Interspecific and Intergeneric Hybridization Between Brassicaceae Species

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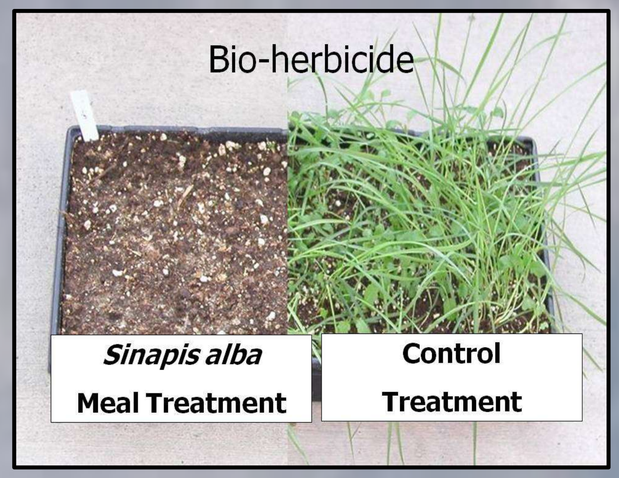
Tarped Methyl Bromide soil fumigation

ABSTRACT

Plants in the Brassicaceae family contain glucosinolates which degrade to toxic compounds that have biopesticides effects. *Brassica napus, B. carinata, B. juncea, B. nigra, Sinapis alba* and *S. arvensis* were inter-crossed to incorporate the different biopesticidal qualities of these species into cultivars which provide alternatives to synthetic soil fumigants. Interspecific hybridization combined with ovary culture, embryo rescue and colchicine-induced chromosome doubling have produced several hybrid combinations that produce the glucosinolate types of both parents.



INTRODUCTION

Restrictions in the registration and use of very effective but environmentally unsound fumigants such as methyl bromide and metam sodium have led to increased interest in the development of more biologically-friendly pest control alternatives. Plants in the mustard (Brassicaceae) family contain glucosinolates that hydrolyze to form highly toxic degradation products that have shown to have pesticidal properties. These toxic secondary products include nitriles, thiocyanates and isothiocyanates. Different Brassicaceae species contain characteristic glucosinolate profiles with varying amounts of specific glucosinolate types, and their degradation products differ in their biopesticidal effects. High glucosinolate content *B. napus* seed meal has been shown to reduce fungi and bacteria in the soil while *B. juncea* seed meal has been shown to effectively control nematodes and insect pests. Seed meal of *S. alba* appears to be most effective in weed control. Related species such as *B. nigra, B. carinata* and *B. rapa* have increased levels of specific glucosinolates that may target a variety of pests. Our goal is to incorporate the biofumigant properties of cultivated and exotic Brassicaceae species into new crops that can be used to replace synthetic fumigants. Using interspecific and intergeneric hybridization aided by ovary culture, embryo rescue and colchicine-induced doubling, we have produced several hybrid combinations. The aim of this study was to determine which species cross combinations would successfully produce viable hybrids.

MATERIAL AND METHODS

Parental species and lines were selected based on glucosinolate profile. Several cycles of planting and culturing have been carried out since the initial planting in August, 2005. Plants were grown in a growth chamber at 15°C day/10°C night temperatures with 16h daylight.

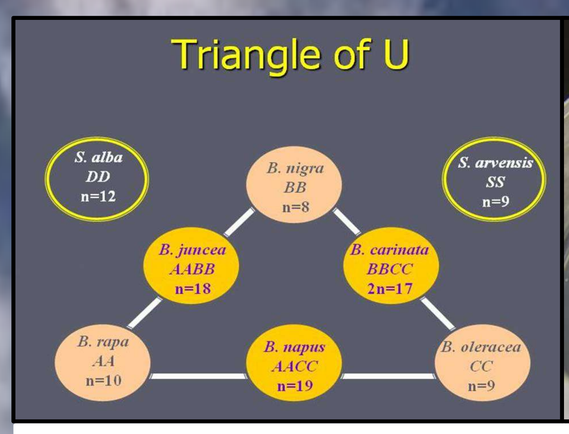


Figure 1. Cross compatibility between Brassicaceae species.

Figure 4. *S. alba* pods being cultured before embryo rescue.

Figure 2. Cross pollination conducted in a glasshouse.

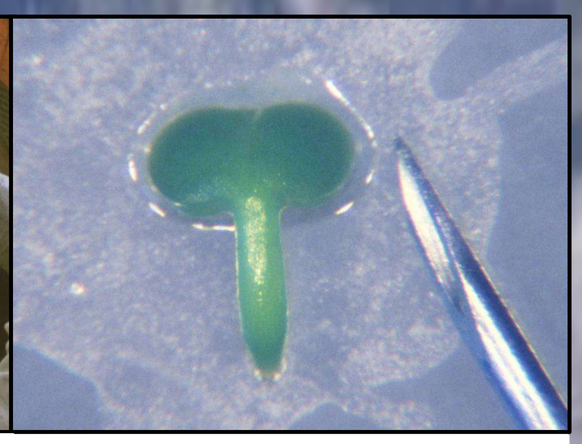


Figure 5. Developing embryo just after embryo rescue.

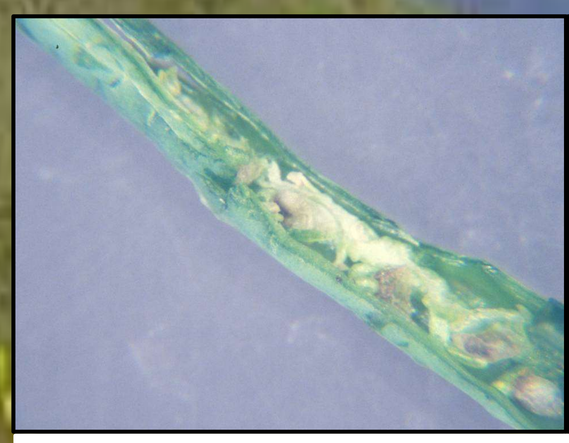


Figure 3. Aborting embryos after interspecific hybridization.

Figure 6. Hybrid embryo developing root *in vitro*.

Species	*S.alb*	*S.arv*	*B.ju*	*B.ra*	*B.ni*	*B.ca*
S. alba	-	√	√			
S. arvensis	√	-		√	√	
B. juncea	√		-	√	√	√
B. rapa	√	√	√	-	√	√
B. nigra					-	
B. carinata	√	√	√	√	√	-

Figure 7. Species combinations where hybrids were sucessfully developed.

Cross	Allyl	3-But	4-Pent	OH-But	OH-Benz
B.carinata x B.nigra	194.4	0.4	-	0.6	-
B.juncea x B.carinata	209.0	0.9	-	-	-
B.carinata x S.arvensis	133.6	9.6	2.1	21.8	15.8
B.carinata x S.arvensis	71.7	4.2	1.2	9.6	55.2

Figure 8. Glucosinolate profile of selected hybrid crosses.

MATERIAL AND METHODS (cont)

Hybridizations were carried out by hand-pollination and crosses were bagged to prevent contamination. Female parents were self-pollinated in addition to the crosses and were treated as protocol controls. Pods were harvested after seven to ten days of development, surface-sterilized and placed on Murashige and Skoog® basal salt media containing 25g/L of sucrose. After 7 plus days, pods were dissected under sterile conditions using a Leica dissecting scope and any embryos were transferred to fresh media. Viable embryos developed into plantlets which were grown to maturity in the greenhouse. Data collected included numbers of crosses made, pods cultured, and stage of embryos.

RESULTS AND DISCUSSION

Interspecific and intergeneric hybridization has resulted in the hybrids shown in Figure 7. More than 50 hybrids have been produced and have survived to maturity and produced seed. Preliminary chromosome studies and glucosinolate profile analyses indicate that these hybrids are allopolyploids. The hybrids have been shown to contain the glucosinolates of both parents. Field trials are in progress to increase seed supply and test for efficacy of the hybrids' biofumigation properties. These results indicate that interspecific hybridization is not without difficulties but hybrid combinations were produced with unique glucosinolate profiles and with increased glucosinolate levels (Figure 8), which could be used in the development of '*Designer Biofumigants*'.

PROBLEMS ENCOUNTERED

- It is difficult to make wide crosses using exotic germplasm as the embryo often fails to develop past the heart stage even if the cross itself is successful.
- Plantlets have limited survival and can be very difficult to transplant.
- Embryos developed roots and cotyledons but failed to develop meristems, leading to embryo death.