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Factors affecting morphogenesis in tissue culture of linseed flax (*Linum usitatissimum* L.)

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Abstract

Factors influencing the morphogenic capability of linseed flax cultivars 'Lirina', 'Barbara' and 'Szaphir' in tissue culture were determined. Two different types of explants (stem segments and hypocotyls) of the three genotypes were cultivated in nutrient media differing in their macro and micro salts concentration and different levels of growth regulators. Our results showed, that callus induction, root formation and shoot regeneration was possible in all types of nutrient media. However, the intensity of morphogenesis was affected not only by the used exogenous growth regulators, but also by the type of explant and genotype. Hypocotyls from all tested genotypes were more responsive in shoot induction than stem segments on all tested media. Calli, shoots and roots were derived at the same time from tested explants in all media depending on the genotype. The best morphogenetic capabilities were demonstrated for cultivar 'Szaphir'. For all cultivars the largest number of shoots was produced on MSB_5 nutrient medium supplemented with cytokinin 6-(y-y-dimethylallylamine)purine (2.0 mg 1^{-1}).

Key words: genotype, growth regulators, flax, media composition, morphogenesis, tissue culture.

Introduction

Flax (*Linum usitatissimum* L.) is a dicotyledonous plant of the family *Linaceae*. Commonly, flax breeders use pedigree selection or bulk breeding methods to create novel lines (Steiss et al.1996). The application of biotechnology have been helpful in accelerating breeding programmes or improving the efficiency of selection, as demonstrated in linseed flax and other oil crop species (Friedt 1990). Tissue culture techniques developed for flax mainly aim to obtain valuable and desirable new traits in flax cultivars (resistance to fungal diseases, oil quality improvement and herbicide tolerance) through somatic hybridization and somaclonal variation (Basiran et al. 1987). Cotyledons, hypocotyls, meristems, and stem segments have been used as explants for culture initiation in flax, but only plant regeneration from hypocotyl segments has proven to be highly efficient (Friedt 1990).

The aim of this research was to determine the effect of genotype, explant type and medium composition on linseed flax morphogenesis *in vitro*.

Materials and methods

The investigation was carried out with three linseed flax (*Linum usitatissimum* L.) cvs. 'Lirina', 'Barbara', 'Szaphir'. Stem segments and hypocotyls were used as explants to

study linseed flax morphogenesis. Tissue culture protocols were the same as described previously (Bretagne et. al. 1994; Blinstrubiene et. al. 2004). Explants were placed on combined medium MSB_5 [MS macro salts (Murashige, Skoog 1962) and B_5 micro salts with vitamins (Gamborg 1968)], $0.75MSB_5$ (75 % MS macro salts and B_5 micro salts with vitamins), $0.5MSB_5$ (50 % MS macro salts and B5 micro salts with vitamins) supplemented with sucrose 30 g l^{-1} and Difco-Bacto agar 6 g l^{-1}. The following growth regulators were used: 1.0 mg l^{-1} 6-furfurylaminopurine (kinetin) + 0.1 mg l^{-1} indole-3-acetic acid (IAA), 1.0 mg l^{-1} 6-benzylaminopurine (BA) + 0.05 mg l^{-1} α-naphthylacetic acid (NAA), 2.0 mg l^{-1} 6-(y-y-dimethylallylamine)purine (2iP), 1.0 mg l^{-1} kinetin. The media pH was 5.7 ± 0.1, illumination – 5000 lx, photoperiod – 16 h, temperature 25 ± 2 °C. Each variant consisted of 50 explants and four replications were used. Explants were transferred to 50 ml fresh medium every 4 weeks into 200 ml glassware.

The morphogenetic potential of tissues was evaluated by analyzing the morphological parameters of the structures formed in the explants. The evaluation was based on the relative frequency of explants forming callus, shoots, and roots.

Significant differences were determined using a computer programme (Tarakanovas 1996) for analysis of variance, grouped by Duncan's criteria $P \leq 0.05$.

Results

The tested genotypes and explant types had different intensities of callus formation (Table 1). The percentage of explants producing callus varied from 48 % on hypocotyls of 'Lirina' in $0.5MSB_5$ with 1.0 kinetin + 0.1 IAA) to 100 %. The affect of medium composition on callogenesis was genotype dependent. Cultivar 'Barbara' had the highest frequency of callus formation in all media. The medium MSB_5 with 1.0 mg l^{-1} kinetin

Table 1. Influence of genotype, explant type and medium composition on callogenesis (%) of linseed flax in tissue culture. Means are significantly different at $P \leq 0.05$ (Duncan's multiple range test)

Nutrient media	'Lirina'		'Barbara'		'Szaphir'	
	Stem segment	Hypocotyl	Stem segment	Hypocotyl	Stem segment	Hypocotyl
MSB_5+1.0 kinetin+0.1 IAA	93^{b}	80^{b}	100^{a}	90^{b}	98^{a}	85^{b}
MSB_5+1.0 kinetin	100^{a}	88^{b}	100^{a}	96^{a}	100^{a}	95^{a}
MSB_5+1.0 BA+ 0.05 NAA	95^{a}	82^{b}	100^{a}	90^{b}	100^{a}	90^{b}
MSB_5+2.0 2iP	97^{a}	85^{b}	100^{a}	93^{b}	100^{a}	92^{b}
$0.75MSB_5$+1.0 kinetin+0.1 IAA	60^{c}	57^{d}	80^{b}	70^{c}	75^{c}	68^{c}
$0.75MSB_5$+1.0 kinetin	70^{c}	68^{c}	78^{c}	70^{c}	75^{c}	70^{c}
$0.75MSB_5$+1.0 BA+ 0.05 NAA	100^{a}	85^{b}	100^{a}	87^{b}	100^{a}	78^{c}
$0.75MSB_5$+2.0 2iP	85^{b}	80^{b}	100^{a}	85^{b}	100^{a}	80^{b}
$0.5MSB_5$+1.0 kinetin+0.1 IAA	53^{d}	48^{d}	68^{c}	60^{c}	65^{c}	57^{d}
$0.5MSB_5$+1.0 kinetin	63^{c}	58^{d}	62^{c}	58^{d}	70^{c}	50^{d}
$0.5MSB_5$+1.0 BA+ 0.05 NAA	100^{a}	68^{c}	100^{a}	94^{b}	100^{a}	85^{b}
$0.5MSB_5$+2.0 2iP	80^{b}	92^{b}	95^{a}	85^{b}	93^{b}	80^{b}

Table 2. Influence of genotype, explant type and medium composition on shoot regeneration (%) of linseed flax in tissue culture. Means are significantly different at $P \leq 0.05$ (Duncan's multiple range test)

Nutrient media	'Lirina'		'Barbara'		'Szaphir'	
	Stem segment	Hypocotyl	Stem segment	Hypocotyl	Stem segment	Hypocotyl
MSB_5+1.0 kinetin+0.1 IAA	60^c	73^c	75^c	80^b	100^a	100^a
MSB_5+1.0 kinetin	60^c	65^c	98^a	98^a	97^a	100^a
MSB_5+1.0 BA+ 0.05 NAA	75^c	80^b	98^a	100^a	85^b	90^b
MSB_5+2.0 2iP	100^a	100^a	100^a	100^a	100^a	100^a
0.75MSB_5+1.0 kinetin+0.1 IAA	8^e	15^d	10^d	18^d	15^d	23^d
0.75MSB_5+1.0 kinetin	10^d	18^d	12^d	26^d	15^d	30^d
0.75MSB_5+1.0 BA+ 0.05 NAA	14^d	20^d	20^d	24^d	30^d	38^d
0.75MSB_5+2.0 2iP	30^d	44^d	30^d	50^c	50^c	62^c
0.5MSB_5+1.0 kinetin+0.1 IAA	2^e	8^e	3^e	10^d	15^d	20^d
0.5MSB_5+1.0 kinetin	5^e	10^d	8^e	12^d	10^d	15^d
0.5MSB_5+1.0 BA+ 0.05 NAA	8^e	12^d	10^d	14^d	10^d	16^b
0.5MSB_5+2.0 2iP	18^d	30^d	25^d	30^d	35^d	42^b

was the most suitable for callus induction for all tested genotypes. On this medium, stem segments and hypocotyls of all tested genotypes showed the highest potential of callogenesis. Hypocotyls from all tested genotypes had lower levels of callus induction than stem segments on all media types.

Morphogenesis of the tested linseed flax cultivars depended on the genotype, explant type and nutrient medium composition (Table 2). Shoot induction was higher on hypocotyls than on stem segments from all tested genotypes and media. The genotype 'Szaphir' demonstrated the highest frequency of morphogenesis on all used types of nutrient media, in comparison with 'Barbara' and 'Lirina'. However, the nutrient medium MSB_5 with cytokinin 2iP was mostly suitable for organogenesis in all genotypes, especially for shoot formation. Also, this medium and hormone combination showed the best rhizogenesis in all of the tested cultivars (data not show). Isolated explants of the cultivars 'Barbara' and 'Lirina' also demonstrated the most intensive morphogenesis in complex MSB_5 medium supplemented with cytokinin 2iP, in comparison with the another medium treatments.

Discussion

It is widely considered that morphogenesis is strongly affected by genetic and exogenous factors (Bhaskaran, Smith 1990; Bjowani, Razdan 1990). Our results illustrate that the genetic background is important both for callus induction and shoot regeneration of in linseed flax tissue culture. The linseed flax cv. 'Szaphir' showed the superior morphogenetic capability. The organogenesis capacity was higher in cv. 'Szaphir' by 1.5 and 1.1 times compared to cvs. 'Lirina' and 'Barbara', respectively.

Depending on the plant species, nutrient media are often modified by adding different compositions of vitamins and growth regulators. The most widely used growth regulators

are the cytokinins BAP, 2iP and kinetin (Bjowani, Razdan 1990), and the auxins IAA and NAA. The effect of medium composition on linseed flax callogenesis, shoot formation and rhizogenesis strongly depended on the genotype and the type of the explant, with the three tested linseed flax cultivars exhibiting different regeneration responses. Hypocotyl segments from the linseed flax cultivar 'Szaphir' gave the best results. It can be assumed that the differences in morphogenetic reaction of different linseed flax genotypes are determined by the balance of endogenous hormones. The combinations of growth regulators optimal for callus induction, root formation and shoot regeneration differed. Our results show that linseed morphogenesis capacity depends not only on growth regulators, but also on the other components (macro salts, micro salts, vitamins). The best medium (MSB_5 + 2.0 mg l^{-1} 2iP) differed from the others in raised quantities of added vitamins and amino acids, which allowed more intensive development of callusogenesis and organogenesis in the linseed flax tissue culture.

In conclusion, linseed flax morphogenesis in tissue culture is influenced by endogenous and exogenous factors: cv. 'Barbara' had the highest frequency of callus formation in all media, but the best morphogenetic capability was demonstrated by the cv. 'Szaphir'; hypocotyls showed better morphogenetical ability in comporison with stem segments; MSB_5 nutrient medium supplemented with cytokinin 2iP (2.0 mg l^{-1}) was the mostly suitable medium for linseed flax organogenesis *in vitro*.

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