Breeding of yellow seeded *Brassica napus* L. var. *oleifera* via wide crosses in *in vivo* and *in vitro* conditions

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Abstract

The crossability in interspecific crosses of male sterile (MS) and fertile *B. napus* var. *oleifera* (2n = AACC = 38) with four yellow and brown seeded *Brassica* species i.e. *B. campestris* ssp. *trilocularis* (yellow seeded turnip rape, 2n = AA = 20), *B. campestris* ssp. *pekinensis* (brown seeded pekinensis cabbage, 2n = AA = 20), *B. hirta* (white mustard, 2n = SS = 24) and *B. carinata* (Ethiopian mustard, 2n = BBCC = 34) in *in* vivo and *in vitro* conditions was investigated. Crossing of over mentioned species was done in the greenhouse and *in vitro* culture laboratory of the Department of Genetics and Plant Breeding of Agricultural University in Poznań, Poland. The crossability between used species was evaluated based on the observation of pollen grains germination, pollen tube growth and seed set in *in vivo* conditions and embryo set in the ovules after *in vitro* pollination. Hybrid F_1 seeds and hybrid F_1 plants regenerated in embryo culture were obtained from four of five cross combinations. Crossing of *B. napus* with white mustard was quite ineffective. The hybrid F_1 plants obtained after crosses of fertile *B. napus* with other *Brassica* species were male fertile, while those emerged after pollination of MS mother line were male sterile. However, they set the seeds after back crossing with paternal initial form. The observation of microsporogenesis in F_1 hybrid plants showed that they formed normal tapetum and archespore tissue but there were disturbances in chromosome conjugation and their distribution into descendant nuclei. Effectivenes of crossing was different in particular cross combinations both in *in vivo* and *in vitro* conditions.

Key words: *Brassica*, seed colour, interspecific crosses, embryo culture

Introduction

Interspecific hybridization is of great importance for the transfer of useful traits into crop plants e.g. male sterility (Wojciechowski 1993, 1995), self incompatibility (Werner and Jennaway 1995), resistance to different diseases (Liu et al. 1995) and seed coat colour (Li et al. 2003, Rahman 2003). The control of seed colour of oilseed rape *(B. napus*, genomes AACC=38 chromosomes) has attracted the attention of many plant breeders and researches because development of yellow-seeded froms would imply an increase in oil and protein content and decrease in fibre content, which are useful agronomic characters (Shirzadegan 1986). In Poland the breeding and study on yellow-seeded *B. napus* began in 1997 (Barcikowska et al.). At our Department the breeding of yellow-seeded *B. napus* has began in 2003.

The aim of the present study was to evaluate the crossability between different brown and yellow-seeded *Brassica* species. Furthermore, it was and it will be also aimed to develop a yellow-seeded *B. napus* through introgression the yellow seed colour genes from allied *Brassica* species into oil seed rape (*B. napus*).

Materials and Methods

Five species of *Brassica* genera were used in interspecific crosses (Table 1). Plants of *Brassica* species were grown in pots in a greenhouse of Department of Genetics and Plant Breeding, Agricultural University in Poznan. Crosses were made in 2003 and 2005. The young flower buds were emasculated two days before opening. The flowers were pollinated immediately after emasculation and pollination was repated two days later. The most of pollinated pistils were left on the plants till full ripening and some of them were used for embryo *in vitro* culture. In the case of embryo culture, pistils of all crosses were excised 15-20 days after pollination, surface sterilized with 70% and 95% ethyl alkohol (1 minute in each) and rinsed two times with sterile water for 5 min. Isolated embryos at the early torpedo and torpedo shape were cultured on White's (1963) basal medium supplemented with coconout milk. The seedlings obtained from the cultured embryos were transplanted into small pots and grown under glasshouse conditions.

F_1 plants were grown in glasshouse conditions and F_2 plants were grown in an open field conditions. On these materials morphological and cytological observations were made. Morphological observations concerned to: the leaf shape, presence of hair, waxines, flower colour and morphotype. The parental and hybrids traits were compared.

For observation of meiosis young flower buds of different sizes were fixed in the solution of acetic acid and 95% ethanol (AA - 1:1). Emasculated anthers were stained in 2% aceto-orcein and examined under a light microscope. The mitosis was investigated in root tips collected from the plants growing in pots. The roots were fixed in AA and stained in 4% nigrozine.

Results and Discussion

Hybrid F_1 seeds from the maternal plants ripped in the glass house (Table 2) and hybrid F_1 plants regenerated by applying embryo culture technique (Table 3) were obtained from the most cross combinations. Crossing of *B. napus* MS line and

culivars Lisek and Westar with white mustard (*B. hirta*) was quite ineffective (Table 2 and 3). However, Brown et al. (1997) and Mathias (1991) obtained hybrids from the crosses of *B. hirta*×*B. napus* by means of embryo in vitro culture technique but not in the reciprocal one. From the investigations of Momotaz et al.(1998) it is concluded that for the production of interspecific hybrids from the cross *B. napus*×*B. hirta* more effective is ovary in vitro culture technique. The hybrid F_1 plants obtained both from the seeds and regenerated from the cultured embryos were male sterile (similar to maternal form). However, they set seeds after pollination with pollen of paternal species used in the experiment. Allmost all the hybrids were intermediate to their respective parents in many of the morphological traits such as leaf shape, presence of hairs and waxiness on the leaf surface, inflorescence and flowers characters. The colour of F_1 seeds was in the most cases typical to the seed colour of mother plant. Yellow and brown seeds segregated occasionally.

Table 1. Materials used in the experiment

Species	Genome: 2n	Common name	Cultivar
B. hirta (*S. alba* L.)	SS= 24	white mustard	Borowska
B. campestris (L.) ssp. *pekinensis*	AA = 20	pekinese cabbage	Unknown
B. campestris (L.) ssp. *trilocularis*	AA = 20	Yellow sarson	Unknown
B. carinata (Braun.)	BBCC = 34	Ethiopian mustard	Unknown
B. napus (L.) (f. *biennis*)	AACC = 38	Winter oilseed rape	MS- line
		Winter oilseed rape	Lisek
B. napus (L.) (f. *annuua*)	AACC = 38	Spring oilseed rape	Westar

Table 2. Results of interspecific crosses within *Brassica* genera

Cross combination		Number of:			S/F[%]	seeds mean no./ silique
Maternal form	Paternal form	flowers pollinated(F)	siliqua set (S)	seeds obtained		
B. napus f. *biennis* MS line	*B. hirta*	885	79	0	8.9	0.0
	B. c. ssp. *pekinensis*	1825	1253	17388	68.7	13.9
	B. c. ssp. *trilocularis*	786	337	3154	42.9	9.4
	B. carinata	939	518	1351	55.2	2.6
	B. napus f. biennis	691	634	8541	91.8	13.5
	B. napus f. annuua	70	66	786	94.3	11.9
B. napus f. *biennis* cv. Lisek	*B. hirta*	103	7	0	6.8	0.0
	B. c. ssp. *pekinensis*	101	48	273	47.5	5.7
	B. c. ssp. *trilocularis*	99	46	387	46.5	8.4
	B. carinata	100	2	2	2.0	1.0
	B. napus f. *biennis*	102	98	1379	97.1	14.1
	B. napus f. *annuua*					
B. napus f. *annuua* cv. Westar	*B. hirta*	93	14	0	15.1	0.0
	B. c. ssp. *pekinensis*	100	67	215	67.0	3.2
	B. c. ssp. *trilocularis*	102	1	1	1.0	1.0
	B. carinata	95	35	138	36.8	3.9
	B. napus f. *annuua*	100	95	1248	95.0	13.1

The observation of microsporogenesis in F_1 hybrid plants showed that they formed normal tapetum and archespore tissue but there were disturbances in chromosome conjugation and their distribution into descendant nuclei. The chromosome number evaluated by the observation of mitosis was determined in 10 F_1 plants from each cross combinations. F_1 hybrids *B. napus* (MS line)×*B. campestris* ssp. *sarson* were found to have 28 to 32 chromosome, hybrids *B. napus* (MS line)×*B. campestris* ssp. *sarson* from 27 to 34 and hybrids *B. napus* (MS line) ×*B. carinata* from 35 to 37 chromosomes. It is interesting that no one hybrid showed 2n chromosomes number typical to the parent plants. Li et al. (1998) crossing *B. juncea and B. carinata* with O*rychphragmus violaceus* obtained hybrids which showed different somatic chromosome numbers but the most frequent were parental chromosome numbers. According to Li et al (1998) such big variation in chromosome number may be caused by chromosome elimination during mitotic divisions.

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Table3: Results of interspecific crosses within *Brassica* genera by means of embryo culture techique

Cross combination		Number of:			Mean no. of well developed ovules/ ovary	E/O [%]
Maternal form	Paternal form	siliqua	Ovules (O)	embryos (E)		
B. napus f. *biennis* MS line	*B. hirta*	17	326	0	19	-
	B. c. ssp. *pekinensis*	15	272	209	18	76.8
	B. c. ssp. *trilocularis*	14	304	180	22	59.2
	B. carinata	24	602	163	25	27.1
	B. napus f. *annuua*	2	48	48	24	100.0

Table 4. Results of backcrossing of F_1 interspecific hybrids with paternal initial form

Cross combination		Number of:			S/F [%]	seeds mean no./ silique
Maternal form (F_1)	Paternal form	flowers pollinated (F)	siliqua set (S)	seeds obtained		
B. napus MS line × *B. c.* ssp. *pekinensis*	*B. c.* ssp. *pekinensis*	415	76	52	18.3	0.68
	B. napus f. *annuua*	519	90	71	17.3	0,79
B. napus MS line×*B. c.* ssp.*trilocularis*	*B. c. trilocularis*	193	54	7	28.0	0.13
	B. napus f. *annuua*	596	174	17	29.2	0,10
B. napus MS line×*B. carinata*	*B. carinata*	95	35	0	36.8	0.23
	B. napus f. *annuua*	902	276	32	30.5	0.11
B. napus MS line×*B. napus* f. *annuua*	*B. carinata*	25	5	12	20.0	2.40
	B. c. ssp. *pekinensis*	76	32	52	42,1	1.62
	B. c. ssp. *trilocularis*	102	23	24	22.5	1.04
	B. napus f. *annuua*	176	33	230	18.7	6.97
	B. napus f. *biennis*	102	23	24	22.5	1.4