

Development of semi-RS doubled haploid lines of winter oilseed rape with zero erucic acid and low glucosinolates content

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The production of oilseed rape has increased rapidly over the past decade not only due to a major increase in production area, but also high yielding winter oilseed rape varieties

However, the level of genetic diversity in double-low genotypes is relatively low. It is caused above all by intensive selection of genotypes in terms of two features: associated with improving oil, first of all eliminating erucic acid and improving meal through the reduction of glucosinolates content. Today, oilseed rape breeders are seeking genetic diversity in their breeding programmes. One of the source of genetic diversity of germplasm for breeding is resynthesis (RS) of *Brassica napus* from interspecific crosses between the two highly polymorphic diploid ancestors *Brassica rapa* and *Brassica oleracea*.

B. rapa AA=20 → *B. napus* AACC=38 ← *B. oleracea* CC=18



In this study resynthesized oilseed rape was obtained as a result of crosses between *Brassica rapa* ssp. *chinensis* var. *chinensis* (Pak Choy), and *Brassica oleracea* ssp. *acephala* var. *sabellica* (curly kale) using embryo rescue technique. As expected, the RS lines obtained in the study have been characterized by a high content of erucic acid in oil and high glucosinolates in seeds. Several resynthesized oilseed rape lines were crossed with lines of double low quality winter oilseed rape. Populations of large number of androgenic plants (semi-RS) were developed from F1 hybrids using isolated microspore *in vitro* culture method. (Fig. 1)

Table 1: The number of DH semi-RS lines obtained from F1 hybrid DH R x DHRS

Microspore donor plant F ₁ hybrid	No of androgenic plants	No of plants after first selection	Semi-RS DH lines
12r DH-R1 x DH-RS1	414	127	104
17r DH-R2 x DH-RS2	395	159	133
29r DH-R3 x DH-RS3	246	79	56
total	1055	365	293

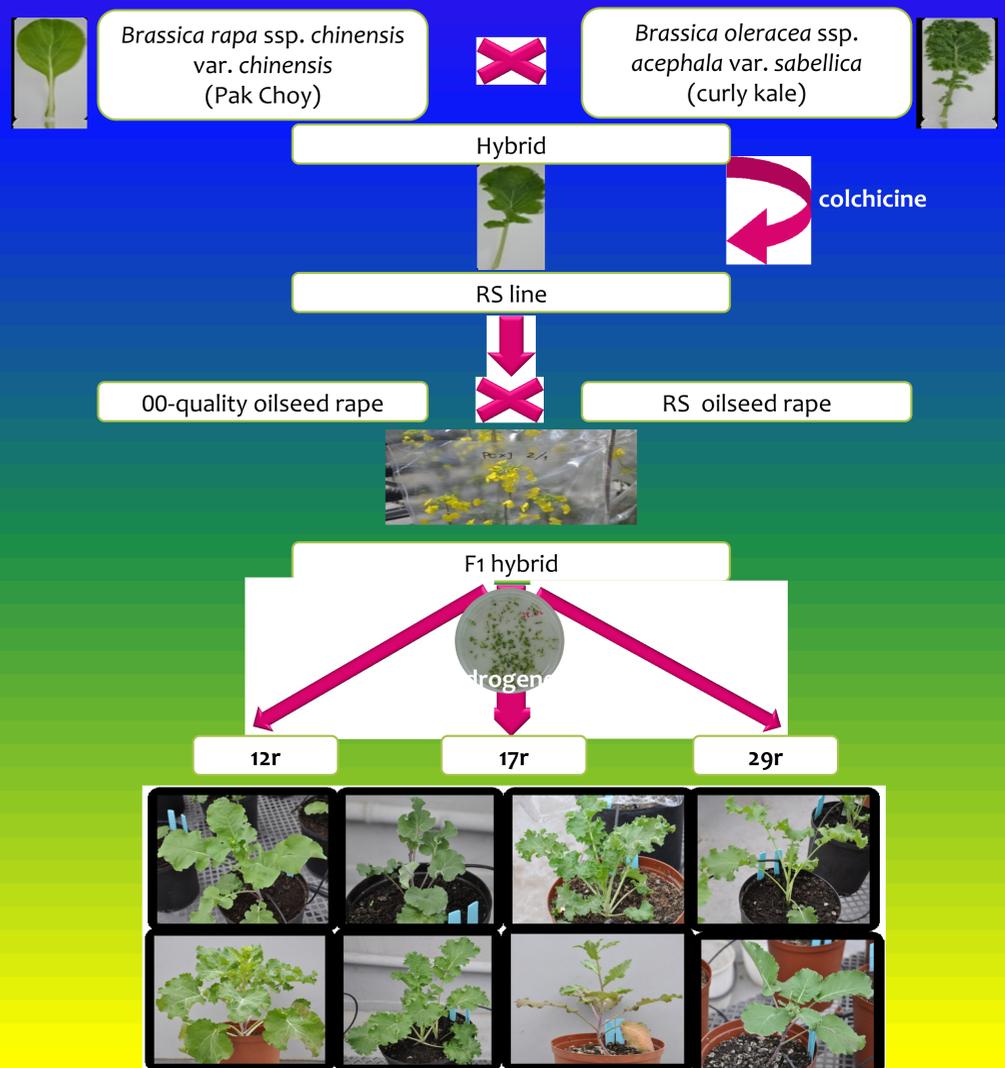
DH R1-3 – double low oilseed rape; DH RS1-3 (*B. rapa* x *B. oleracea*)

The seeds of all obtained semi-RS DH lines were analyzed biochemically with regard to 00-quality of seeds. (Table 2)

Table 2: Erucic acid and glucosinolates content in seeds of DH lines of semi-RS

Microspore donor plant F ₁ hybrid (DH R x DH RS)	Erucic acid %		Glucosinolates (µmol/g)		No of DH semi-RS double low quality (00)
	range %	No of zero erucic acid DH semi-RS	range (µmol/g)	No of DH semi RS with ≤ 17,4 µmol/g	
12r C _{22:1} 29,4%; GLS. 31,1 µmol/g	0-58,3 (29 DH)	5 DH	10,1-61,6 (8 DH)	4 DH	-
17r C _{22:1} 26,8% GLS. 23 µmol/g	0-51,2 (52 DH)	4 DH	8,1-100,2 (25 DH)	2 DH	2 DH
29r C _{22:1} 31,6% GLS 11,6 µmol/g	30,1-48,8 (4 DH)	-	28,9-50,9 (2 DH)	-	-

Figure 1: Schematic resynthesis of winter oilseed rape



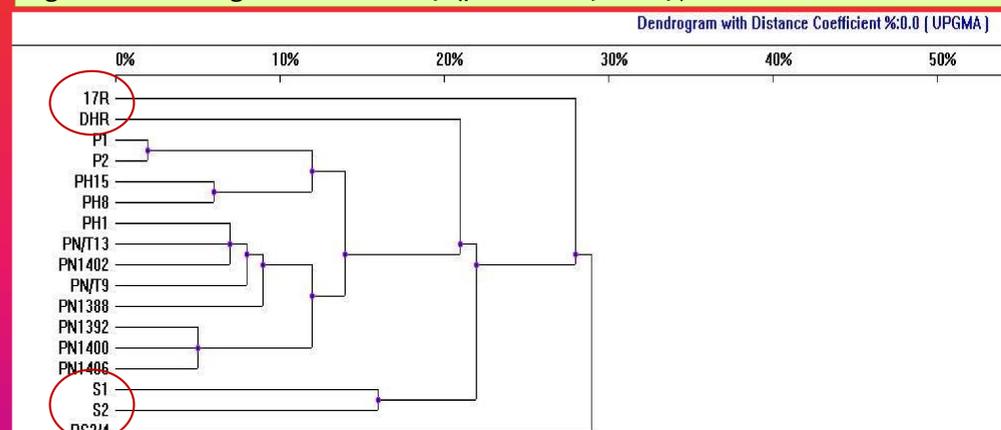
Among the population of doubled haploids the semi-RS DH lines of winter oilseed rape with very low or zero erucic acid content and low amount of glucosinolates were selected. (Table 3)

Table 3: Biochemical analyses of seeds of two semi-RS DH lines of double low winter oilseed rape

DH line	Fatty acids content (%)							GLS (µmol/g)
	C16:0	C18:0	C18:1	C18:2	C18:3	C20:1	C22:1	
S1	4,4	1,1	55,8	23,4	10,4	2,5	2,4	11,5
S2	4,4	1,1	69,8	16,2	7,1	1,4	0,0	11,6

The dendrogram of genetic distance was created based on RAPD markers. The selected two semi-RS DH lines S1, S2, with 00 quality, and donor F1 hybrids as well as other double low winter oilseed rape were compared. Semi-RS DH lines S1 and S2 have demonstrated the significantly high genetic distance towards other studied genotypes. (Fig. 2)

Figure 2: Dendrogram of similarity (preliminary study)



RS2 / 4 – DH RS, DH-R - 00 winter oilseed rape DH line, 17R - F1 hybrid (RS2 / 4 x DHR)
S1 and S2 - DH lines semi-RS 00 winter oilseed rape,
PH, PN1, P1, P2 - other lines of winter oilseed rape