RESYNTHESIS OF AMPHIDIPLOID BRASSICA SPECIES AND THEIR CLUBROOT DISEASE REACTIONS

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INTRODUCTION

Resynthesis is a promising way to enhance the natural variability of allopolyploid crop species. In the genus Brassica, three amphidiploid species, Brassica napus, B. juncea and B. carinata can be resynthesised by interspecific hybridizations between the ancestral diploid species B. campestris, B. oleracea and B. nigra. Our aim is to combine different sources of resistance against the race-forming pathogen Plasmodiophora brassicae in resynthesised amphidiploid hybrids. In the case of B. napus resynthesis should lead to a combination of race-specific resistance from B. campestris with non-differential resistance originating in B. oleracea. Also in B. nigra clubroot resistance occurs which seems to be race-specific. It is of general interest to see whether resistance is expressed in the same manner on a different genetical background or if it is possible to combine the advantages of different types of resistance for practical use.

As interspecific crosses are complicated crosses, cross efficiency has to be enhanced by tissue culture means. In the genus Brassica different methods have been applied by several workers (reviewed by Takeshita et al. 1980). Here we report on comparisons between ovule and embryo culture and the influence of abscissic acid (ABA) on cross efficiency and embryo development.

MATERIALS AND METHODS

P. brassicae-isolates were collected from german rape seed or turnip growing areas. They were maintained in clubbed roots of chinese cabbage and stored at -20°C.

For interspecific crosses and pathogen description the lines of the European Clubroot Differential Set (ECD-Set) were used (Buczacki et al. 1975). The following lines resp. cultivars were included in the crosses:

B. campestris: ECD-01, ECD-02, ECD-03, ECD-04, Mosa, Aarselia, Chinese Cabbage (susceptible).

B. oleracea: ECD-11, ECD-12, ECD-13, ECD-14 (susceptibel), ECD-15, Böhmerwaldkohl, Frosty.

B. nigra: 460, 2051, 16220 (Sacristan et al. 1989).

With the exception of Chinese Cabbage and ECD-14, all cross parents show a relative or absolute resistance against at least one isolate.

Reciprocal crosses and ovule culture were carried out according to Sacristan and Gerdemann (1986). In general,

ovules were cultured on hormone free MS-medium supplemented with 1% sucrose. Some crosses were included in a comparison of the following culture variants:

I : Ovule Culture on hormone free MS-medium (OC/MS)

II : Embryo Culture on hormone free MS-medium (EC/MS)

III : Ovule Culture on MS-medium plus 1 µM ABA (OC/ABA)

IV : Embryo Culture on MS-medium plus 1 µM ABA (EC/ABA)

For embryo dissection a microscope (10x) was used. Racemic ABA was solved in 50% DMSO and sterile filtered. After ten days embryos resp. ovules of the variants III and IV were transferred onto hormone free MS-medium. Germinated embryos were subcultered on the same MS-medium in larger vessels. After another six weeks in culture, they were classified as well developed plantlets (true leaves with shoot apex) or teratomous embryos (supernumerary and/or hypertrophic cotyledons resp. hypocotyls, no shoot apex).

After transplanting into soil interspecific hybrid lines have been maintained via colchicine treatment and bud pollinations. Chromosome counts in root tip metaphases revealed their hybrid character.

For resistance tests resting spores of P. brassicae were isolated from clubbed roots according to Sacristan and Hoffmann (1979). Spore suspensions of 10^7 or 10^8 spores/ml were used to inoculate five days old seedlings one day after transplanting. Four ml suspension were applicated at the stem base using a pipette. The plants were grown in a peat/soil/sand mixture and were kept under conditions of high soil humidity for the first two weeks. Seven weeks after inoculation the soil was removed from the roots and the disease reactions were notated on a four graded scale. The disease index (DI) was calculated according to Williamson and McRitchie (1981).

A field trial was carried out at a naturally infested field site (Schleswig-Schuby), where heavy infections on oil seed rape had been observed. The lines were sown on the 18th of August 1989 and disease assessment could be made on the 1st of November. At least 30 seeds per line were sown, for the most lines 100 seeds in each of four replications could be sown.

RESULTS

Former results of the interspecific hybridizations have been published elsewhere (Diederichsen and Sacristan, 1988). More recent crosses included highly resistant cultivars like 'Böhmerwaldkohl' and the stubble turnip 'Mosa'. Table 1 summarizes the results of these crosses with no regard to different culturing variants (see next page).

Crosses with 'Böhmerwaldkohl' as the maternal parent lead to high yields of hybrids. As all plants from this line belonged to the same clone, paternal influences on the cross

efficiency are visible. Crosses with Chinese Cabbage were most efficient, also the reciprocal cross lead to good hybrid yields. The lowest efficiency occured in reciprocal crosses with 'Mosa'.

Table 1: Recent results of B. napus-resynthesis followed by different tissue culturing methods.

Cross combination	X No. of pollin. buds	Y No. of hybrid plants	Efficiency Y·100/X
Böhmerw. x ECD-04	53	75	142
Böhmerw. x Chin.Cab.	8	26	325
Böhmerw. x Mosa	111	35	32
Chin.Cab. x Böhmerw.	14	34	243
Mosa x Böhmerw.	77	5	6

Table 2: Effect of different culture methods on germination rate and development of interspecific hybrid embryos

Method	diss. ovules A	Number of terat. embryos B	develop. plantlets C	Proportion of terat. embryos B·100/B+C	Germin. rate* (B+C)·100/A
OC/MS	282	134	39	78%	61%
EC/MS	90	7	13	35%	22%
OC/ABA	278	68	25	73%	34%
EC/ABA	90	8	4	67%	13%

* on hormone free medium

Though the ovule culture on hormone free medium lead to the highest proportion of teratomous embryos, the number of well developed plantlets is much higher than for the other variants (Table 2). 61% of the ovules on hormone free medium germinated. In both comparisons the ovule culture lead to higher germination rates than the embryo culture. Treatments including ABA reduced germination and had no clear influence on the development. The culture of excised embryos lead to slightly lower proportions of teratomous embryos, but lowered the germination rate. Without any tissue culture support no hybrids could be obtained.

After chromosome doubling several synthetic lines of B. napus, B. juncea and B. carinata have been maintained. The lines 15/04 and Bwk/04 are descendents from crosses between ECD-04 and ECD-15 resp. 'Böhmerwaldkohl'. Resistance from B. nigra is also incorporated in the lines 04/2051 resp. 04/460 (synth. B. juncea) and in the lines 460/12 resp. 12/2051 (synth. B. carinata).

Green house trials were carried out with different P.brassicae-isolates (Table 3). The B. napus-hosts of the ECD-set (06 - 10) were heavily attacked by all isolates. Isolate f is more virulent for B. oleracea than isolate e, which is more virulent for B. campestris. The artificial mixture of equal amounts of both spore suspensions lead to a broader virulence, though dilution effects are possible.

Table 3: Clubroot reactions of the ECD-set and a synthetic rape seed line with the P.b.-isolates e and f and an artificial mixture of both (10^8 Spores/ml).

Host line	Isolate f		Mix (e+f)		Isolate e	
	%*	DI	%	DI	%	DI
ECD-01	0	0	27	11	52	28
ECD-02	0	0	86	59	79	59
ECD-03	0	0	31	8	63	21
ECD-04	0	0	21	13	69	32
ECD-05	100	100	100	100	100	100
ECD-06	19	16	100	76	100	100
ECD-08	100	100	100	100	100	100
ECD-09	100	100	100	100	100	99
ECD-10	90	38	97	82	100	99
ECD-11	100	97	100	97	64	33
ECD-12	97	79	100	90	89	49
ECD-13	100	100	100	100	80	50
ECD-15	87	51	93	44	3	1
15/04	0	0	0	0	4	1

* = percentage of inf. plants, 100% = 30 plants

Table 4: Disease reactions of the B. napus ECD-lines and synthetic rape seed lines in a field trial and a green house test with the in situ collected isolate.

Line	Field		Green House (10^8 Sp/ml)	
	%	DI	%	DI
ECD-06	98	96	100	100
ECD-08	97	94	100	95
ECD-09	95	93	100	78
ECD-10	95	94	100	100
Bwk/04 C31	-	-	0	0
Bwk/04 2	0	0	0	0
Bwk/04 20	0	0	10	3
Bwk/04 A10	0	0	10	10
15/04 A10 2	0	0	38	21
04/15 3 2	2	1	7	5
ECD-04/ECD-11	26	16	33	24
ECD-13/ECD-04	20	14	50	33

The synthetic line 15/04 showed no attack with the isolates e and f, which are able to overcome one of the

combined resistances. The mixed isolate could not attack this line as well. It was possible to reproduce this reaction in a second independent trial (data not shown).

Different progenies from the four cross combinations were tested (table 4). Infection in the green house trial with the collected isolate was slightly higher, but revealed the same tendencies than the field trial. The synthetic lines were significantly less infected than the ECD-hosts which represent the natural variability in B. napus for clubroot resistance. Synthetic lines that are descendents from B. oleracea-parents with lower levels of resistance (see table 3) than ECD-15 or Böhmerwaldkohl were moderate infected. Progenies from the mentioned B. oleracea parents revealed an effective clubroot resistance in the field as well as in the greenhouse.

Table 5: Clubroot reaction of synthetic B. juncea, B. carinata and trigenomic hybrids (AACCBB) with the P.brassicae isolate a (10^7 sp/ml).

Line (Genome)	n	% inf. plants	DI
ECD-04 (AA)	34	0	0
ECD-05 (AA)	697	100	100
ECD-12 (CC)	28	93	65
460 (BB)	25	0	0
2051 (BB)	31	3	1
Brutor (AACC)	44	100	100
RC-B.juncea (AABB)	20	100	100
B.carinata (CCBB)	27	96	94
ECD-04/460 (AABB)	18	72	43
ECD-04/2051(AABB)	27	100	89
460/ECD-12 (BBCC)	22	100	97
ECD-12/2051 (BBCC)	17	100	98
Brutor/460 (AABBCC)	13	100	49

In several replicated tests isolate a has been shown to be not virulent for the B. nigra-lines 460 and 2051 (data not shown). However, interspecific hybrids from crosses with these lines did not express clubroot resistance against the isolate a (table 5). Also descendents from ECD-04, which is resistant against isolate a too, were susceptible.

DISCUSSION AND CONCLUSIONS

Ovule culture on hormone free MS-medium has been shown to be the most promising method to support sexual hybridizations between different Brassica-species. Microscopical observations showed that globular and early heart shaped embryos did not continue their development and died, whereas early cotyledon embryos germinated rapidly. A possible explanation for the higher germination rate after ovule culture might be, that nourishment of the ovule allows a better, physiologically more adapted supply of the embryo inside. This might lead to further development of also very early embryo stages.

Most of the excised embryos were early heart shaped. As interspecific hybrid embryos tend to exhibit an abnormal, teratomous morphology, this might be due to precocious germination. Finkelstein and Crouch (1984) observed this phenomenon for ordinary rape seed embryos. Plümper (1990) pointed out, that the proportion of teratomous embryos from interspecific hybridizations decreases with later germination times. Finkelstein et al. (1985) could suppress precocious germination of early rape seed embryos by exogenous ABA in vitro, which induced also the continued expression of embryo-specific storage protein genes. In our experiments exogenous ABA could delay the time of germination (data not shown), but did not influence embryo development in direction to higher germination rates or a more normally morphological development.

The synthetic B. napus-lines Bwk/04 and 15/04 expressed a very effective clubroot resistance. Therefore they are now included in a practical breeding programme on rape seed. The disease reactions of these lines followed the expectation of a complementary genetic interaction. This was different for the interspecific hybrids with B. nigra. Clubroot resistance was not expressed, which could be due to epistatic genetic interactions or gene dosage dilution effects. May be for the same reason, clubroot resistance has so far never been reported for natural B. juncea or B. carinata.

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