

# Analysis of genetic components of winter oilseed rape (*Brassica napus* ssp. *oleifera*) regeneration ability under *in vitro* culture

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## Abstract

This paper presents the study on the genetic components of regeneration ability of winter oilseed rape (*Brassica napus* ssp. *oleifera*) explants cultured *in vitro*. The general combining ability (GCA) and specific combining ability (SCA) effects and the heritability in the broad and narrow sense were determined for four traits: the efficiency of shoot regeneration from hypocotyls, the number of regenerated shoots per single hypocotyl, the efficiency of shoot regeneration from cotyledons with a petiole, and the number of regenerated shoots per a single cotyledon with a petiole. For the study, explants originating from winter oilseed rape doubled haploid (DH) lines (W15, W69, W70, W78, and W131) and their F1 hybrids were used. Conducted analysis revealed that most of GCA effects were insignificant. None of the DH lines showed positive GCA effect, while lines W15 and W131 exhibited significant negative GCA effects for three of four analyzed traits. There were no significant SCA effects for any of the F1 hybrids. Calculated values of heritability in the broad and narrow sense were very low. The results obtained in the study confirmed that quantitative traits connected with plant regeneration under *in vitro* conditions are characterized by high variability. Although the results have widened knowledge of the genetic determinants of regeneration ability trait, further studies and observations are still required.

**Key words:** oilseed rape, *in vitro* culture, regeneration ability, GCA, SCA, heritability

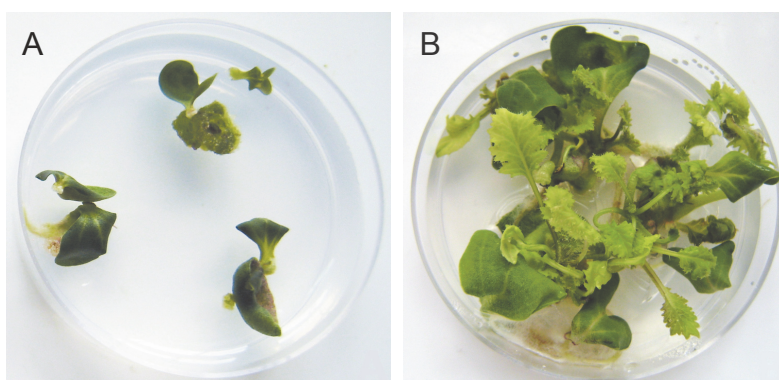
## Introduction

Winter oilseed rape (*Brassica napus* ssp. *oleifera*) is usually described as a good source of plant material to conduct *in vitro* cultures, but the species reveals a considerable genotypic diversity concerning its regeneration ability. Regeneration is a prerequisite in obtaining valuable plant material under *in vitro* conditions. Genotypes with a high regeneration ratio are a significant tool in biotechnological research, especially in genetic engineering. Despite the progress in research, many important aspects of *in vitro* plant regeneration are poorly known. The present study attempts to investigate the genetic background of the regeneration of rapeseed plants by using a classical genetic analysis. The genetic research and breeding programs depend on the proper diagnosis of the conditions of quantitative trait inheritance. During the selection process, the information about the combining ability of parental components used for crossbreeding is very important. This knowledge is essential for

proper selection of suitable parents in identifying promising hybrids. A common method used in a classical genetic analysis is diallel crossing applied to evaluate the combining ability of parents and progeny generations. One possible way to analyze diallel crosses is the method proposed by Griffing (1956a), which divides the total genetic variance for the GCA of parents and the SCA of obtained hybrids. The GCA determines the ability of the tested line of providing an abundant offspring when crossing it with many other lines, while the SCA is characterized by the ability of two different lines of giving an abundant offspring after their mutual crossbreeding (Sprague and Tatum, 1942). The assessment of the general and specific combining abilities allows determining the additive and non-additive gene action as well (Falconer, 1967). Thus, the knowledge of combinatorial abilities helps to understand the nature of the action of genes involved in the expression of quantitative traits and predicts the value of further generations (Machikowa et al., 2011). How

**Table 1.** DH lines of winter oilseed rape used in the study and F1 hybrids resulting from diallel crosses

DH line	F1 hybrid	DH line	F1 hybrid	DH line	F1 hybrid	DH line	F1 hybrid	DH line	F1 hybrid
W15	W15×W69	W69	W69×W15	W70	W70×W15	W78	W78×W15	W131	W131×W15
	W15×W70		W69×W70		W70×W69		W78×W69		W131×W69
	W15×W78		W69×W78		W70×W78		W78×W70		W131×W70
	W15×W131		W69×W131		W70×W131		W78×W131		W131×W78

**Fig. 1.** Hypocotyls and cotyledons of winter oilseed rape doubled haploids cultured *in vitro*: A) line W15 with low regeneration effectiveness, B) line W78 with high regeneration effectiveness

ever, it is important to know not only the value of general and specific combining abilities, but also how the values obtained are reproducible in subsequent generations, that is, what is their heritability. The broad and narrow sense heritability coefficients are often reported in the literature and are not much applicable for breeding. For the selection process, it is more important to know the heritability in the narrow sense because the additive variance is a measure of similarity between parents and offspring (Falconer, 1974), which allows predicting a response to the selection (Bos and Caligari, 1995).

With regard to the above, the aim of the research was to analyze the effects of GCA and the SCA, and the heritability in the broad and narrow sense on the characteristics that make up the overall picture of the regeneration capability of winter oilseed rape (*Brassica napus* ssp. *oleifera*) explants cultured *in vitro*.

### Materials and methods

The plant material included five doubled haploid (DH) lines of winter oilseed rape (*Brassica napus* ssp. *oleifera*), showing different regeneration effectiveness

under *in vitro* conditions. In addition, F1 progeny obtained from diallel crosses was also used. DHs included lines with high regeneration effectiveness (W69, W70, and W78) and lines with low regeneration effectiveness (W15 and W131) when cultured *in vitro*. DH lines were obtained in cultures of isolated microspores, in cooperation with the Institute of Plant Breeding and Acclimatization, Department of Oilseed Crops in Poznan. Details concerning DH lines and F1 hybrids are shown in Table 1; explant samples of lines W15 and W78 are depicted in Figure 1.

The effects of the GCA and the SCA and the heritability in a broad and narrow sense were estimated by using the classical two-factor model (Mađry et al., 2010), and the second method of Griffing (1956a), with the assumptions that there is no maternal effect, action of non-allelic genes is independent, multiple alleles do not exist, parents are homozygous, segregation of genes between parents is independent, and the breeding coefficient is equal to 1 (Frankenberger et al., 1981; Griffing, 1956b).

Heritability in the broad sense was defined by the formula:

$$h_{bs}^2 = \text{Var}(G)/\text{Var}(P) \quad (1)$$

where

$h_{bs}^2$  is heritability in the broad sense,  $\text{Var}(G)$  is the genotypic variance,  $\text{Var}(P)$  is the total phenotypic variance.

Heritability in the narrow sense was defined by the formula:

$$h_{ns}^2 = \text{Var}(A)/\text{Var}(P) \quad (2)$$

where

$h_{ns}^2$  is the heritability in the narrow sense,  $\text{Var}(A)$  is the additive variance,  $\text{Var}(P)$  is the total phenotypic variance.

The significance of the effects of the GCA and the SCA was determined using a Fisher-test with the significance level  $\alpha = 0.05$  and  $\alpha = 0.01$ . GCA effects were estimated for the DH lines in the system "each with each".

The valuation of the genetic components described above was performed for four traits which make up the overall picture of rapeseed explant regeneration capability under *in vitro* conditions. These were as follows: 1) the efficiency of shoot regeneration from hypocotyls, 2) the efficiency of shoot regeneration from cotyledons with a petiole, 3) the number of regenerated shoots per single hypocotyl and the number of regenerated shoots per single cotyledon with a petiole.

All the analyses were performed using the statistical package R (R Core Team, 2012).

## Results and discussion

The evaluation of the general combining ability effects revealed that the majority of the GCA effects were not statistically significant. There were no positive GCA effects for any of the DH lines, while significant negative effects of the general combining ability were demonstrated for lines W15 and W131 and three of four analyzed traits. These were as follows: the effectiveness of shoot regeneration from hypocotyls (lines W15 and W131), the number of shoots regenerated per a single hypocotyl (lines W15 and W131), and the number of regenerated shoots per a single cotyledon with a petiole (line W131). The negative values for all DH lines were obtained probably due to the very high variability of the analyzed traits, which also points to the uselessness of these lines as a form of parenting. None of the F1 crossing combinations showed any statistically significant SCA effects for the analyzed traits either. The calculated values of heritability in the broad and narrow sense proved to be very low. The significance of the effects of GCA and SCA for F1 hybrids is shown in Tables 2 and 3, while the values

of heritability in the broad and narrow sense are presented in Table 4.

The SCA variance higher than the GCA variance reveals an overwhelming effect of the dominant genes in determining the studied traits. Conversely, a higher GCA variance suggests that the effects of additive genes are involved in determining these traits. If none of the variances is significant, the existence of an epistatic gene effects is indicated (Marinković et al., 2000; Škorić et al., 2000). The results could therefore indicate a little effect of the additive genes in determining the regeneration ability trait. However, a vast majority of statistically insignificant GCA and SCA effects may suggest the existence of effects of other genes. Nevertheless, the calculated GCA effects point that the DH lines showing significant negative effects (W15 and W131) may affect the decrease in value of analyzed traits in the progeny.

A study presented in this paper demonstrates very low heritability values of analyzed traits in the broad and narrow sense. The efficiency of shoot regeneration from hypocotyl heritability in the broad and narrow sense amounted to 0.074 and 0.06, and the efficiency of shoot regeneration from cotyledons with a petiole was 0.035 and 0.008, respectively. The number of regenerated shoots per single hypocotyl was characterized by slightly higher values of heritability, which amounted to 0.11 in a broad sense and 0.085 in the narrow sense. The numbers of regenerated shoots per single cotyledon with a petiole were 0.057 and 0.037 in the broad and narrow sense, respectively. Despite such low values of heritability in both broad and narrow sense, within the F1 hybrids, higher values of the regeneration efficiency were observed, when comparing them to weaker one of their parents. This may prove that the dominant gene effect was higher than the additive effect of genes, and the additive genetic variance could be low in this population when considered separately.

Ono and Takahata (2000) studied the genetic background of winter oilseed rape shoot regeneration using diallel crosses. In those studies, shoot regeneration from cotyledonous petioles was associated with the additive and dominant effect of genes, and the additive effect outweighed the effect of dominance. Heritability in the broad and narrow sense was analyzed, respectively, amounting to 0.973 and 0.819 values.

Akasaka-Kennedy et al. (2005) demonstrated a genetic control of the shoot formation ability for winter oil-

**Table 2.** The significance of the effects of GCA of three DH lines of winter oilseed rape

DH Line	The efficiency of shoot regeneration from hypocotyls	The efficiency of shoot regeneration from cotyledons with a petiole	The number of regenerated shoots per single hypocotyl	The number of regenerated shoots per single cotyledon with a petiole
W15	-0.177**	-0.772	-0.211**	-1.153
W69	-0.132	-0.644	-0.128	-1.037
W70	-0.138	-0.689	-0.156	-1.003
W78	-0.143*	-0.622	-0.167*	-0.959
W131	-0.199**	-0.744	-0.228**	-1.314*

The significance of the effects on levels of 0.05 - \* and 0.01 - \*\*

**Table 3.** The significance of the effects of SCA for F1 hybrids of winter oilseed rape

F1 hybrid	The efficiency of shoot regeneration from hypocotyls	The efficiency of shoot regeneration from cotyledons with a petiole	The number of regenerated shoots per single hypocotyl	The number of regenerated shoots per single cotyledon with a petiole
W15 × W69	-0.020	0.061	-0.039	0.132
W15 × W70	0.024	-0.061	0.028	-0.146
W15 × W78	-0.026	0.050	-0.028	0.032
W15 × W131	0.086	0.500	0.100	0.771
W69 × W15	0.058	0.000	0.083	0.066
W69 × W70	-0.020	0.033	-0.011	0.027
W69 × W78	0.019	0.100	0.022	0.182
W69 × W131	0.063	0.506	0.039	0.743
W70 × W15	-0.026	0.044	-0.044	0.099
W70 × W69	0.330	-0.200	0.572	-0.662
W70 × W78	0.002	-0.078	0.006	0.016
W70 × W131	0.069	0.639	0.067	0.932
W78 × W15	-0.020	-0.044	-0.033	-0.101
W78 × W69	0.013	0.044	0.006	-0.018
W78 × W70	0.036	0.011	0.050	0.149
W78 × W131	0.074	0.550	0.078	0.910
W131 × W15	-0.031	-0.078	-0.039	-0.123
W131 × W69	-0.042	-0.078	-0.022	-0.040
W131 × W70	0.280	-0.278	0.422	-0.473
W131 × W78	0.019	0.000	0.011	-0.096

**Table 4.** Heritability in the broad and narrow sense obtained for the four studied traits

Trait	Heritability in the broad sense	Heritability in the narrow sense
The efficiency of shoot regeneration from hypocotyls	0.074	0.060
The efficiency of shoot regeneration from cotyledons with a petiole	0.035	0.008
The number of regenerated shoots per single hypocotyl	0.110	0.085
The number of regenerated shoots per single cotyledon with a petiole	0.057	0.037

seed rape leaf explants, and a strong correlation between the number of shoots formed on a single explant and the efficiency of shoot formation. However, after the comparison of these data with the results of Ono and Takahata (2000), no correlation between the shoot formation from leaves and shoot formation from the cotyledonous petioles was shown. This could either be due to the differences in phytohormones used to conduct the *in vitro* culture in both experiments or the existence of variations in genes controlling the regeneration capacity of leaves and cotyledons.

Sparrow et al. (2004) used a diallel analysis to investigate the genetic control of cabbage (*Brassica oleracea*) shoot regeneration. According to the results, the regeneration of shoots from the cotyledonous petioles of *B. oleracea* was found to be strongly influenced by genes. About 85% of the variance was accounted for genetic variation and the rest was due to environmental factors. In these studies, most of the genetic variation was due to the additive gene action (71%) while a high efficiency of shoot regeneration was observed as a dominant trait over the low ability to regenerate shoots.

Łuczkiwicz et al. (2006) analyzed several quantitative traits associated with the *in vitro* regeneration of *Camelina sativa*. Six different (in terms of analyzed traits) genotypes were crossed using diallel crossing. Cotyledon-hypocotylous explants obtained from F1 hybrid and parental plants were cultured *in vitro*, and the mass of callus and regenerated plant parts and the number of regenerated shoots were evaluated. The evaluation of the GCA and the SCA effects revealed that most of them were not statistically significant. No statistically significant SCA effect on the mass of the callus and regenerated plant parts was proven. The values of heritability were not evaluated at all due to the large error caused by too high quantitative trait variation. The additive effects of genes were not as yet been demonstrated.

Etedali and Khandan (2012) studied the genetic control of callus growth and shoot regeneration of oilseed winter rape mature embryos. Callus growth was recognized using two parameters: the callus diameter measured in millimeters and the fresh weight of callus measured in grams. The GCA effects estimated by researchers were found to be insignificant for all the studied traits, while significant SCA effects were estimated only for the callus concerning traits. In addition, Etedali and Khandan obtained high values of broad sense heritability of stu-

died traits, which amounted to 0.68 for shoot regeneration, 0.89 for callus diameter, and 0.82 for the weight of callus. Conversely, they obtained very low values of heritability in the narrow sense, which were, respectively, 0.03 for shoot regeneration, 0.04 for callus diameter, and 0.03 for the weight of callus. The authors concluded that high values of broad sense heritability of the studied traits indicate that callus growth in oilseed winter rape is genetically controlled; however, low values of narrow sense heritability estimated to be these traits suggest the effect of dominant genes exceeded onto an additive gene action. The researchers found that the genes which control the growth of callus are likely to be different from those controlling the regeneration of shoots.

Our results and of those reported in literature indicate that quantitative traits associated with the regeneration ability under *in vitro* conditions, such as shoot regeneration frequency and the number of shoots per explant, are highly variable, which hinders the genetic analysis of these characteristics. The results of the evaluation of the four traits investigated in this work, which compose the general picture of rapeseed plant regeneration ability, are markedly different from the results of Ono and Takahata (2000) and Sparrow et al. (2004). They do, however, remain in accordance with the results of Łuczkiwicz et al. (2006) and Etedali and Khandan (2012). The differences may have been due to or connected with a sampling error, other genotypes used for testing, or different types of media used in the *in vitro* culture. It is also possible that a certain clonal or epigenetic variation occurred. Although the obtained results undoubtedly contribute to the knowledge of genetic determinants of oilseed rape regeneration ability trait, further studies and observations are still required.

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