertilized ovules was found due to growth condition (15°C vs. 20°C) while using *B. napus* as the female parent. However, using *B. oleracea* as the female parent, a significantly higher number of fertilized ovules per silique were obtained at a lower temperature (4.4 ± 1.9 vs. 9.8 ± 3.4; $t=4.78$, $p \leqslant 0.01$; Table 1). This was also evident from self-pollination of *B. olerecea*.

Table 1. Number of fertilized ovules for siliques harvested from 6-16 DAP following reciprocal interspecific crosses between *Brassica napus* and *B. oleracea* var. *alboglabra*

Cross	Temp. (°C)	No. pollinations	No. fertilized ovules	No. fertilized ovules / poll. (Mean ± S.E.)
HiQ×*B. oleracea* var. *alboglabra*	20/15	91	304	3.3 (± 3.2)
	15/10	115	309	2.7 (± 1.4)
A01-104NA×*B.oleracea* var. *alboglabra*	20/15	114	340	3.0 (± 1.5)
	15/10	116	715	6.2 (± 3.8)
B. oleracea var. *alboglabra*×HiQ	20/15	88	330	3.8 (± 2.3)
	15/10	108	1002	9.3 (± 4.4)
B. oleracea var. *alboglabra*×A01-104NA	20/15	92	454	4.9 (± 2.2)
	15/10	123	1251	10.2 (± 3.2)
Selfed *B. napus*	20/15	29	522	18.0 (± 3.1)
	15/10	34	550	16.2 (± 5.9)
Selfed *B. oleracea* var. *alboglabra*	20/15	27	172	6.4 (± 3.4)
	15/10	30	388	12.9 (± 5.5)

Table 2. Number of hybrid embryos from siliques harvested between 6-16 DAP and developed under two temperature conditions for reciprocal crosses between *B. napus* and *B. oleracea* var. *alboglabra*

Cross	*B. napus*×*B.oleracea* var. *alboglabra*		*B. oleracea* var. *alboglabra*×*B. napus*	
Temp. (°C)	20/15	15/10	20/15	15/10
No. pollinations	205	231	180	231
No. embryos produced	11	17	29	5
No. embryos / pollination	0.05	0.07	0.16	0.02

Embryo yield was extremely low/zero at 6-8 DAP in all crosses. A significant interaction was found between temperature and DAP ($\chi^2=9.46$, $P \leqslant 0.05$) for embryo yield. Overall, using *B. napus* as the female parent, the greatest efficiency of embryo rescue occurred when siliques were harvested at 14 DAP and developed under 15°/10°C temperature, where 0.26 embryos per pollination were rescued (Figure 1). On the other hand, using *B. oleracea* as the female parent, the yield of rescueable embryos was significantly greater at a higher temperature than at a lower temperature ($\chi^2=17.22$, $P \leqslant 0.01$; Table 2). For this cross, only 0.7 % of fertilized ovules at a lower temperature yielded rescueable embryos, compared to 5.6% of fertilized ovules at a higher temperature. The highest number of embryos per pollination was obtained at 16 DAP under 20°/15°C, yielding 0.36 rescueable embryos per pollination (Figure 1). Furthermore, 97% of all embryos were obtained at 12-16 DAP for this cross.

Discussion

Several investigations have been done to improve the hybridization efficiency between relatives in the *Brassica*ceae family (Rahman, 2004; Zhang et al., 2004; Inomata, 1993; Ayotte et al., 1987; Bajaj et al., 1986; Takeshita et al., 1980). These studies focused primarily on the method of embryo rescue, time of harvest of hybrid embryos (DAP), and type of culture media. To the best of our knowledge, no studies have been done on the effect of growing temperature and age of siliques on the efficiency of embryo rescue for the production of interspecific hybrids. The data presented in this paper suggest that the efficiency of embryo rescue in a *B. napus* and *B. oleracea* var. *alboglabra* interspecific cross depends greatly on the interaction between maternal genotype and temperature. The slower growing species *B. oleracea* var. *alboglabra* yielded the greatest number of hybrid embryos under higher temperatures, and at 12-16 DAP. On the other hand, the relatively rapid growing species *B. napus* yielded the greatest number of hybrid embryos under lower temperatures, and at 14-16 DAP. The results of this research can be applied for efficient production of interspecific hybrids of *B. napus*×*B. oleracea* crosses.

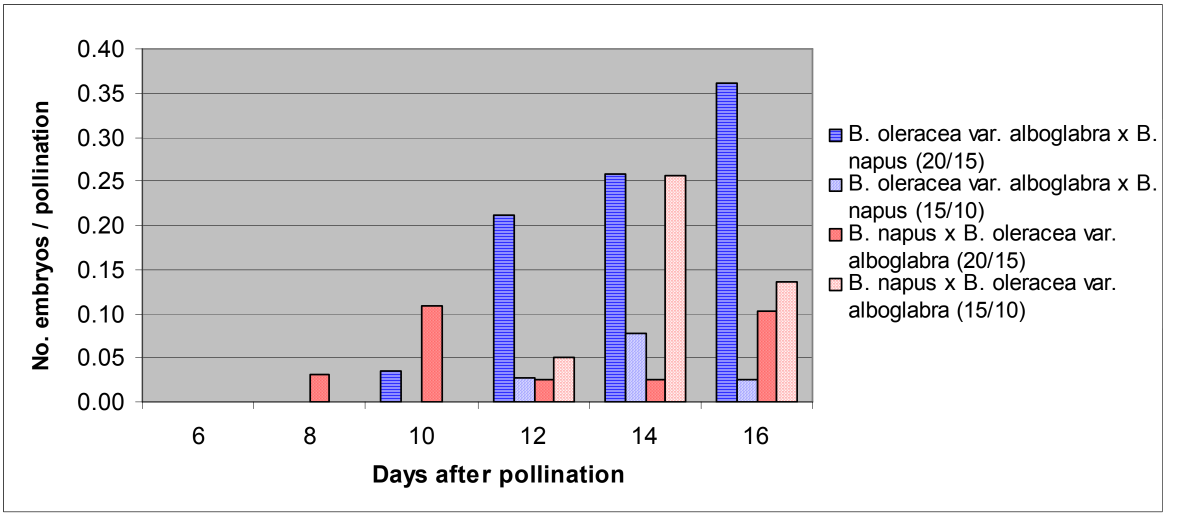

Figure 1: Hybrid embryo yield from two temperatures and different harvest dates for reciprocal crosses of *B. napus* and *B. oleracea* var. *alboglabra* (average values of *B. napus*, HiQ and A01-104NA, shown)

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