# Selection for double low quality semi-resynthesized DH lines of oilseed rape (Brassica napus L.)



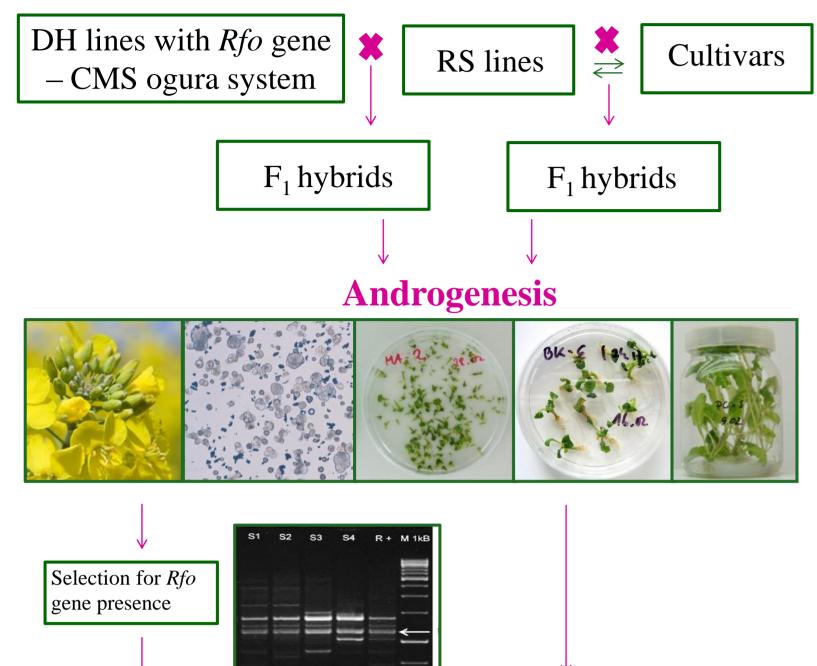
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Novel *B. napus* genotype (AACC genome) resynthesized (RS) by interspecific crosses between the two diploid ancestors, namely *B. rapa* (AA genome) and *B. oleracea* (CC genome), represents a comparatively genetically divergent source of germplasm for open-pollinated and hybrid breeding. The undesirable features of resynthesized oilseed rape like low seed yield and not canola quality ("00"), stimulate attempts to improve RS by developing of semi-RS-lines (Szała et al., 2016). The significant genetic separateness of the semi-resynthesized lines of *B. napus* from commonly cultivated double-low winter oilseed rape (WOSR) cultivars enables a broadening of the variability within *Brassica* (Sosnowska et al., 2017, Liersch et al., 2019). One of the ways to create double-low semi-RS lines is crossing the RS lines and double-low natural oilseed rape genotypes and selected "00" DH lines as a result of androgenesis *in vitro* of F<sub>1</sub> hybrids, **Fig.1**.

Fig.1. Development of double-low semi-RS DH lines



# Results

The resynthesized lines were received by using *in vitro* pollination and embryo rescue methods (Sosnowska and Cegielska-Taras, 2014), from the parental components: *B. oleracea*: curly kale cv. Vitessa, cv. Kapral and *B. rapa*: turnip cv. Premium "00", cv. Salut "00" and Chinese cabbage cv. Kilakin, **Table 1**.

### Table 1. Origin of resynthesized DH lines of WOSR

<b>RS</b> line	B. oleracea × B. rapa	B. rapa × B. oleracea
<b>63 and</b>	curly kale cv. Vitessa × turnip cv.	
66	Premium "00"	
67		turnip cv. Salut × curly kale cv. Kapral
68		Chinese cabbage cv. Kilakin × curly kale cv. Vitessa
69	curly kale cv. Vitessa × Chinese cabbage cv. Kilakin	



Selection for double-low semi-RS DH lines

RS plants were crossed with double-low ("00") WOSR cultivars or lines containing the *Rfo* gene for Ogura cytoplasmic male sterility (CMS *ogura*) system. Populations of doubled haploids (DH) were developed from these  $F_1$  hybrids using the microspore *in vitro* culture method. Only a part of these DH plants formed proper seeds. The seeds of DH lines obtained from nine different populations were analyzed with regard to erucic acid content in the oil and glucosinolate content in the seeds (**Table 3**).

#### Table 3. Origin of microspore donors in process of androgenesis

Symbol of DH semi-RS population	<b>Cross combination</b>
<b>D</b> 10	$\mathbf{R}_2$ ( <i>Rfo</i> ) × RS 67
D 11	$\mathbf{R}_2 (Rfo)  imes \mathbf{RS} \ 66$
D 12	$\mathbf{R}_{6}$ ( <i>Rfo</i> ) × RS 63
D 14	PH 16/2 ( <i>Rfo</i> ) × RS 68
D 18	PH 17/2 ( <i>Rfo</i> ) × RS 69
D 22	<b>R</b> <sub>7</sub> ( <i>Rfo</i> ) × <b>RS 69</b>
D 23	cv. Arot × RS 69
D 24	RS 69 × cv. Arot
D 25	cv. Lohana × RS 63
D 27	cv. Tosca × RS 68

Biochemical analysis showed that among the 5 tested resynthesized oilseed rape, the two RS lines from the crosses involving double-low *B. rapa* cv. Premium did not erucic acid in oil (**Table 2**).

Table 2. The content of erucic acid  $(C_{22:1})$  and glucosinolates (GLS) in seeds of resynthesized *Brassica napus* L.

	Content of		
<b>RS line</b>	C <sub>22:1</sub> (%)	GLS (µmol g <sup>-1</sup> )	
<b>RS 63</b>	0.0	119.2	
<b>RS 66</b>	0.0	78.8	
<b>RS 67</b>	15.5	75.6	
<b>RS 68</b>	39.6	95.9	
<b>RS 69</b>	32.4	75.9	

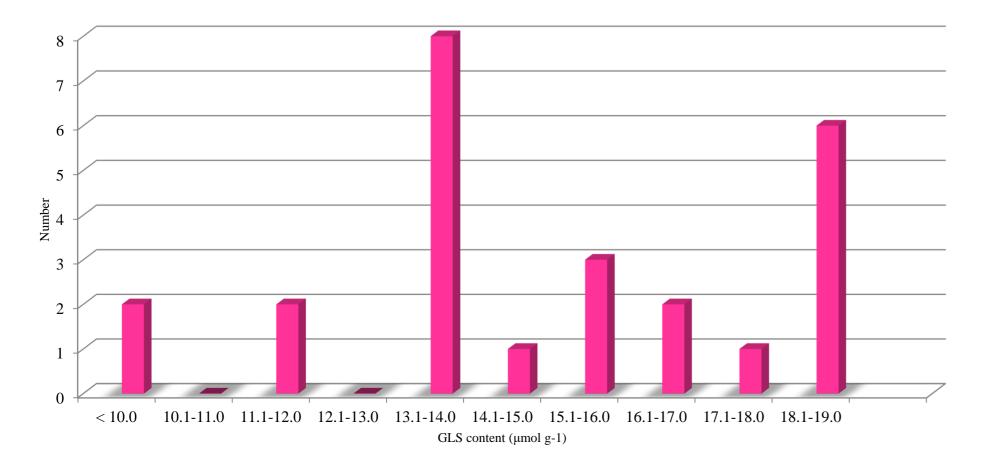
Among 223 semi-RS DH lines 53 semi-RS DH lines did not possess erucic acid and 25 semi-RS DH lines possess less than 18.5  $\mu$ mol g<sup>-1</sup> glucosinolates. The majority of semi-RS doubled haploids were characterized by high glucosinolate and erucic acid content in seeds (**Table 4**).

# Table 4. The content of erucic acid and glucosinolates in seeds of nine populations of semi-RS DH lines

Population	No of DH	No of DH semi-	No of DH semi-RS	No
	semi-RS lines	<b>RS</b> lines	lines with GLS	of DH semi-RS
	analyzed*	without C <sub>22:1</sub>	content < 18 µmol g <sup>-1</sup>	lines "00"
<b>D</b> 10	24	8	3	0
D 11	17	6	4	3
D 12	35	14	3	0
D 14	19	3	3	0
D 18	14	3	0	0
D 22	4	0	1	0
D 23	44	9	1	0
D 24	36	3	4	0
D 25	7	3	2	1
D 27	23	4	4	1
Total	223	53	25	5

Finally, from all studied doubled haploids, selection of five semi-RS DH lines with 00-quality including three semi-RS lines with *Rfo* gene (restorer lines) was possible. Distribution of 25 semi-RS DH lines according to glucosinolate content was from 4.7  $\mu$ mol g<sup>-1</sup> to 18.5  $\mu$ mol g<sup>-1</sup> (**Fig. 2**).

Fig. 2. Distribution of semi-RS DH lines according to glucosinolate content



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\*during the analysis

Currently, the double-low quality semi-RS DH lines are used for development of CMS *ogu* hybrids of WOSR. The introduction of 50% of a resynthesized oilseed rape genotype to natural restorer lines is sufficient to deliver a high heterosis effect (Szała et al., 2019).

## Conclusion

This research demonstrated the feasibility of developing a semi-RS DH line with double-low quality as a components for hybrid breeding and to create new openpollinated cultivars of winter oilseed rape.

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