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THE EFFECT OF INDOLE-3-BUTERIC ACID ON REGENERATION CAPABILITY OF COTYLEDONOUS AND HYPOCOTYLOUS EXPLANTS OF WINTER RAPESEED (*BRASSICA NAPUS* SSP. *OLEIFERA*) DOUBLED HAPLOID LINES

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A b s t r a c t

The division and growth of most types of plant cells kept under *in vitro* culture requires that the medium has an outside source of auxin. Phytohormones stimulate morphology and are essential ingredients of the medium used in the *in vitro* culture. This article presents an experiment whose aim was to evaluate the impact of indole-3-butyric acid (IBA) on the regeneration capability of explants of six doubled haploid lines of rapeseed (*Brassica napus* ssp. *oleifera*) kept under *in vitro* conditions. The cotyledons and hypocotyls were incubated on an MS medium with the addition of 10 mg/l indole-3-butyric acid (IBA).The regeneration assessment was made after 28 days of culture. For all the tested DH lines we observed both substantial genotype differentiation concerning shoot regeneration effectiveness and considerable impact on shoot regeneration of adding the regulator to the medium. The average shoot regeneration effectiveness of the explants incubated on the medium containing auxin was 19.3% with the range of variability falling in between 6.7% and 42.2%.

WPŁYW KWASU INDOLILO-3-MASŁOWEGO NA ZDOLNOŚCI REGENERACYJNE EKSPLANTATÓW LIŚCIENIOWYCH I HYPOKOTYLOWYCH PODWOJONYCH HAPLOIDÓW RZEPAKU OZIMEGO (*BRASSICA NAPUS* SSP. *OLEIFERA*)

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S ł o w a k l u c z o w e: auksyny, regeneracja, podwojone haploidy, rzepak, *Brassica napus* ssp. *oleifera.*

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Abstrakt

Podział i wzrost większości typów komórek roślinnych utrzymywanych w warunkach kultury *in vitro* wymaga zewnętrznego źródła dopływu auksyn do pożywki. Fitohormony stymulują morfologię i są istotnym składnikiem pożywek stosowanych w kulturach *in vitro*. Celem doświadczenia była ocena wpływu kwasu indolilo-3-masłowego (IBA) na zdolności regeneracyjne eksplantatów z 6 linii podwojonych haploidów rzepaku ozimego (*Brassica napus* ssp. *oleifera*) w kulturze *in vitro*. Eksplantaty liścieniowe i hypokotylowe inkubowano na pożywce MS z dodatkiem 10 mg/l kwasu indolilo-3-masłowego (IBA). Zdolność regeneracyjną oceniono po 28 dniach kultury. Pośród analizowanych linii DH zaobserwowano istotne genotypowe zróżnicowanie dotyczące efektywności regeneracji pędów oraz stwierdzono istotny wpływ dodatku regulatora na regenerację pędów wszystkich testowanych linii DH. Średnia efektywność regeneracji pędów na pożywce zawierającej auksynę wyniosła 19,3% dla zakresu zmienności wynoszącego od 6,7% do 42,2%.

Introduction

Phytohormones are compounds which plants need in order to grow and expand. Auxins are plant hormones which stimulate the growth of cells and take part in the differentiation of the latter (WOŹNY and PRZYBYŁ 2004). The division and growth of most types of plant cells kept under the *in vitro* culture require that the medium has an outside source of auxin (PETRASEK et al. 2002). The type of auxin used in the medium influences the morphogenesis of the culture's explants (HOFMANN et al. 2004). Synthetic auxins are important ingredients of the mediums used in the *in vitro* culture (ŚLESAK et. al. 2005). When added to the medium in appropriately high concentration, they induce formation of callus and adventitious roots (WOŹNY and PRZYBYŁ 2004) and stimulate the process of somatic embryogenesis (VIKRANT and RASHID 2003).

The aim of the experiment was to examine whether adding artificial auxin (indole-3-butyric acid) to the medium had impact on the shoot regeneration process of hypocotylous and cotyledonous explants from six doubled haploid lines of rapeseed kept under *in vitro* conditions.

Materials and Methods

The plant material used in the research included hypocotylous and cotyledonous explants from six doubled haploid lines of winter rapeseed obtained from isolated cultures of microspores from Bor and Wotan varieties (Table 1).

In order to obtain explants, the rapeseed seeds were decontaminated and incubated on the basic MS medium (MURASHIGE and SKOOG 1962) according to the standard *in vitro* sterilization and breeding procedures. The seeds were kept in a breeding room under 16-hours fotoperiod conditions at light source intensity of 3800 lux and temperature of 24 degrees Celsius. After 5 days

Table 1

The origin of the DH lines used in the study

Plant material	DH lines
Bor Cultivar	B-18
	B-21
Wotan Cultivar	W-15
	W-69
	W-70
	W-78

cotyledons and hypocotyls were collected from young seedlings. These explants were incubated on MS medium with the addition of indole-3-butyric acid (IBA) in 10 mg/l concentration. MS medium without the regulator was used as a control. Three explants coming from one seedling including two cotyledons and one hypocotyle were incubated on each plate. The experiment was repeated 3 times with each DH line requiring 15 plates per repetition, i.e. 45 explants. Altogether, 810 explants were collected, out of which 270 were hypocotylous and 540 cotyledonous explants.

The regeneration assessment was made after 28 days of culture. The regeneration effectiveness of each DH line was defined as the ratio of the number of explants regenerating shoots to the total number of explants which were collected, according to the formula:

$$\mathrm{E} = \frac{R}{T} \cdot 100\%,$$

where:
E – stands for regeneration effectiveness,
R – is the number of explants regenerating shoots,
T – is the total number of collected explants (ZANDECKA-DZIUBAK, ŁUCZKIEWICZ 2000).

In order to examine the impact of IBA addition on explants regeneration effectiveness a statistic *t*-student test was carried out with the use of a statistic R platform. The null hypothesis assumed mean identity for both trials, that is no significant differences in shoot regeneration effectiveness between the two examined medium types, while an alternative hypothesis assumed that the means for both trials would differed significantly. The IBA impact on shoot regeneration effectiveness of winter rapeseed DH lines was considered as truly significant when the p value was lower than 0.05.

Results

Among the analyzed DH lines we observed varied impact of the medium type on the shoot regeneration process in the plant parts collected from rapeseed seedlings. Shoot regeneration in explants occurred indirectly via callus and through direct formation of shoots and roots. Callus was formed on each type of explants, yet its formation was limited to the cut parts of plants tissue and, what's more, it spread only in the later stages of culture. The formation of callus and roots was observed mainly in the basal part of hypocotylous explants while the apical end of hypocotyl was more likely to form shoots. Only the explants from W-15 line, incubated on the medium containing IBA, did not create callus. Moreover, in the control medium the formation of callus was not observed in hypocotylous explants from B-21 line and on in cotyledonous explants from W-15 line. It is, however, notable that in the control medium callus was created only occasionally (roughly in 17% of explants) while on in the medium containing auxin it was formed in approximately 67% of the incubated explants.

With the exception of the explants from B-18 line, which did not create roots, rhizogenesis occurred in each line of the cotyledonous explants incubated on the control medium, though to a limited extent. In the medium containing IBA acid root regeneration was observed in all explants, except for the mentioned W-15 line, whose explants did not formed roots. Rhizogenesis was observed in approximately 81% of the explants incubated on the medium with the hormone and in 24% of the explants from the control medium.

In comparison, shoot formation occurred in a smaller number of explants. The range of variability of shoot regeneration effectiveness for the medium containing IBA was between 6.7% and 42.2% for the first repetition of the experiment, 6.7% and 37.8% for the second repetition and 6.7% and 40.0% for the third one. The minimum and maximum values for the control medium were 0% and 6.7% for the first repetition, 0% and 8.9% for the second repetition and 0% and 6.7% for the third repetition. The results of shoot regeneration in the control medium and in the medium containing phytohormone for the three repetitions of the experiments are presented in Figure 1 and Figure 2. The average effectiveness of shoot regeneration in the explants growing on the medium with the regulator reached 19.3% and was higher than the average shoot regeneration in the control medium explants. In the latter group, shoot regeneration was observed only for cotyledonous explants. In the medium containing IBA, shoot regeneration in cotyledonous explants occurred in nearly all DH lines. In comparison, the hypocotylous explants that were incubated on the medium containing IBA were regenerating shoots only in 2% of cases. For both types of the medium, the highest shoot regeneration effectiveness was observed for the W78 line.

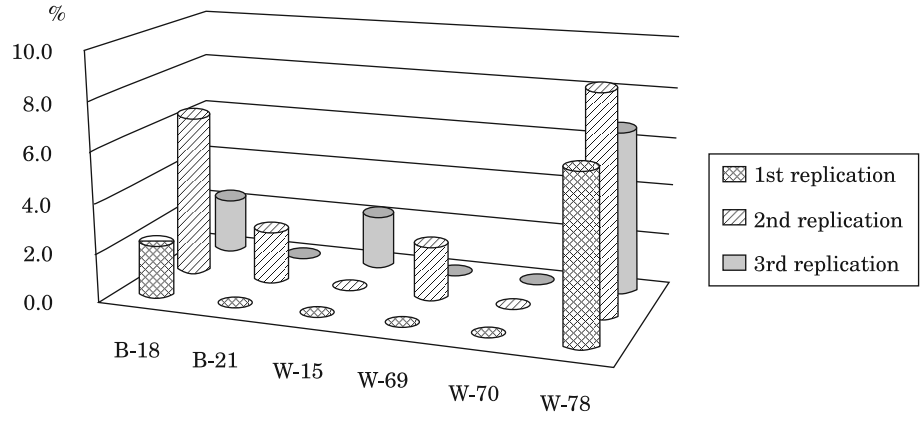

Fig. 1. Shoot regeneration effectiveness [%] of the examined DH winter rapeseed lines in three replications of the experiment in the control medium

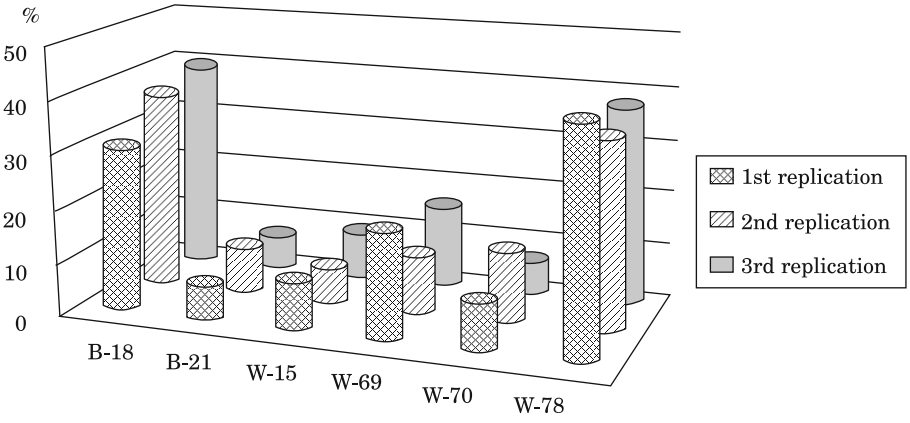

Fig. 2. Shoot regeneration effectiveness [%] of the examined DH winter rapeseed lines in three replications of the experiment in the medium containing IBA phytohormone in 10 mg/l concentration

The student-t test conducted on the samples incubated on the medium with IBA and on the one without it confirmed that IBA had significant impact on shoot regeneration effectiveness in all the tested DH lines. The p-values obtained in the t-student test are presented in Table 2.

Table 2

Impact of IBA regulator on shoot regeneration of DH winter rapeseed lines

The differences in shoot regeneration effectiveness of the explants incubated on the IBA-containing medium as compared to the control medium						
DH lines	B-18	B-21	W-15	W-69	W-70	W-78
p values	0.001*	0.003*	0.002*	0.022*	0.039*	0.001*

* differences significant for $p < 0.05$

Discussion

Regeneration is an important step in the process of obtaining valuable plant material in the *in vitro* culture. As it was mentioned, auxins induce the formation of callus and adventitious roots (WOŹNY and PRZYBYŁ 2004) as well as the process of somatic embryogenesis, provided that they are added to the medium in appropriately high concentration (VIKRANT and RASHID 2003). However, the reaction of explants to the *in vitro* culture conditions may be very different (WOJCIECHOWSKI 1998). Moreover, the type of auxin used in the medium impacts the morphogenesis of the culture (HOFMANN et al. 2004).

In the experiment the formation of callus was observed only in the cut parts of hypocotyls and cotyledons of DH rapeseed lines. An intensive growth occurred only in the later stages of culture, which goes in line with the earlier reports on callus regeneration in *Brassica napus* after the addition of exogenous auxin to the medium (ŚLESAK et al. 2005, ULLAH et al. 2004). The observed formation of roots on the apical side of hypocotyls and of callus and roots on their basal side is also consistent with the results achieved by Ślesak and others in *Brassica napus* cv. Kana.

In the studies conducted by ŚLESAK et al. (2005) and BOGUNIA and PRZYWARA (2000), a 28-day exposition of explants to 2 mg/l 2,4-D exogenous auxin caused inhibition of rhizogenesis and shoot formation. Callus occurred in the majority of the hypocotylous and cotyledonous explants exposed to the regulator, though in very small amounts. For the control medium considerable root regeneration was observed but the callus occurred only from time to time (ŚLESAK et al. 2005). In a study on *Brassica napus* cv. Oscar regeneration (KHAN et al. 2002) higher concentration of 2,4-D regulator suppressed proliferation of callus and adding 8 mg/l of this regulator to the medium resulted in the total absence of callus.

In the current study the presence of callus was observed in both types of explants incubated on the medium containing auxin. Its rare formation was also found in explants incubated on the control medium. These results are consistent with the results obtained by ŚLESAK et al. (2005) and BOGUNIA and

PRZYWARA (2000). In this experiment rhizogenesis was observed in 75% of the explants incubated on the medium with auxin, which does not confirm the results reported by ŚLESAK et al. (2005) and by BOGUNIA and PRZYWARA (2000). In the case of the control medium roots regeneration was definitely less effective than the rhizogenesis observed in the experiments conducted by ŚLESAK et al. (2005). Contrary to the results obtained by previous authors, root regeneration was observed mainly in the cotyledonous explants incubated on the MS medium with IBA. The dissimilarity of the obtained results probably comes from using different types of regulators to initiate shoot regeneration process.

The differences in regeneration effectiveness observed in the experiment may be due to genotype differences of the tested DH lines as the experiment was fully controlled with regard to the temperature, lightening and chemical composition and similar results were obtained in the three replications of the experiment. The obtained results may thus indicate an important role of genotype in determining regeneration capability.

Conclusion

The capability of cotyledonous explants to regenerate is a complex feature which can be affected by genetic and environmental factors. The obtained results confirm that the indole-3-butyric acid can have a positive effect on regeneration capability of rapeseed explants and point to a crucial role of genotype in determining the *in vitro* culture regeneration capability.

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