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Evaluation of different embryogenic systems for production of true somatic embryos in *Arabidopsis*

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Abstract

Somatic embryogenesis (SE) in *Arabidopsis* was induced using various systems, including auxin treatment of *in vitro* cultured explants (immature zygotic embryos, IZEs) and transgenic plants overexpressing embryogenesis-related transcription factors, *e.g*. LEC2 together with the *GUS* reporter gene under control of the auxin-induced DR5 promoter. The study indicated that the SE-systems used gave different embryogenic capacities for the production of true embryos. The highest ratio of true embryos (75 %) was found among embryo-like structures in transgenic seedlings overexpressing *LEC2*. Analysis of *in vitro* induced SE systems indicated that in somatic embryos produced in response to exogenous auxin treatment the formation of root poles is frequently disturbed. A lack of a properly formed root meristem was observed in 35 - 80 % of *in vitro* induced somatic embryos, in dependence on auxin concentration and duration of treatment.

Additional key words: DR5::GUS, *LEC2* overexpression, reporter gene analysis, root formation *in vitro*.

Introduction

Since the first work on somatic embryo formation in carrot culture (Steward *et al.* 1958) numerous protocols have been published on plant regeneration *via* somatic embryogenesis (George and Debergh 2008). Morphological similarities of the developing *in vitro* structures to zygotic embryos have been routinely used as the basic and sufficient criterion to classify them as somatic embryos. By definition, somatic embryos are bipolar structures, and thus the presence of shoot (SAM) and a root (RAM) apical meristems is recommended as the main marker of the true embryonic nature of the regenerated structure. Unfortunately, due to laborious and time consuming histological analysis, the formation of two poles in the regenerated embryo-like structures has been documented only occasionally. Defects in proper root formation have been suggested when the problems with rooting of the SE-derived plants have been encountered (Barry-Etienne *et al.* 2002, Carredoira *et al.* 2003). Only rarely developmental disorders in the formation of somatic embryo roots were convincingly documented at the histological level (Vasil *et al.* 1985, Faure *et al.* 1996, Griga 2002).

A lack of the root pole in otherwise embryo-shaped structures was observed in *Pelargonium* × *hortorum* (Madden *et al.* 2005) and *Linum usitatissimum* (Salaj *et al.* 2005) cultures, indicating that the morphology of the regenerated structures is not sufficient to classified them as true somatic embryos. Accordingly, the presence of the basal root pole, frequently questionable or neglected in somatic embryo characterization, has been proposed to identify complete and true somatic embryos (Sharma and Millam 2004, Madden *et al.* 2005, Salaj *et al.* 2005). Recently, the transgenic plants expressing reporter genes provide simple and fast analytical methods to mark the identity of different organs, tissues or cells (Sabatini *et al.* 1999, Nolan *et al.* 2009, Su *et al.* 2009, Debnath *et al.* 2010). Among others, the reporter *GUS* gene under an auxin controlled promoter marks auxin accumulation in plant tissues (Ulmasov *et al.* 1997, Sabatini *et al.* 1999). In the DR5::GUS construct a bacterial *β-glucuronidase* (*GUS*) reporter gene is controlled by several copies of a synthetic auxin response element positioned before a minimal promoter (Ulmasov *et al.* 1997). This construct was used for visualization of

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Abbreviations: CIM - callus induction medium; 2,4-D - 2,4-dichlorophenoxyacetic acid; DSE - direct somatic embryogenesis; ISE - indirect somatic embryogenesis; IZE - immature zygotic embryo; LEC2 - LEAFY COTYLEDON2; RAM - root apical meristem; SAM - shoot apical meristem.
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cell with highly active auxin-dependent transcriptional responses, and "auxin response maxima" (Sabatini *et al.* 1999). Tracking of auxin maxima in the regenerated structures allows true somatic embryos to be distinguished, due to the signal expected in the properly formed root pole (Bassuner *et al.* 2007).

In *Arabidopsis*, somatic embryo formation under *in vitro* conditions is induced on auxin supplemented media. Various protocols have been developed enabling direct (Gaj 2001) and indirect (Pillon *et al.* 1996, Mordhorst *et al.* 1998, Ikeda-Iwai *et al.* 2002, Su *et al.* 2009) SE induction in the culture of immature zygotic embryos (IZEs). Beside *in vitro* culture in *Arabidopsis,* somatic embryos can also occur *in planta* in transgenic plants overexpressing embryogenesis-related transcription factors, including WUS (Zuo *et al.* 2002), AGL15 (Harding *et al.* 2003), MYB (Wang *et al.* 2008) and BBM (Boutilier *et al.* 2002). The overexpression of master regulators of zygotic embryogenesis, *LEC1* (Lotan *et al.* 1998) and *LEC2* (Stone *et al.* 2001) genes, can also induce efficient development of embryo-like structures in transgenic seedlings.

Histological analysis has confirmed somatic embryo formation in *Arabidopsis* IZE explants cultured *in vitro* under auxin treatment (Mordhorst *et al.* 1998, Pillon *et al.* 1996, Raghavan 2004, Kurczyńska *et al.* 2007), and SEM analysis demonstrated the embryonic nature of the structures developed *in planta* (Stone *et al.* 2001). However, the global evaluation of different *Arabidopsis* SE-systems in terms of the frequency of complete, true somatic embryos, has not yet been reported. Such analysis seems to be especially valuable in respect of the central position of *Arabidopsis* SE-systems in genomic studies on plant embryogenesis and totipotency (Zimmerman 1993, Tzafrir *et al.* 2004, Casson *et al.* 2005).

Thus in the present work, the frequency of true somatic embryos was evaluated in various SE systems in *Arabidopsis*. The embryo-like structures produced *in vitro via* direct and indirect SE pathways, and *in planta* on transgenic seedlings overexpressing *LEC2* gene were analyzed with the use of DR5::GUS reporter gene to detect true embryos marked by properly formed root poles. The influence of exogenous auxin concentration and treatment period on true somatic embryo production was investigated.

Materials and methods

Arabidopsis thaliana (L.) Heynh. Columbia (Col-0) ecotype from NASC (The Nottingham Arabidopsis Stock Centre) was used in the study. Transgenic plants carrying the DR5::GUS were kindly provided by Dr. J. Murfett (Division of Biological Sciences, University of Missouri, Columbia). The DR5::GUS and 35S::LEC2-GR (Ledwon and Gaj 2009) lines were crossed, and the plants with 35S::LEC2-GR DR5::GUS construct produced. Immature zygotic embryos of the DR5::GUS and Col-0 control genotypes were used as explants for SE induction *in vitro*. The explants at late-cotyledonary stage were isolated and cultured according to the standard protocol (Gaj 2011).

To induce direct SE (DSE) we used E solid media derived from basal B5 medium (Gamborg *et al.* 1968), supplemented with 20 g dm^{-3} sucrose and 4 g dm^{-3} *Phytagel*. The SE-induction media with different 2,4-dichlorophenoxyacetic acid (2,4-D) concentrations were tested, namely a standard E5 medium with 5.0 µM (Gaj 2001) and E1 medium with 2,4-D concentration lowered to 1.0 µM.

In the indirect somatic embryogenesis (ISE), callus formation proceeds development of somatic embryos. In the ISE-E5 system, the explants were induced for 4 d in callus induction liquid medium (CIM) with 2.26 µM 2,4-D and 0.46 µM kinetin (Feldman and Marks 1986), and then transferred onto E5 solid medium for indirect somatic embryo production. The ISE-E9 system followed the method of Ikeda-Iwai *et al.* (2002), with modifications according to Su *et al.* (2009). The IZE explants were induced for 14 d on solid E medium containing 4.5 µM 2,4-D and 500 mg dm^{-3} casein hydrolysate and then explants with primary somatic embryos were kept for 10 d on ECIM liquid medium with 9.0 µM 2,4-D to induce embryogenic callus. The embryonic callus was then transferred to liquid auxin-free SEIM medium to promote development of secondary somatic embryos.

To evaluate the influence of 2,4-D treatment period on somatic embryo production the explants in the DSE and ISE-E5 systems were cultured on auxin media (E5 or E1) for various periods (10, 12 and 15 d). Thirty explants in three biological replicates were analyzed for each culture combination tested.

Morphological inspection of regenerated structures was used to estimate the embryogenic capacity of different SE systems. The SE efficiency (the percentage of IZE explants or transgenic seedlings producing somatic embryos) and productivity (an average number of the somatic embryos produced by an explant or seedling) were scored in each system. The analyses were carried out at 15 d cultures on E5 or E1 medium (DSE-E5, DSE-E1 and ISE-E5 systems), and in the ISE-E9 system at 8 d cultures on auxin-free medium.

To produce somatic embryos without exogenous auxin treatment the 35S::LEC2-GR DR5::GUS transgenic line was used. Nuclear activity of the overexpressed *LEC2* transcription factor was induced *in planta* by dexamethasone (DEX) treatment of the transgenic plants (Ledwon and Gaj 2009). The seeds were surface-sterilized for 15 min in 20 % solution of sodium hypochloride, rinsed in sterile water and germinated on MS20 solid medium (Murashige and Skoog 1962), with 20 g dm^{-3} sucrose and 30 µM DEX (*Sigma*, St. Louis,

MO, USA). After 21 d, the seedlings were inspected under a stereomicroscope to score the plants developing embryo-like structures, and to calculate SE efficiency and productivity.

The regenerated embryo-like structures produced in the different treatment combinations were isolated at the time of SE capacity evaluation, and then transferred to MS20 solid medium. After 21 d the percentage of complete plants, *i.e*. those developing shoots and roots, was calculated. The conversion ratio of embryo-like structures was calculated in three independent experiments, and thirty explants were analyzed in each repetition of each combination.

To identify a GUS signal, the embryo-like structures regenerated in the SE systems were cultured on MS20 solid medium for 10 d, and then a histochemical assay for GUS enzyme activity was performed as described by Bassuner *et al.* (2007). The embryo-like structures were stained in a standard X-Glu solution at 37 °C for 12 h. The experiments were repeated three times for each combination studied, and at least fifty somatic embryos were subjected to GUS signal detection in each repetition. The morphology of the analyzed structures and the GUS staining pattern were analyzed using a stereomicroscope *Zeiss STEMI 2000-C* (Jena, Germany) integrated with a digital camera *Canon Power Shot G6*.

The plants delivering IZE explants for *in vitro* culture, and those used in crosses were grown in *Jiffy-7* (Jiffy, Norway) pots in a walk-in type greenhouse at 20 - 22 °C, 16-h photoperiod and irradiance of 100 µmol m^{-2} s^{-1}.

In vitro induced explants and derived cultures, the transgenic 35S::LEC2-GR; DR5::GUS seedlings, and the embryo-like structures subjected to a conversion test were cultured at 23 °C, 16- h photoperiod and irradiance of 55 µmol m^{-2} s^{-1}.

Results

The culture of IZE explants on media supplemented with different auxin concentrations led to somatic embryo development *via* a direct or indirect morphogenic pathway, while spontaneous somatic embryo formation was observed *in planta* on transgenic seedlings overexpressing *LEC2* (Fig 1).

To induce DSE in IZE culture solid induction media containing 1.0 (E1) or 5.0 µM (E5) of 2,4-D were used. The first green protuberance were visible at 10 d on the adaxial surface of the IZE cotyledons (Fig. 1*A*,*C*). By 15 d of culture numerous embryo-like structures developed on the cotyledon part of the IZE explant (Fig. 1*B*,*D*), and the explants were then transferred onto auxin-free medium for plant development. The systems applied to induce ISE morphogenic pathways included a sequence of liquid and solid media supplemented with different 2,4-D concentrations. In the ISE-E5 system, the IZEs were pre-treated in a low-auxin liquid CIM medium. Following 4 d of treatment, expansion and swelling of the cotyledons were noticed, and abundant callus formation was then observed upon explant transfer into E5 solid medium (Fig. 1*E*). During the subsequent 10 - 15 d, embryo-like structures were formed on the callus tissue, indicating their indirect origin (Fig. 1*F-H*).

In the ISE-E9 system, IZEs were induced after 14 d on solid medium with 4.5 µM 2,4-D, and the explants developing embryo-like structures on cotyledons (Fig. 1*I*) were then sub-cultured into liquid medium with 9.0 µM 2,4-D. A 2-week culture in this medium resulted in embryogenic callus formation (Fig. 1*J*), and somatic embryos were then produced in this tissue upon transfer onto auxin free medium (Fig. 1*K*).

Overexpression of *LEC2* induced in 35S::LEC2-GR DR5::GUS plants resulted in distinct developmental changes in seedling morphology. The transgenic seedlings were smaller than non-transgenic ones, and embryo-like structures were formed on their leaves and in proximity of the shoot apical meristem (SAM). Moreover, callus tissue was produced on shoots and roots (Fig. 1*L*).

The embryogenic capacity of the SE-systems was evaluated in terms of SE efficiency and productivity. Embryogenic capacity of the transgenic DR5::GUS line was found similar to control Col-0 genotype (Table 1). Among *in vitro* embryogenic systems, the DSE-E5 system was found to be the most effective in respect to high percentage (86 %) of responding explants (Table 1). Relatively efficient embryogenic response (77 %) was also observed in two other *in vitro* systems, enabling direct (DSE-E1) and indirect (ISE-E5) embryo development. The lowest SE-efficiency, only 50 % of embryogenic cultures, was in ISE-E9 system involving prolonged treatment (24 d) with increased 2,4-D concentration (up to 9.0 µM). However, this system was found to provide the highest average number of somatic embryos per embryogenic explant (Table 1).

In planta almost all (93 %) of the transgenic seedlings overexpressing *LEC2* were found to produce embryo-like structures. This system was also very effective in terms of the number of produced embryo-like structures, and on average 10 of these structures were identified per transgenic seedling (Table 1).

Beside embryo-like, leafy-like structures could also be observed especially after short treatment (10 d) of explants with a low 2,4-D concentration (DSE-E1). The leafy-like structures were observed in about 60 % of explants induced for 15 d on E1 medium. In the other systems, including *LEC2*-induced SE, the leafy structures were detected only incidentally.

Given that the embryo-like shape of a regenerative structure is insufficient to prove its complete bipolar development, we used DR5::GUS transgenic plants to mark auxin signal expected in properly formed root meristem. Analysis indicated that in all of these embryo-

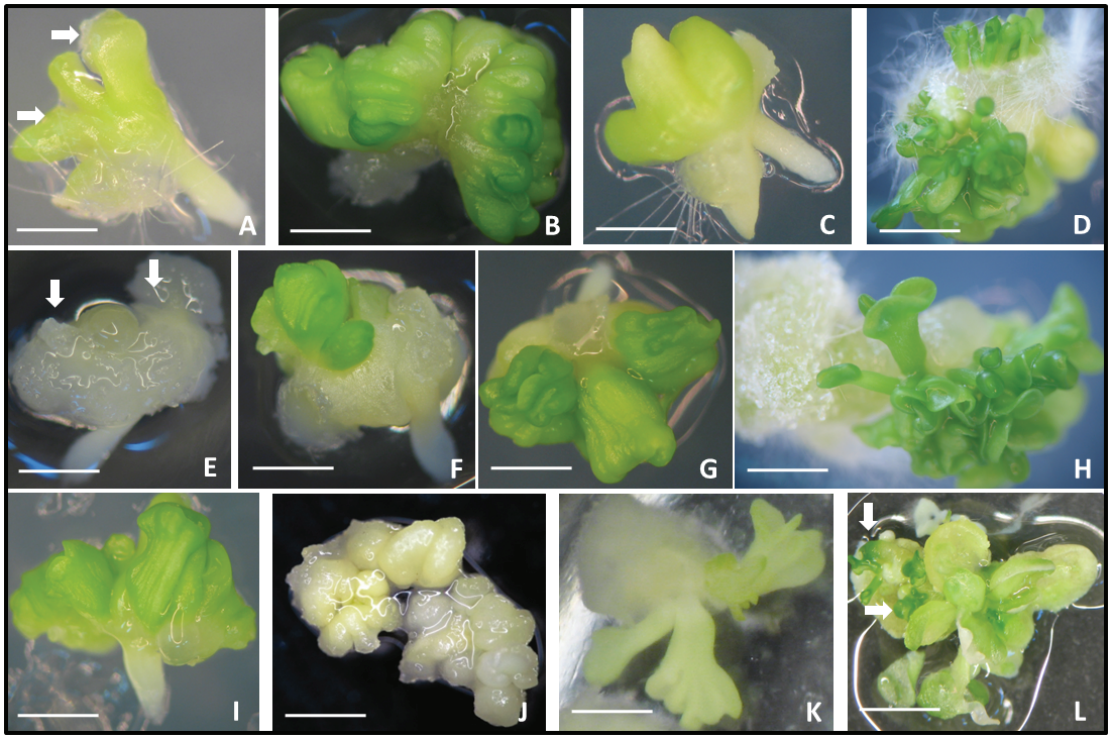

Fig. 1. Development of somatic embryos under *in vitro* culture of immature zygotic embryos, IZEs and *in planta* in transgenic seedlings (35S::LEC2-GR) of *Arabidopsis*. Direct SE induced on medium with 1.0 µM 2,4-D (DSE-E1; *A-B*) and 5.0 µM 2,4-D (DSE-E5; *C-D*). Indirect SE induced on medium with 5.0 µM 2,4-D (ISE-E5; *E-H*) and 9.0 µM 2,4-D (ISE-E9; *I-K*). *In planta* induced SE in 35S::LEC2-GR seedling (*L*). *A* - enlargement of cotyledon part (*arrows*) of IZE after 10 d; *B* - numerous embryo-like structures at 15 d; *C* - IZE explant after 10 d on E5; *D* - numerous embryo-like structures after 15 d; *E* - callus formation by cotyledons (*arrows*) of IZE explant pre-incubated in CIM medium and cultured for 3 d on E5; *F* - embryo-like protuberances after 10 d on E5; *G* - emergence of somatic-like structures after 12 d on E5; *H* - numerous somatic-like structures after 15 d on E5; *I* - primary somatic embryos developing at 14 d of culture on medium with 4.5 µM 2,4-D; *J* - embryonic callus after 10 d of culture on liquid E9 medium; *K* - somatic embryos originated from callus after 8 d of culture on auxin-free medium; *L* - numerous somatic embryo-like structures (*arrows*) and callus after 21 d on DEX medium. *Bars*: 0.5 mm (*A*, *C*); 1.0 mm (*E-G*, *I,J*); 2.0 mm (*B*, *D*, *H*, *K*, *L*).

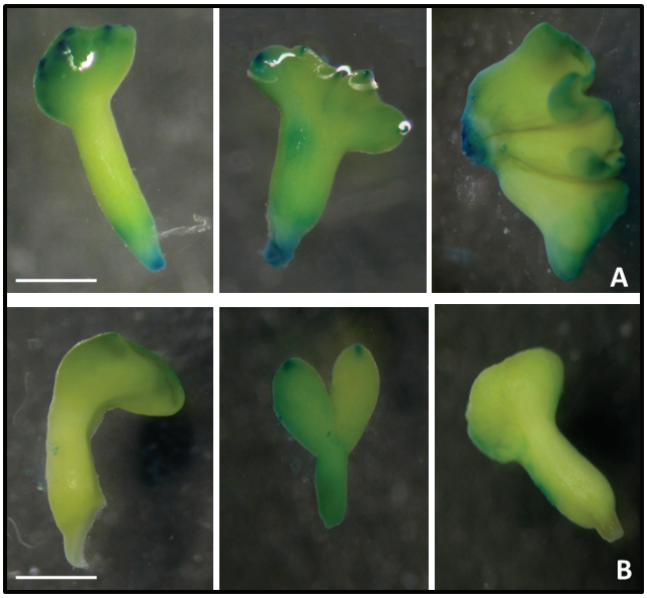

Fig. 2. GUS analysis in DR5::GUS line-derived embryo-like structures to identify true somatic embryos. *A* - GUS signal in apical and basal parts of the true somatic embryos; *B* - a lack of GUS signal in embryo-like structures indicating a defective root pole. *Bars*: 0.2 mm

shaped structures the GUS signal could be detected at the tips of cotyledons. In contrast, the staining of the basal pole was observed in only part of the embryo-like structures. Thus, the analysis of GUS expression made possible to distinguish the true somatic embryos with properly formed root poles (Fig. 2*A*), as distinct from morphologically similar embryos defective in the formation of a regular root meristem (Fig. 2*B*).

Table 1. Frequency of explants producing somatic embryos and average number of somatic embryos produced by explant in different *Arabidopsis* somatic embryogenesis (SE) systems. Means ± SD, $n = 3$.

SE system		Genotype	Frequency [%]	Number [explant^{-1}]
in planta		Col-0	0 ± 0.0	0.0 ± 0.0
	35S::LEC2-GR	DR5::GUS	93 ± 4.9	10.0 ± 1.2
in vitro	DSE-E1	Col-0	76 ± 4.0	3.8 ± 1.3
		DR5::GUS	77 ± 5.0	4.0 ± 0.9
	DSE-E5	Col-0	87 ± 3.0	4.1 ± 0.83
		DR5::GUS	86 ± 4.0	3.9 ± 0.93
	ISE-E5	Col-0	78 ± 6.0	3.5 ± 0.92
		DR5::GUS	77 ± 8.0	3.2 ± 0.62
	ISE-E9	Col-0	56 ± 9.0	6.6 ± 1.1
		DR5::GUS	50 ± 7.8	5.6 ± 0.92

The analysis showed distinct differences between SE systems in respect to the frequency of true embryos produced (Table 2). The highest ratio (75 %) of true embryos was found among those produced on seedlings overexpressing *LEC2* (35S::LEC2-GR) *in planta*. True somatic embryos were found less frequently in auxin-induced SE systems established *in vitro*. Among these systems the highest proportion (over 60 %) of true embryos was detected in explants induced on E5 medium, regardless the direct (DSE-E5) or callus-mediated (ISE-E5) type of morphogenesis induced. Strikingly, the duration of auxin treatment strongly influenced the percentage of true embryos, and a proportion of the somatic embryos with root pole increased in parallel with a prolonged exposure of the culture to auxin. It was observed that up to 3-fold higher frequency of true embryos could be induced when the explants were exposed to 2,4-D for 15 instead of 10 d (Table 2). On the other hand, a prolonged auxin treatment up to 24 d employed in ISE-E9 system was found to impair the frequency of true somatic embryos, as less than a half (48 %) of the structures produced in this system displayed a root pole.

Table 2. The frequency of true somatic embryo formation and somatic embryo conversion in different SE-systems. DR5::GUS explants were used to induce SE *in vitro* (DSE-E1, DSE-E5, ISE-E5 and ISE-E9) and *in planta* (35S::LEC2-GR). Duration of auxin treatment period (10, 12, 15 and 24 d) is indicated for each of the system studied. Means ± SD, $n = 3$.

SE system		Auxin treatment [d]	Frequency of true somatic embryos [%]	Frequency of conversion [%]
in planta	35S::LEC2-GR	0	75 ± 1.0	82 ± 8.0
in vitro	DSE-E1	10	30 ± 1.0	82 ± 1.0
		12	45 ± 13.0	82 ± 3.0
		15	48 ± 9.0	79 ± 4.0
	DSE-E5	10	41 ± 2.0	88 ± 1.0
		12	59 ± 3.0	88 ± 2.0
		15	65 ± 1.0	88 ± 1.0
	ISE-E5	10	20 ± 1.0	60 ± 1.0
		12	47 ± 4.0	60 ± 3.0
		15	62 ± 3.0	69 ± 4.0
	ISE-E9	24	48 ± 2.0	87 ± 3.0

In addition to auxin treatment period, also the concentration of auxin in the induction medium was noticed to influence true embryo frequency. Accordingly, regardless of duration of auxin treatment, the frequency of true somatic embryos was found to be noticeably lower in explants treated with 1.0 μM of 2,4-D (DSE-E1; up to 48 %), than in those induced in presence of 5.0 μM of 2,4-D (DSE-E5; up to 65 %).

The SE systems were also evaluated in terms of their capacity to produce completely developed plants, *i.e.*, with shoots and roots. Hence, the embryo-like structures were maintained on auxin-free medium for 21 d, and the conversion rate was calculated for different SE systems and auxin combinations tested (Table 2). The results showed that the frequency of embryo-like structures developing rooted shoots were higher than the frequency of the true embryos, as estimated by the DR5::GUS marker system. Accordingly, the conversion rates approached 90 % in the most effective systems. Surprisingly, the high frequency (60 - 90 %) of root development was also found among embryo-like structures derived from 10 d auxin treatment, and characterized by the low frequency (20 - 40 %) of true embryos.

Discussion

In this study we have taken advantage of the DR5::GUS reporter line and evaluated different SE-systems of *Arabidopsis* in respect of the ratio of true somatic embryos produced. The systems differed considerably in terms of the SE induction factor applied (exogenous auxin treatment *in vitro* versus *LEC2* overexpression *in planta*), type of the morphogenic pathway involved (direct *versus* indirect) and concentration and duration of 2,4-D action.

In order to distinguish true somatic embryos produced in the *Arabidopsis* SE systems of different embryogenic capacity, the expression of the auxin-controlled *GUS* gene in the basal pole of the developing embryo-like structures was analyzed. The results show that the ratio of true somatic embryos varied between the SE systems, and is dependent on the presence of auxin and its concentration and the duration of action.

True somatic embryos were the most frequent (75 %) among the structures formed without exogenous auxin treatment *i.e*. *in planta*, in seedlings overexpressing *LEC2*. The ectopic production of somatic embryos in the transgenic 35S::LEC2 plants is believed to result from an increase in the endogenous auxin level, as LEC2 was found to control expression of auxin biosynthesis genes, *YUCCA2* and *YUCCA4* (Stone *et al.* 2008). In agreement with this suggestion, the increased concentration of IAA was recently shown in tissues in a transgenic line overexpressing *LEC2* (BW and MDG unpublished). Thus, it seems that increased *LEC2*-driven content of endogenous auxin is not only inductive for embryogenic switch in somatic seedling cells, but also positively influences the proper formation of root poles in developing somatic embryos.

In contrast, exogenous auxin applied *in vitro* was found to be less effective in the establishment of a functional root meristem in regenerated embryo-like structures and a clear influence of 2,4-D concentration and time of exposure on the ratio of true somatic embryos was noticed. Among the key factors influencing the establishment and maintenance of proper root meristems and rooting of regnerants, auxin should be considered (Jiang and Feldman 2005a,b, Mendes *et al*. 2011). Treatments influencing auxin transport or signaling were reported to severely affect root meristem morphogenesis (Hadfi *et al.* 1998), including a decay of the auxin maximum in root tips of primary roots (Koprivova *et al.* 2010). On this basis, it is expected that auxin treatment used *in vitro* to induce SE disturbs auxin homeostasis, and is responsible for developmental defects in meristem root formation manifested by disappearance of the properly organized auxin maximum.

Auxin is believed to be a key regulator of zygotic embryogenesis (ZE), acting from the earliest stages of zygote development, and involved in the formation of both, shoot and root apical meristems (Friml *et al.* 2003, Smith and Long 2010). In SE, auxin was widely documented to induce an embryogenic switch in somatic plant cells (Feher *et al.* 2003), and to control development of shoot meristems in embryogenic callus (Su *et al.* 2009). Likewise, the present results, together with the earlier suggestions (Raghavan 2004, 2005), clearly indicate that auxin also regulates root development in somatic embryos, and that the establishment of a fully developed root pole is severely influenced by auxin treatment. Thus far the conclusion on the effects of auxin mediated formation of embryonic roots in somatic embryos is based on indirect evidence. It remains to be elucidated if the molecular mechanisms of auxin-controlled embryonic pole formation during SE are similar to those recognized in ZE, including the establishment of a fully organized RAM marked with the auxin maximum, as was described in zygotic embryos of *Arabidopsis* (Benfey *et al.* 2010).

The presently indicated frequent formation of somatic embryos with defective root meristems should be also viewed in the context of the usefulness of *Arabidopsis in vitro* systems in studies on plant embryogenesis. Among others, the present analysis indicates that the majority of the embryos produced in the ISE-E9 system recommended for studies on basic molecular events in plant embryogenesis (Ikeda-Iwai *et al.* 2002) form defective roots. However, this system was successfully applied to elucidate the auxin-controlled molecular mechanisms involved in determination of the shoot apical meristem in embryogenic callus (Su *et al.* 2009) suggesting that similarly to ZE (Werner *et al.* 2003, Kyozuka 2007) also in SE separate organ-specific mechanisms seem to control proper shoot and root meristem formation.

The development of defective root poles in somatic embryos is expected to impair the rooting of the SE-derived plants, and in consequence to limit the usefulness of SE-system in micropropagation of commercial species (George and Debergh 2008). The present results indicated that the ratio of embryo-like structures converting into plants distinctly surpasses the frequency of true somatic embryos and thus suggest that the developmental defects in embryonic roots could not negate production of rooted regenerants. Therefore, it remains to be determined if the embryonic root pole lacking a properly formed auxin maximum is able to elongate and develop into a functional root, or whether adventitious roots are formed by the embryo-like structures defective in the root poles.

In summary, our study has shown that somatic embryos produced in various *Arabidopsis* systems are frequently defective in root development and a concentration and duration of auxin treatment seem to strongly influence the establishment of embryonic root meristem during *in vitro* culture. Among the various *Arabidopsis* SE systems available, the treatment of IZE explants with 5.0 μM of 2,4-D, for at least 15 d can be recommended for the efficient production of somatic embryos among which the majority develops a proper root meristem.

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