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CAPACITY FOR SOMATIC EMBRYOGENESIS IN DIFFERENT PEA CULTIVARS

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A b s t r a c t

Using the method described by GRIGA (1998), embryoids were obtained (through direct or indirect somatic embryogenesis) in cultures of shoot apical meristems of the following pea cultivars: Bankier, Dino, Hazard, Iłówiecki, Konserwowy IHAR, Kosynier, Makler, Oskar, Pegaz, as well as unregistered line HM-6. With cultivars Izolda and Lantra the efforts at somatic embryogenesis (SE) induction remained unsuccessful. The highest responsiveness to SE induction was observed (after 14 days of treatment with a relatively low concentration of picloram – 2.5 µM) in cultivars Oskar, Hazard and line HM-6, in which embryoids were formed with frequencies of 31, 15.9 and 12.5%, respectively. Increasing picloram level to 5 µM and extending period of induction to 28 days, it was possible to obtain SE efficiency above 10% in cultivars Konserwowy IHAR, Dino and Kosynier. Photoperiod affected SE efficiency and the degree and direction of this influence greatly depended on pea cultivar.

ZDOLNOŚĆ DO SOMATYCZNEJ EMBRIOGENEZY U WYBRANYCH ODMIAN GROCHU

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S ł o w a k l u c z o w e*:* groch, somatyczna embriogeneza, pikloram, mikrorozmnażanie.

A b s t r a k t

Posługując się metodą GRIGI (1998), uzyskano zarodki somatyczne (na drodze embriogenezy bezpośredniej lub pośredniej) w hodowlach wierzchołków pędów następujących odmian grochu: Bankier, Dino, Hazard, Iłówiecki, Konserwowy IHAR, Kosynier, Makler, Oskar, Pegaz. Próby uzyskania somatycznej embriogenezy u odmian Izolda i Lantra się nie powiodły. U odmian Oskar i Hazard, jak również niezarejestrowanej linii HM-6, indukcja somatycznej

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embriogenezy zachodziła najwydajniej – po 14-dniowym pobudzaniu pikloramem o stosunkowo niskim stężeniu (2,5 µM) zarodki tworzyły się z częstością odpowiednio: 31, 15,9 i 12.5%. Podwyższenie stężenia pikloramu do 5 µM i wydłużenie czasu indukcji do 28 dni pozwoliło na uzyskanie częstości SE przekraczającej 10% u odmian Konserwowy IHAR, Dino i Kosynier. Fotoperiod wpływał na wydajność SE, a stopień i kierunek tego oddziaływania silnie zależały od odmiany grochu.

Introduction

Somatic embryogenesis (SE) raises great interests for both theoretical and practical reasons. It is a very useful system for experiments in plant development and potentially an efficient way of plant vegetative propagation. The responsiveness to SE induction treatments differs markedly across plant species and carrot, cucumber, alfalfa, wheat and maize are among the plants reacting most readily to SE inducers (DUNCAN et al. 2003, HITA et al. 2003, MALINOWSKI et al. 2004, YASUDA et al. 2000, WANG and WEI 2004). Grain legumes (including such important crops as soybean, bean, and pea) are considered rather refractory with respect to SE induction (LAKSHMANAN, TAJI 2000). Pea (*Pisum sativum* L.) is not only an important food legume (FAOSTAT 2007), but it also serves as a favorite object of physiological and molecular research (SCHROEDER et al. 1993). However, somatic embryogenesis in this species is rather time-consuming and the frequencies of embryo formation are often quite low (SCHROEDER et al. 1993, SANAGO et al. 1996). A reliable system for somatic embryogenesis in pea is still missing. In this work we report intervarietal differences in pea competence for somatic embryogenesis.

Materials and Methods

Plant material

Pea seeds were surface sterilized in aqueous solution of chloramine B (5%) for 15 minutes (smooth seeds) or 30 minutes (wrinkled seeds), followed by 3 washes with sterile distilled water. After sterilization, seeds were placed in tubes (25 cm^3 capacity) on a moist cotton wool. After germination (in darkness at 25-26ºC for 4-7 days) shoot apical meristems were excised and transferred to SE induction medium as primary explants.

Seeds of pea cultivars Bankier, Dino, Hazard, Iłówiecki, Konserwowy IHAR, Izolda, Kosynier, Lantra, Makler, Pegaz were bought from PLANTICO corporation (Poland) and seeds of pea cv. Oskar and line HM-6 were kindly provided by AGRITECH Ltd.(Czech Republic).

Somatic embryogenesis induction

Two experimental systems were tested:

System I. Shoot apices excised from 4-day-old, etiolated, axenically grown seedlings were subjected to 14-days induction on basal medium described by GRIGA (1998), that contained MS salts (MURASHIGE, SKOOG 1962), Gamborg's B5 vitamins (GAMBORG et al. 1968), 3% sucrose and 2,5 μM picloram. During induction, cultures were kept in growing chamber under 16:8 photoperiod (light:darkness) and temperatures of 23–24ºC at day and 19–20ºC at night.

System II. It was adopted for those cultivars that did not form embryos under conditions of System I (see Results). Shoot apices excised from 7-days-old, etiolated, axenically grown seedlings were subjected to 28-days induction on basal medium as used in system I, except picloram concentration raised to 5 μM. During the time of induction cultures were kept in growing chamber in darkness at 26–27ºC, or under 16:8 hours photoperiod (light:darkness) at temperatures of 23–24ºC at day and 19–20ºC at night.

Somatic embryos differentiation

After induction all cultures were transplanted to the differentiation medium (basal medium with no phytohormones) and cultures were kept in growing chamber under 16:8 hours photoperiod with temperatures of 23–24ºC at day and 19–20ºC at night. Physiological state of cultures, their morphogenetic reactions and efficiency of somatic embryogenesis were evaluated 3 weeks after transfer to the differentiation medium. Observations were made every 7–10 days.

The efficiency of somatic embryogenesis was defined as average number of somatic embryos per explant and expressed as per cent.

Statistical analysis

The effects of cultivars on efficiency of somatic embryogenesis induction were analyzed by one-way analysis of variance (ANOVAs). Means and standard errors (± SE) were calculated for five series of data with four replicates in each series. The means were grouped using Duncan's multiple range test at $P \leq 0.05$.

The Microsoft Excel 2007 and STATISTICA 8.0 computer programs were used.

Results

At the beginning of culture period explants excised from Konserwowy IHAR and Pegaz seedlings increased their size considerably (to 2–3 mm) without changing their overall shape. Somatic embryogenesis was successfully induced in 10 out of 12 tested cultivars/lines of *Pisum sativum* L. In experiment I direct somatic embryogenesis was observed. Normal somatic embryos with two cotyledons and shoot apex were obtained after 5 weeks of induction culture on explants of Oskar, Bankier, Hazard, Pegaz and HM-6 cultivars. The highest efficiency of somatic embryogenesis was observed in cultures of cv. Oskar and the lowest efficiency of somatic embryogenesis was observed in cultures of cv. Bankier (Table 1). Numerous embryoids in cotyledonary stage were noticed in cultures of line HM-6 (70%), Oskar (55%) or Hazard and Pegaz cultivars (50%). Only one somatic embryo was obtained from all cultures of Lantra cultivar. In cultures of other cultivars no somatic embryos were observed under conditions of experiment I.

Table 1

The efficiency of somatic embryogenesis in 12 pea cultivars under photoperiodic (16:8 h) conditions (mean ± S.E) after 80 days incubation

Cultivar	Somatic embryogenesis efficiency (%)
Makler	0.0^{a}
Izolda	0.0^{a}
Konserwowy IHAR	0.0^{a}
Dino	0.0^{a}
Kosynier	0.0^{a}
Iłówiecki	0.0^{a}
Oskar	31.0 ± 6.8^{d}
Lantra	0.0^{a}
HM-6	12.5 ± 5.8^{b}
Bankier	5.5 ± 2.3^{c}
Hazard	15.9 ± 7.8^{b}
Pegaz	7.4 ± 3.3^{c}

Values followed by the same superscript are not significantly different at 5% level

In the second experimental system (somatic embryogenesis induction with increased level of picloram at constant darkness), indirect somatic embryogenesis was noticed in cultures of all tested pea cultivars, except Izolda and Iłówiecki (Table 2). The highest efficiency of somatic embryogenesis was observed in cultures of cv. Kosynier, and the lowest efficiency of somatic embryogenesis was observed in cultures of cv. Makler. When somatic embryos induction was carried out under 16-hours

photoperiod conditions the rise of somatic embryogenesis efficiency was observed in cultures of Makler, Iłówiecki and Konserwowy IHAR cultivars. There was no somatic embryogenesis efficiency changes in cultures of Dino cultivar. In cultures of Kosynier cultivar decrease of somatic embryogenesis efficiency (approximately 50%) was noticed. During the first five weeks of culture callus (approximately 2–5 mm wide) developed around the edges of the explants of cultivars Iłówiecki, Izolda and Konserwowy IHAR. Shoot tips isolated from Kosynier, Makler and Dino cultivars produced larger calli, approx. 20 mm wide.

Table 2

The efficiency of somatic embryogenesis in 6 pea cultivars subjected to 28 days induction with 5 μM picloram under constant darkness or photoperiodic conditions (mean ± S.E)

Cultivar	Somatic embryogenesis efficiency (%)	
	darkness	photoperiod 16/8h day/night
Makler	1.8±1.8^{d}	4.4±0.3^{a}
Izolda	0.0^{c}	0.0^{c}
Konserwowy IHAR	4.0±2.0^{a}	12.2±1.4^{b}
Dino	11.5±4.8^{b}	11.9±4.2^{b}
Kosynier	24.8±6.2	13.0±5.8^{b}
Iłówiecki	0.0^{c}	5.8±2.9^{a}

Values followed by the same superscript are not significantly different at 5% level

The obtained embryos showed many developmental anomalies. Most of them reached only the early torpedo stage. Approximately 15–20% of embryos produced cotyledons, however their further development was disrupted (data not shown). The efficiency of somatic embryogenesis in both experimental systems did not depend on the age of seedlings (4- or 7-day-old) at the time of explant isolation (data not shown). The rate of embryo formation however, i.e. the moment when the first embryos appeared, was strongly affected by duration of the induction culture and picloram concentration. When cultures were exposed for 28 days to higher concentration of picloram (5 μM), callus developed and the induction of embryogenesis was retarded. First somatic embryos were observed on explants after 3 weeks of induction culture of Oskar and HM-6 cultivars (experimental system I) or after 7 weeks culture (3 weeks after transfer to hormone-free medium) of Konserwowy IHAR, Dino, Kosynier i Iłówiecki (experimental system II). Explants of Makler, Konserwowy IHAR, Dino, Kosynier and Iłówiecki cultivars subjected to 28-days induction culture under standard photoperiod conditions generated embryos after 6 weeks of culture (2 weeks after transfer to hormone-free medium).

On some explants multishoots were observed along with somatic embryos. The level of shoot development varied and depended on cultivar. On explants of Kosynier, Hazard and Oskar cultivars shoot clumps clearly

derived from callus tissue. In the other cultures shoots formed from primary apical meristems. Callus formation occurred chiefly on the basal part of the explants.

Discussion

Many plant species can readily form somatic embryos under *in vitro* culture. It is known, however, that plant species, and even cultivars (PODWYSZYŃSKA 1997) and lines (GAIN et al. 1998) differ widely in responsiveness to somatic embryogenesis induction. Legumes are considered rather tenacious in this respect and the obtained frequencies of somatic embryo formation according to literature range from zero to dozens per cent (NADOLSKA-ORCZYK 1992, BRODA, TORZ 1997, GRIGA 1998, WALKER, PARROT 2001, TAMLIN et al. 2002). The potential for normal germination of somatic embryos is also correlated with cultivar and genotype of explant cells (SALAJOVA et al. 1999, BELMONTE et al. 2007).

In this report significant differences were observed in numbers of somatic embryos across twelve pea cultivars. Somatic embryogenesis efficiency under standard photoperiodic conditions ranged from 0 to 31%. Oskar cultivar seemed most efficient and line HM-6 proved quite good, which corroborates results obtained by GRIGA (1998). Cultivars Hazard, Pegaz and Kosynier also turned out quite responsive to somatic embryogenesis induction. They may thus be added to the list of SE-prone cultivars/lines including, among others, Belman, Brite (LEHMINGER-MERTENS, JACOBSEN 1989), R 4111, PF 5/81, Belinda (KYSELY, JACOBSEN 1990) and Sugar Ann and Patriot (SANAGO et al. 1996).

The efficiency of somatic embryogenesis depends on source of explant, its tissue composition and age of seedling at the time of explant isolation. Fragments of young seedlings or zygotic embryos are usually used as explants in somatic embryos induction cultures of legumes (LAKSHMANAN, TAJI 2000, CHHABRA et al. 2008, JOSHI et al. 2008). Reports by LOISEAU et.al. (1995) and GRIGA (1998) show that shoot apical meristems may also be applicable and our results further support this suggestion.

Type and concentration of plant growth regulators used for induction of somatic embryogenesis have to be adjusted for any specific plant and type of explant. In legumes, with a few exceptions, somatic embryogenesis was induced with the following auxins: 2,4-D; NAA; picloram; IAA and dicamba (LAKHMANAN, TAJI 2000, JOSHI et al. 2008). in pea SE was mainly induced with 2,4-D or NAA at 2–4 μM concentration (KYSELY, JACOBSEN 1990; OZCAN et al. 1993, GRIGA 1998, LAKHMANAN, TAJI 2000). High concentrations of auxins are believed to increase somatic embryogenesis efficiency, however, exceedingly high levels of auxins may also have inhibitory effect on embryo formation (TOMLIN i in. 2002).

Conclusions

The results show that the choice of cultivar may be critically important for efficient induction of somatic embryogenesis in pea. Among tested cultivars Oskar, Hazard and line HM-6 were most amenable for induction of somatic embryogenesis. With cultivars less prone to form embryoids, the increase of auxin concentration in induction medium and extension of the induction period proved helpful.

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