Final report

Chromatin-regulated transcriptional reprogramming in somatic plant cells during the acquisition of totipotency

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Introduction

The goal of the current research project was to understand the mechanisms that control the initiation of the process of somatic embryogenesis (SE) in differentiated plant cells. We planned to use the model plant *Arabidopsis thaliana* in order to identify and follow the dynamics of transcriptional reprogramming. We aimed to define a transitory "totipotent" state of induced cells when they already express pluripotency transcription factors but still not express those associated with embryo development. Arabidopsis was selected based on the availability of molecular/genetic markers and tools allowing thorough investigation. However, in Arabidopsis efficient somatic embryogenesis had been previously achieved only with zygotic embryos as explants. Therefore, our first aim was to investigate the possibility to establish a more efficient embryogenic tissue/cell culture system of Arabidopsis where the process can be initiated in fully differentiated vegetative tissues providing unlimited source for high-throughput analyses. This approach was based on early publications on Arabidopsis plant regeneration from cultured root explants. During the establishment, problems with the in vitro root-culture system, the efficiency of regeneration and a difficulty to separate shoot organogenesis from somatic embryogenesis were encountered. This hampered the high efficiency synchronous initiation of somatic embryogenesis, a basic prerequisite for detailed studies using marker genes and transcriptomics. Meanwhile, based on theoretical considerations and experimental observations we raised the hypothesis that somatic embryogenesis can proceed through various pathways not all requiring a transient totipotent state. Moreover, system optimization approaches led to the observation that plant regeneration from roots is hindered by shoot-derived auxin and this can be alleviated by exogenous polyamine application. Therefore, although our primary goals could not be achieved, we made original discoveries and laid down new hypotheses considering somatic embryo development from differentiated plant tissues.

Details of the used experimental approaches and their results

Optimizations of the Arabidopsis root regeneration system

Although studying somatic embryogenesis of immature zygotic embryos of Arabidopsis resulted in significant insights into the process (Gaj 2004; Nowak et al. 2012), to generalize the findings the use of other explants (preferably the ones having more differentiated tissues than immature zygotes) would be beneficial. Early tissue culture works with Arabidopsis root explants often reported regeneration via somatic embryogenesis or at least via embryo-like structures (Márton and Browse 1991; Mathur et al. 1995; Wenck and Márton 1995). Regeneration of Arabidopsis shoots from root explants is generally achieved via the direct conversion of lateral root primordia into shoot meristems or via the intermediate formation of callus tissue. In the latter system, culturing the roots on the callus induction medium (balanced auxin-to-cytokinin ratio) is followed by shoot primordium formation due to a high cytokinin/low auxin ratio in the shoot induction medium). Márton and Browse reported (Márton and Browse 1991) that shortening the callus induction phase (ARMI medium) resulted in improved regeneration of root explants via somatic embryo formation on the subsequently used high cytokinin regeneration medium (ARMIIr). We tried to follow their culture regime as closely as possible, however, we found, extensive lateral root development and green callus formation under these conditions (Fig. 1).

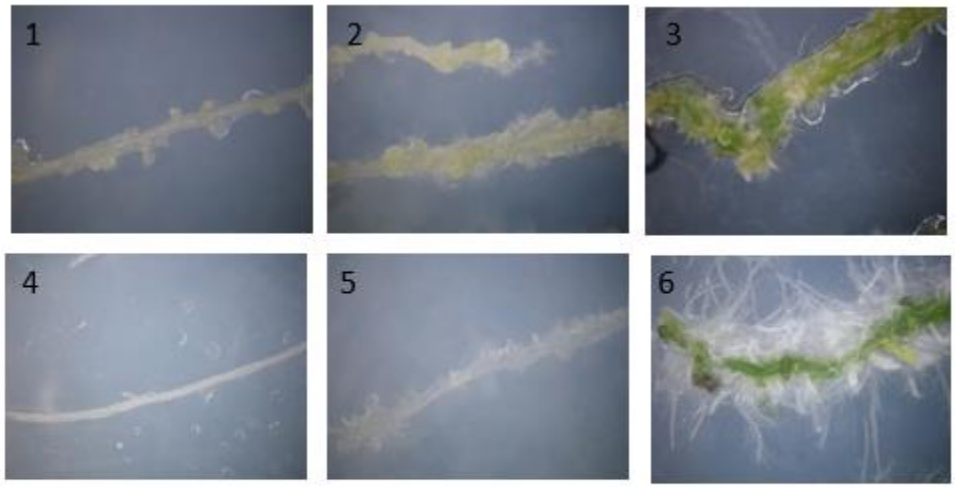


Fig. 1. Experiments following the methods of (Márton and Browse 1991; Wenck and Márton 1995). Segments of in vitro cultured roots were used as explants (one week in the presence of 1 mg/l NAA followed by one week hormone-free culture before transfering to ARMI followed by ARMII). 1-3 Columbia, 4-6 RLD. 1,4 – explants cultured on ARMI for 10 days; 2,5 - ARMI 10 days, ARMII 2 days; 3,6 - ARMI 10 days, ARMII 10 days.

Various auxin treatment conditions (type, concentration, length) were tested to limit lateral root formation on ARMI as well as to increase regeneration on ARMII media. Cold and drought

stresses were also applied to increase regeneration efficiency as recommended in the original publications. Moreover, beside the Columbia-0 genotype, the RLD genotype considered to be more amenable for somatic embryogenesis was also tested. It was established that modifying the media and limiting the time on the ARMI medium to three days promoted the regeneration efficiency, although lateral root formation was still considerable (Fig. 2).

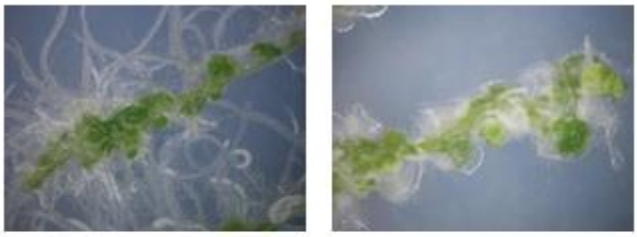


Fig. 2. Result of the improved protocol. Segments of in vitro cultured roots were used as explants (one week in the presence of 1 mg/l NAA followed by one week hormone-free culture before transfering to ARMI followed by ARMII). 1-Columbia, 2 - RLD. The explants were cultured on modified ARMI for 3 days and on modified ARMII for 10 days.

In order to decide whether the green regeneration foci represent embryogenic structures, we took a series of stereomicroscopic as well as cross sectioned microscopic images and collected daily samples until 10 days on ARMIIr for mRNA isolation and cDNA synthesis. Specific RT-QPCR primers were planned and get synthesized towards various embryogenic marker genes including LEAFY COTYLEDON 1 (LEC1), FUSCA3 (FUS3), SOMATIC EMBYOGENESIS RECEPTOR KINASE (SERK), AGAMOUS-LIKE 15, and a newly described transcription factor with potential role in somatic embryogenesis, bHLH109. Using the Genevestigator gene expression database (https://genevestigator.com/gv/), two reference genes with unaltered expression levels in a wide range of conditions including the auxin induced regeneration of root explants were selected and used for RT-QPCR primer synthesis. We carried out the gene expression assays on the cDNA samples representing a time series of cultured roots. However, microscopic and gene expression data were ambiguous. Although the increased expression of the marker genes could be observed when the greening (differentiation) of the root-derived calli started (LEC1 expression is shown as an example in Fig. 3), it often disappeared at later stages in accordance with the microscopic investigations that indicated re-callusing of the differentiating structures.

In order to avoid re-callusing, the root explants were transferred from the ARMIIr medium to hormone free conditions after 2-5 days. The appearance of green embryo-like globular structures was observed on the root surfaces of both the Col and RLD ecotypes from the 4. day onward in hormone-free medium. However, the RLD genotype was found to be more efficient in plant regeneration. Using the embryogenesis defective *lec1* mutant in the COL background,

the formation of green foci on the root was restricted indicating that the green structures on the COL roots mostly represented the initiation of embryogenesis (Fig. 4 A).

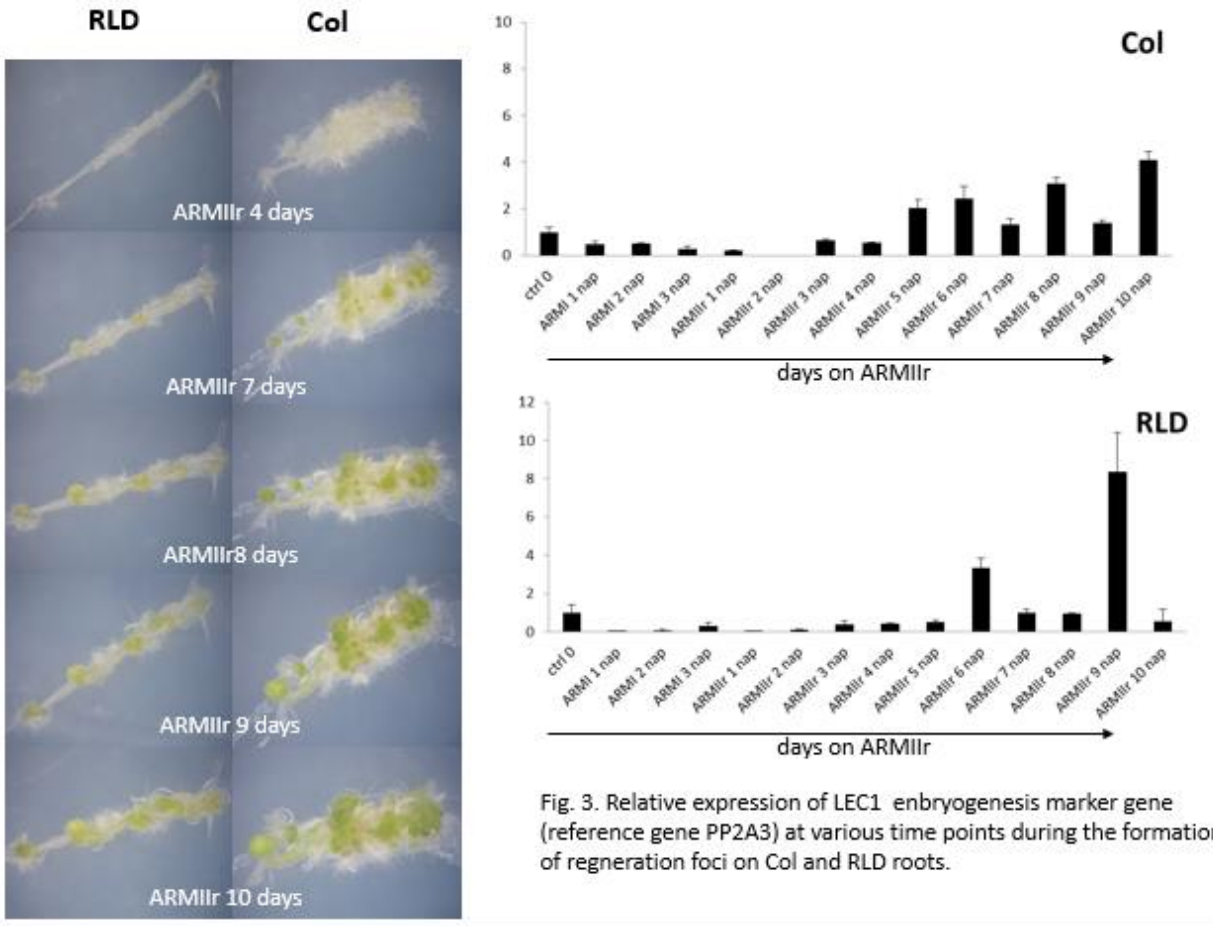


Fig. 3. Relative expression of LEC1 enbryogenesis marker gene (reference gene PP2A3) at various time points during the formation of regneration foci on Col and RLD roots.

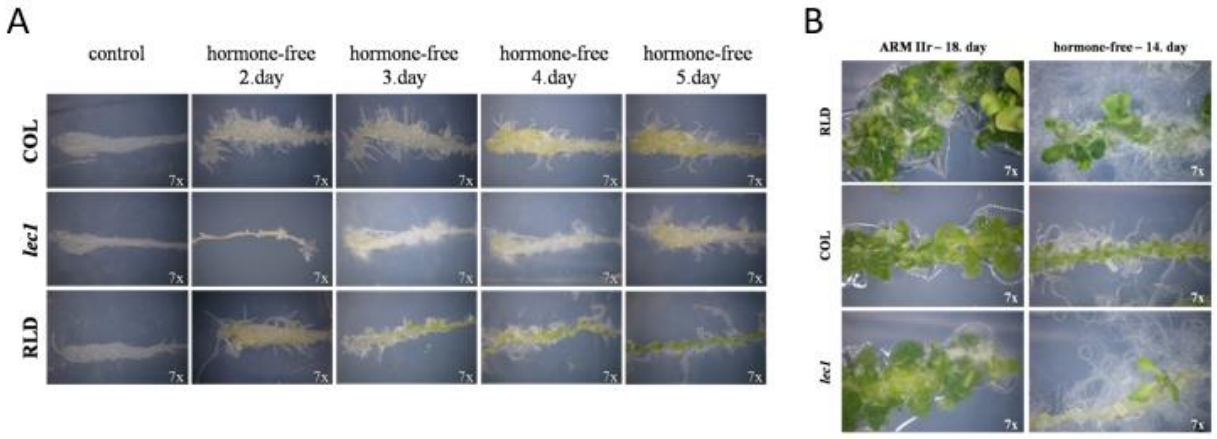


Fig. 4 Somatic embryo induction in the root explants of RLD/COL/*lec1* plants. A. Appearance of embryo-like structures under hormone-free conditions following a transient culture on ARMIIr medium. B. Plant regeneration on the roots two weeks later.

Culturing the appropriately pre-cultured root explants for app. three weeks on the high cytokinin ARMIIr medium, shoots appeared on the surface of root explants (Fig. 4 B). The regenerates had trichome-bearing first leaves indicating their formation via shoot organogenesis. The absence of trichomes is often used as a morphological marker for somatic embryo formation since somatic embryos develop cotyledon-like trichome-less first leaves.

Embryogenic marker genes, e.g. LEC1 and ATS1, exhibited a strong induction following the transfer of roots onto hormone-free media (Fig. 5).

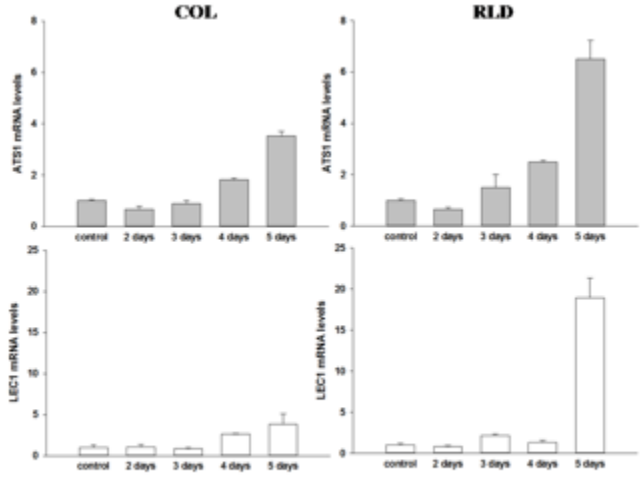


Fig. 5. Relative mRNA levels of *ATS1* and *LEC1* in COL/RLD root explants under control and SE induction process. Days indicate the time on the hormone-free medium.

Meanwhile, it was realized that using in vitro root culture explants result in inconsistent regeneration frequencies, and contamination of the cultures often caused severe delays in the experiments. Therefore, the use of roots of 7-days-old seedlings were tested in the same regeneration system. More consistent and efficient regeneration could be achieved; therefore, this system was used for further optimization and analysis. In this system, the frequency of regeneration was similar in the Col and RLD genotypes, therefore we made most experiments with Col since we had already some markers available in this background.

In this system, the timing on the high cytokinin medium was evaluated in respect of regeneration and especially somatic embryogenesis efficiency (Fig. 6). Four-days on the ARMIIr medium was found to be optimal.

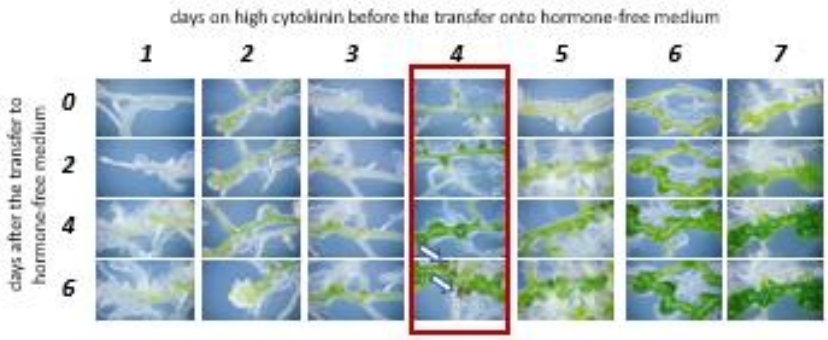


Figure 6. Callus formation and plant regeneration on root explants cultured for the indicated periods (1-7 days) on the high cytokinin shoot induction medium (ARMIIr) before transferring them to hormone-free conditions. Pictures were taken 0, 2, 4 or 6 days after the transfer to the hormone-free medium as indicated. White arrows point to the regenerated somatic embryos on roots cultured for four days on the high cytokinin medium followed by 6 days on the hormone-free one (red box).

Regeneration in the continuous presence and following a 4-day-long transient application of high cytokinin concentration (ARMIIr) was compared. It was found that the continuous presence of cytokinin prevented the formation of somatic embryos but allowed shoot regeneration. On the hormone-free medium following a 4-days-long cytokinin induction both shoot regeneration and somatic embryogenesis could take place (Table 1.; Fig. 7). The *lec1* mutant could not regenerate somatic embryos, only shoots. Shoot and somatic embryo regeneration occurred at about 50-50% frequencies. This was an interesting finding that allowed to draw a conclusion that shoot regeneration and somatic embryogenesis might share the initial steps but later the presence of cytokinin does not allow somatic embryo development (e.g. by blocking root meristem formation) but promote shoot organogenesis.

Since the regeneration pathways are mixed, and likely the regeneration of somatic embryos not started in single totipotent cells but rather the parallel formation of shoot and root poles from groups of cells take place in this system, we had to realize that our primary goals cannot be achieved using this system. Nevertheless, several attempts were made to use this system efficiently to understand plant regeneration processes.

Description of the culture system formed part of a manuscript that has been submitted to Plant Cell Reports (Q1, Plant Sciences).

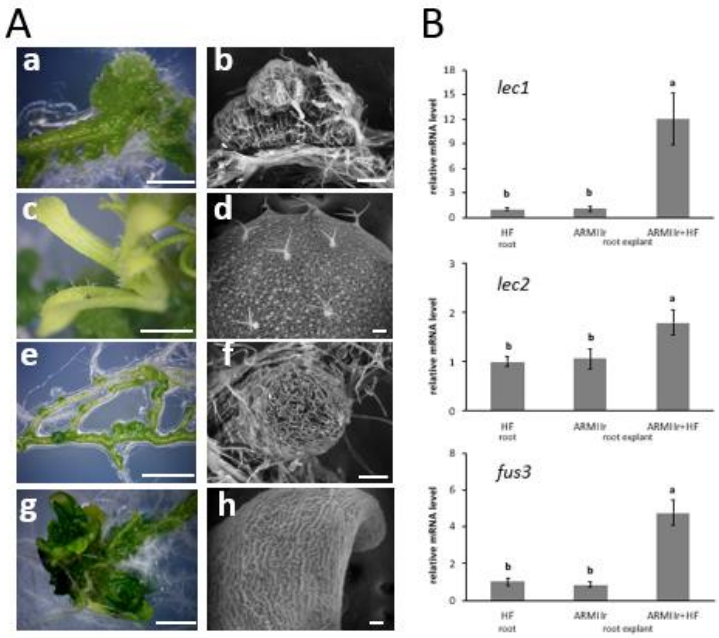


Fig. 7. Plant regeneration from seedling root explants. A. Plant regeneration of root explants in the continuous presence of high cytokinin concentration (a-d) or after moving them to hormone-free conditions following a period of 4 days on the high cytokinin medium (e-h). The first leaves of the regenerated plantlets were investigated by scanning electron microscopy (c, d and g, h). The bars represent 1 mm (a, c, e, g), and 100 µm (b, d, f, h), respectively. B. Relative expression of the embryogenesis marker genes lec1, lec2 and fus3 under both conditions (ARMIIr and ARMIIr+HF, respectively).

Table 1. Regeneration efficiency and type of wild type (*col.*) and *lec1* mutant seedlings/root explants with or without TIBA treatment.

Plant material	TIBA	No. of regeneration foci/root[a]	% of regenerated shoots[b]	% of shoots with trichomeless leaves[c]	% of shoots with trichome-bearing leaves[c]
***col.* root explant**	**-**	**8+-0,8**	**65,6+-3,7**	**56,9+-4,7**	**43,1+-4,7**
***lec1* root explant**	**-**	**3,9+-0,4**	**23,8+-6,9**	**0,0+-0,0**	**100+-0,0**
***col.* seedling**	**-**	**0,0+-0,0**	**0,0+-0,0**	**0,0+-0,0**	**0,0+-0,0**
***col.* seedling**	**+**	**6,8+-0,8**	**43,8+-4,5**	**48,3+-3,9**	**51,7+-3,9**
***lec1* seedling**	**-**	**0,0+-0,0**	**0,0+-0,0**	**0,0+-0,0**	**0,0+-0,0**
***lec1* seedling**	**+**	**3,3+-0,4**	**15,0+-5,1**	**0,0+-0,0**	**100,0+-0,0**

[a] Green morphogenic foci per root explant (counted after 5 days on the hormone-free medium). Average and standard error.

[b] Percentage of the potential morphogenic foci regenerated into normal shoots per explant. Average and standard error.

[c] Percentage of regenerated plantlets having leaves/cotyledons with/without trichomes. Average and standard error.

The role of wounding and shoot-derived auxin in plant regeneration from Arabidopsis roots

During the above experiments it was observed that the above described regeneration system was inefficient when whole seedlings were used instead of root explants (Fig. 8, Table 1.). Roots of whole seedlings cultured on the high cytokinin medium thickened and become green but failed to regenerate calli and shoots (Fig. 8a; Table 1). When the seedlings were removed to hormone-free medium after 4 days of cytokinin treatment, the thickening and greening of the root did not take place (Fig. 8b). The potential role of shoot-derived auxin on the regeneration potential of the root was tested using the auxin transport inhibitor TIBA. 5 µM TIBA in a low gelling temperature agarose drop was applied to the shoot-to-root junction of seedlings at the time they were transferred to the high cytokinin medium. TIBA application restored the regeneration potential of the seedling roots (Fig. 8 c, d; Table 1.). Similarly, to the excised root explants, the roots of TIBA-treated seedlings regenerated only trichome-bearing shoots on the high cytokinin medium and approximately 50-50% shoots with or without trichomes in the case of transient (4 days) cytokinin treatment followed by hormone-free culture. The trichome-less plantlets regenerated on the TIBA-treated whole seedling roots are likely the result of somatic embryogenesis. It is supported by the increased relative expression of the three embryogenesis markers in these roots (Fig. 9). Moreover, the regeneration of TIBA-treated *lec1* seedlings didn't result in trichome-less plantlets (Table 1).

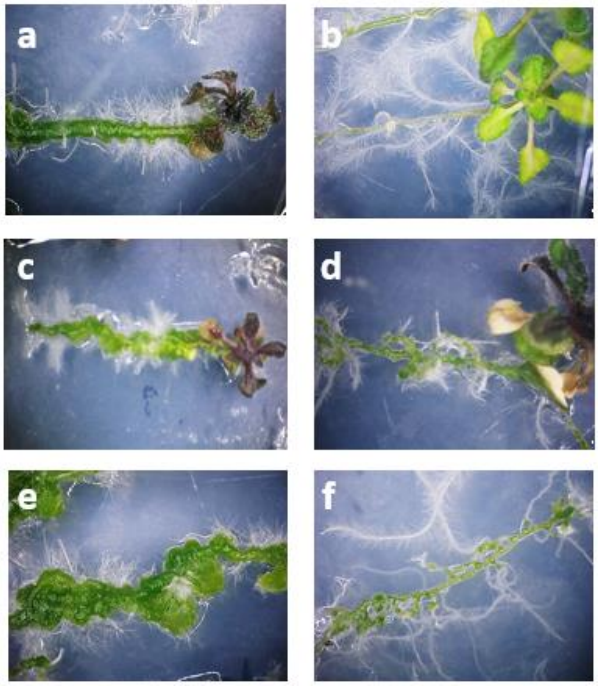


Fig. 8. Plant regeneration of the roots of whole seedlings (a-d) and root explants as controls (e-f) cultured in the continuous presence of high cytokinin concentration (a, c, e) or after moving them to hormone-free conditions following a period of 4 days on the high cytokinin medium (b, d, f). In the case of whole seedlings, some of them was treated by applying 5 µM TIBA in an agarose droplet to the shoot-root junction (c, d). Pictures were taken 9 days after the transfer to the high cytokinin ARMIIr medium (a, c, e) and 5 days after the transfer to hormone-free medium following the previous period of 4 days on the ARMIIr medium (b, d, f), respectively. The bars represent 1 mm.

Wounding and the wound-induced expression of the WIND1 transcription factor are considered to have a central role in the increased regeneration potential of root explants as compared to the roots of intact seedlings (Iwase et al. 2015). Therefore, the expression WIND1 in the roots of TIBA-treated seedlings was investigated. It could be established that blocking the shoot-to-root auxin transport by a TIBA-containing agarose droplet resulted in increased WIND1 expression in the root (Fig. 9). Our observation indicates that either the removal of the shoot as the auxin source or blocking the shoot-to-root auxin transport result in increased WIND1 expression and enhanced competence for callus/shoot/embryo regeneration under appropriate inductive conditions. Shoot-derived auxin somehow interfere with this process. The results were summarized in a manuscript that was submitted to Plant Cell Reports (Q1, Plant Sciences).

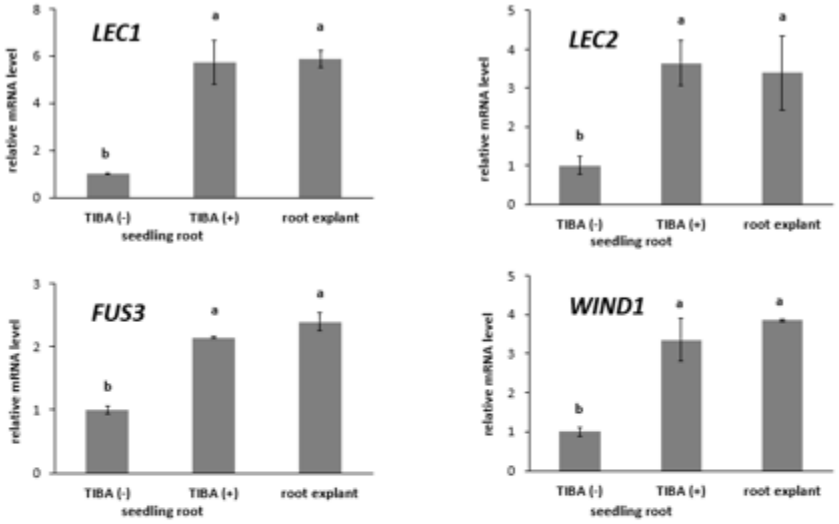


Fig. 9. Real-time quantitative PCR analysis of the relative gene expression of embryogenic marker genes (LEC1, LEC2 and FUS3) and the wound-induced transcription factor (WIND1)
in untreated (TIBA-) and TIBA-treated (TIBA+) seedling roots and in untreated root explants cultured for 4 days on the ARMIIr medium followed by a hormone-free culture for 5 days . Expression in untreated seedling root was used as reference. The expression of the AT2G41960 gene was used for data normalization.

Production and characterization of gene expression marker lines

To follow the early steps of somatic embryogenesis, the use of fluorescent gene expression markers was planned. Several Arabidopsis lines were obtained from colleagues as well as from the Nottingham Arabidopsis Stock Centre (NASC). These were mostly in the Col ecotype background. At the start of the project the RLD genotype was considered to be more responsive in somatic embryogenesis, therefore we introduced several gene expression markers into this genetic background as well. This was done in parallel with the optimization of the regeneration system and the propagation and primary characterization of the other lines. The lines are listed in Table 2.

Table 2. Marker lines that were collected (Col background) and propagated or produced by us via genetic transformation.

Marker lines (Columbia background)
GFP
DR5Rev-GFP – auxin level
pLEC1::LEC1-GFP /3644/ CZN 1884 - embryogenesis
pLEC1::BBM-GFP /4966/ CZN 2045 - embryogenesis
pLEC1::BBM-GFP /4967/ CZN 2046 - embryogenesis
WUS(3)-GFP – shoot organogenesis/somatic embryogenesis
ARR5-GFP – cytokinin signalling
pTCS-GFP – cytokinin level
pPIN1 (2) – auxin transport
PIN1 PIN1 cDNS - GFP - in Col - auxin transport
CFP
LEC2-CFP - embryogenesis
3X-VENUS
pDR5Rev_3xVenus – auxin level
GUS-staining
ARR::GUS – cytokinin signalling
DR5::GUS – auxin level
35S::GUS – control
Marker lines (RLD background), made by ourselves
I1-pPIN1::PIN1-GFP pART27 -- PIN1_1 (3) – auxin transport
I3-pSTM::stm-VENUS pmLBart -- I3_1 (4) – shoot organogenesis
I7-pDR5::3XVENUS-N7 pmLBart -- I7_3 (7) – auxin level
A3-pWUS::dsRed-N7 pmLBart -- A3_2 (8) – shoot organogenesis/somatic embryogenesis

However, our efforts to detect specific gene expression at the single/few cell level using any of these markers failed. The reasons could be various. Using hormone level/signaling markers likely failed due to the extensive cytokinin treatment (high concentration for four days) on the whole roots masking specific changes and staining problems causing inconsistent results (see Fig. 10 for examples. Detection of cell fate markers likely failed due to the difficulties to identify the few responding cells within a whole root and the mixed nature of the regeneration (shoot organogenesis and embryogenesis) as well as technical limitations (sensitivity issues).

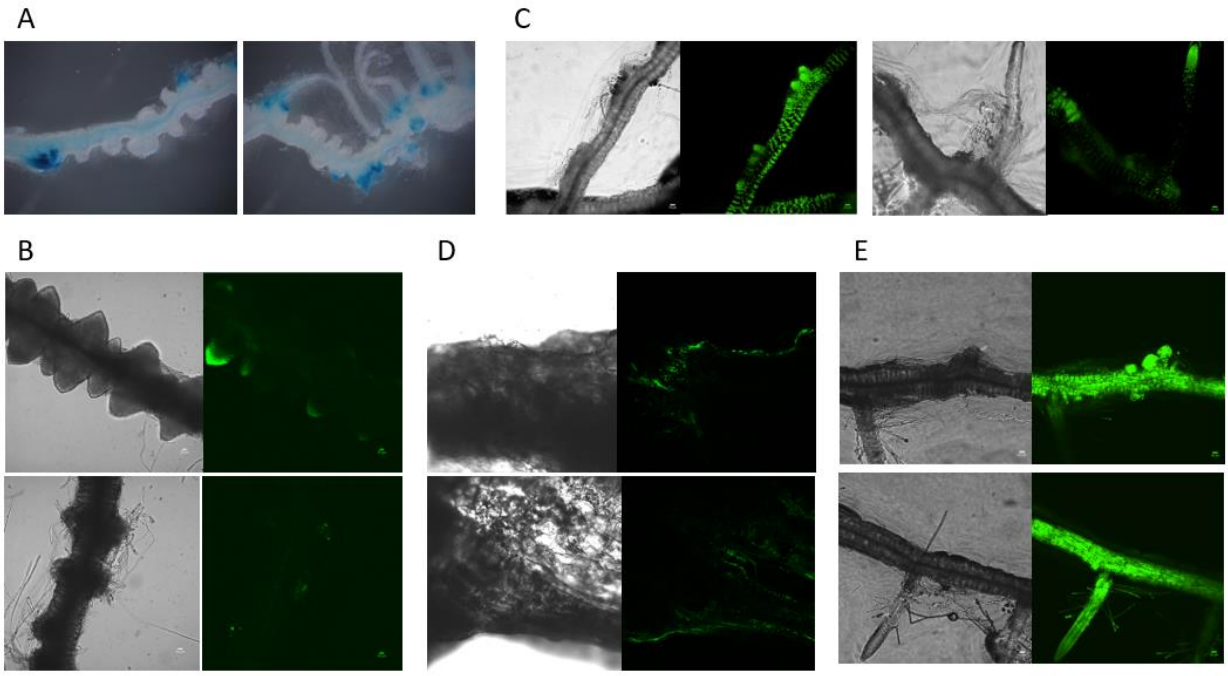


Fig. 10. Inconsistencies in the use of gene expression marker lines. A. DR5:GUS (auxin level); B. DR5:GFP (auxin level); C. PIN1:GFP (auxin transport); D. LEC1:GFP (embryogenesis); E. ARR5:GFP (cytokinin response). Two pictures are shown for each line. The roots were cultured under regeneration conditions (4 dayson ARMII and moved to hormone-free medium (1-4 days).

Chromatin-related experiments

One of the main aims of the proposal was the characterization of gene expression reprogramming at the gene expression level. At the beginning of the project, we summarized our hypotheses about the potential links between chromatin remodelling and the initiation of somatic embryogenesis in a comprehensive review (Fehér 2015).

However, characterizing the experimental system it turned out that the system is not suitable for this (see above). Nevertheless, we made attempts to detect/manipulate chromatin organisation during the regeneration process.

A phosphoSer10 Histone H3 antibody successfully used by us beforehand to label open state chromatin (Bíró et al. 2012). This antibody could not detect any difference between the treatments. Likely the hormone treatment of whole roots masked again the specific responses of the few responding cells of the root. Moreover, discrimination of shoot organogenesis from somatic embryogenesis is likely not possible at this level.

We also tested anti-NRP (Nucleosome-assembly-protein-Related Protein) antibody developed in our laboratory (Bíró et al. 2012). NRP gene expression was previously shown by us to increase during somatic embryogenesis from leaf protoplasts of Medicago sativa (Domoki et al. 2006). However, NRP

protein level did not show an increase in our Arabidopsis root system. It was in agreement with the finding that roots of *Arabidopsis thaliana nrp1-1 nrp2-1* double mutants could regenerate shoots/somatic embryos similarly to the wild type. However, during the propagation of the plant material for the experiments it was recognised that the growth and development of *nrp1-1 nrp2-1* mutant plants altered from that of the wild type, especially during short days. We entered into the detailed characterization of the mutants in collaboration with Balázs Barna (Plant Protection Institute of the Centre for Agricultural Research of the Hungarian Academy of Sciences) who realized that the plants are more sensitive to pathogens. Altogether this research led to the discovery that NRPs coordinate plant growth, development and age-dependent pathogen tolerance in a day-length-dependent way (Barna et al. 2018). This work was also supported by other grants from the Hungarian Ministry for National Economy GINOP-2.3.2-15-2016-00001 and GINOP-2.3.2-15-2016-00039, and the Hungarian Scientific Research Fund (K83615).

To manipulate chromatin reorganization during the induction of the morphogenetic response, sublethal concentrations of the histone deacetylase inhibitors (sodium butyrate, trichostatinA, valproic acid) were used. In the scientific literature, both positive and negative effects have been ascribed to these chemicals considering the induction of the embryogenic response. In our case, all investigated concentrations prevented or decreased the efficiency of morphogenesis (see Fig. 11 as an example). We can not exclude that the effect of the inhibitors might depend on the proper timing of their application. The optimisation of this parameter would require further extensive experimentation considering the complexity of our system (sequential application/removal of auxin and cytokinin).

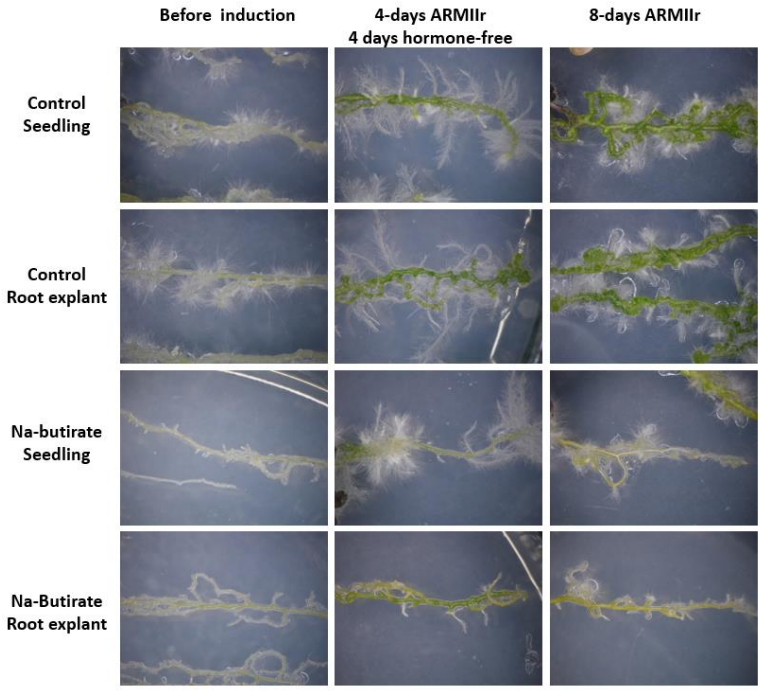


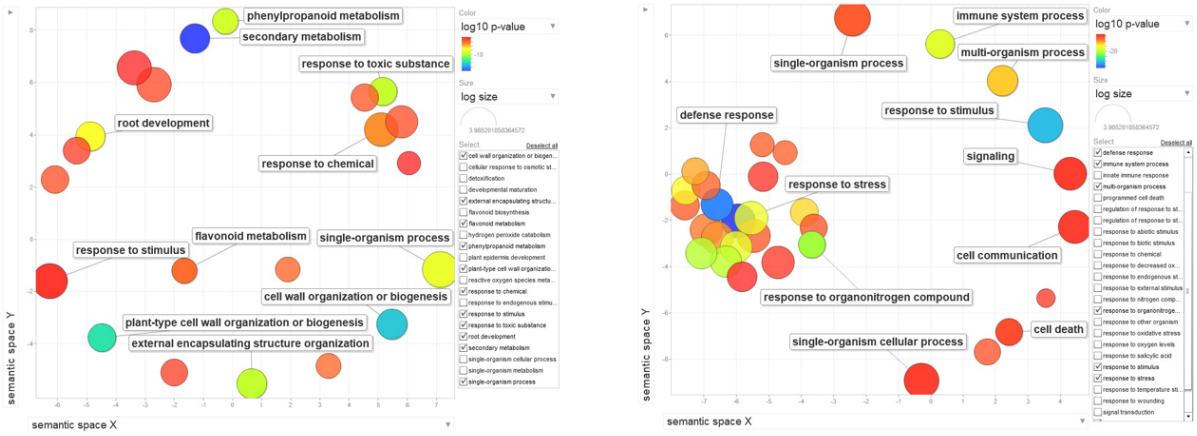
Fig. 11. Effect of sodium-butirate on the regeneration of Arabidopsis roots (roots of whole seedlings or cut root explants were used). Na-butirate known to promote the loosening of chromatin was applied before the induction of regeneration.

Transcriptomic approach

Although we considered the system as unsuitable for a chromatin immunoprecipitation (ChIP) gene expression assay as we originally planned in our proposal, we attempted a transcriptomic analysis via new generation sequencing (NGS). These experiments were carried out in collaboration with Laszlo Bodai (Department of Biochemistry, University of Szeged). Whole seedling and root explants were cultured either on ARMIIr medium for 8 days (ARM) or ARMIIr medium for 4 days followed by hormone-free culture for 4 days (HM). Roots of whole seedlings were selected as controls due to the lack of their regeneration response (see above) and compared to cut root explants. Before sequencing, the samples were tested for the expression of the embryogenesis marker *LEC1*.

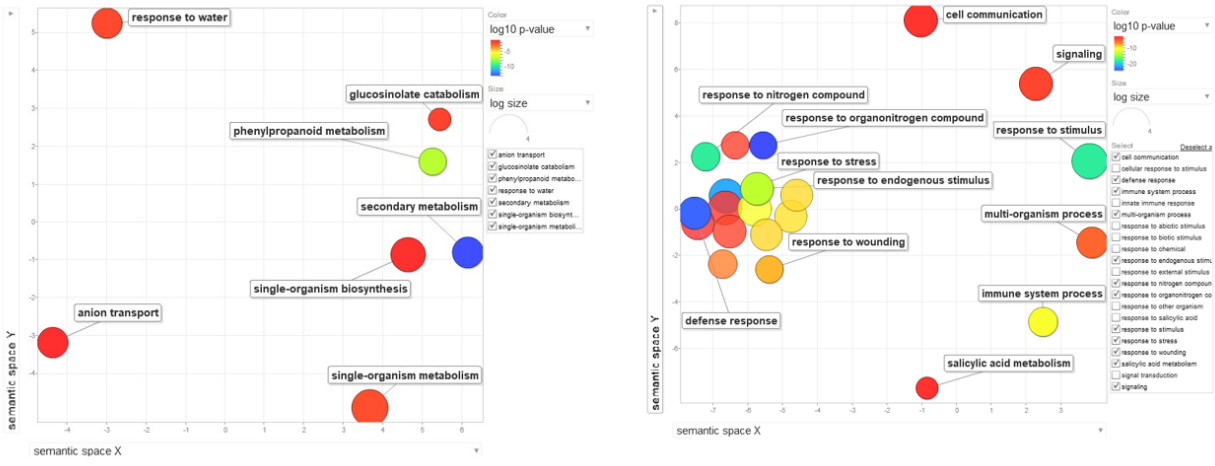
High number of genes exhibited an at least 2-fold change under both conditions but the number of regulated genes, especially the down-regulated ones, was much higher under the hormone-free conditions (HM 2374 genes; ARM 595 genes). 775 genes showed differential expression between the root explants cultured under the ARM or the HM condition, respectively. The detailed results can be found following the link: https://app.box.com/s/ehq2e3ewcmstgopychow2r8tipqsbzb0.

Most of the regulate genes represented gene ontology categories related to stress responses and metabolism (Fig-s. 12-14). None of the significantly regulated genes could be associated, however, with plant regeneration or embryogenesis. This also highlights the drawback of the system: insufficient number of responding cells for this type of analysis.



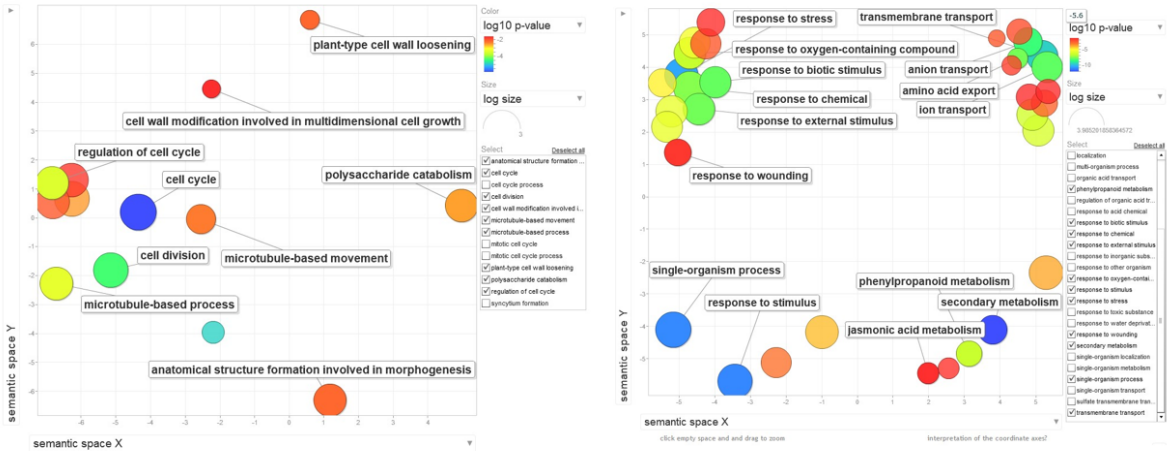
Down-regulated Up-regulated

Fig. 12 Gene ontology (GO) analysis of genes regulated in cut root explants cultured 4 days on ARMIIr medium followed by 4 days under hormone-free conditions as compared to roots of whole seedling cultured on the same way. (Red – highly represented category; Blue – under represented category)



Down-regulated Up-regulated

Fig. 13 Gene ontology (GO) analysis of genes regulated in cut root explants cultured 8 days on ARMIIr medium as compared to roots of whole seedling cultured on the same way. (Red – highly represented category; Blue – under represented category)



Down-regulated Up-regulated

Fig. 14 Gene ontology (GO) analysis of genes regulated in cut root explants cultured 8 days on ARMIIr medium as compared to root explants cultured on 4 days on ARMIIr medium followed by 4 days at hormone-free conditions (Red – highly represented category; Blue – under represented category)

Effect of polyamines on regeneration efficiency

Our attempts to increase regeneration efficiency led us to the discovery that exogenous polyamine application can significantly enhance the regeneration process from Arabidopsis roots. Especially spermidine proved to be effective in this respect (Fig. 15). Based on preliminary data showing that the endogenous polyamine levels and the activity of polyamine metabolizing enzymes are associated with the regeneration process, Katalin Gémes obtained financial support to characterize this phenomenon in more details (NKFIH FK128997 “Improving plant regeneration through the manipulation of polyamine metabolism”).

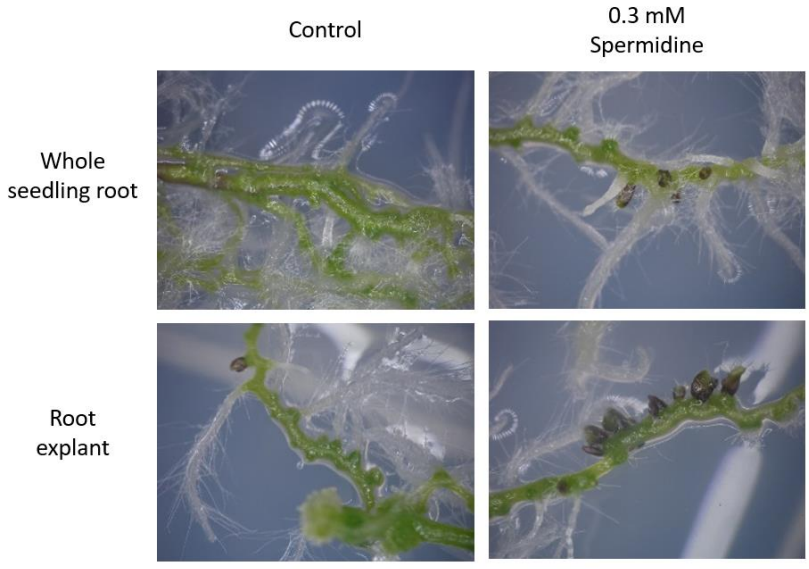


Fig. 15 Exogenous spermidine promotes the shoot regeneration from Arabidopsis roots.

Theoretical considerations

The unexpected project results motivated us to reconsider the potential pathway somatic embryos may form on the root explants. Critical evaluation of the enormous literature of plant somatic embryogenesis led us to realize that the development of embryos from somatic cells can follow several pathways as far as the initial steps are considered. At present, at least there main models can be suggested: 1) direct embryogenesis from single cells through a zygote-like stage; 2) direct embryogenesis dependent on seed/embryo-identity factors (LEC1 and other related embryo identity transcription factors); 3) indirect embryogenesis dependent on WUS and WOX5 (as well as related auxin and cytokinin transport, synthesis and signaling pathways). This hypothesis was published in a book chapter (Fehér et al. 2016). This hypothesis, however, led us to an even more basic questions: does somatic embryogenesis always initiate from totipotent cells?; is the dedifferentiation of somatic cells always involved in the process? The possible answers for these basic questions were given in a review discussing the basic processes of plant tissue culture and plant regeneration pointing out that we have to reconsider many dogmatic statements of this research field. The manuscript is under review in Frontiers in Plant Science (Q1, Plant science). The final conclusions of this review are the following. Dedifferentiation is a transient process and the dedifferentiated cell state is relative. Dedifferentiation in a strict sense is the reversion of the differentiation process and thus can be interpreted within a given cell lineage only. In plant cell and tissue culture and developmental biology, however, this term is often used in a wider sense as a type of transdifferentiation leading to cells or tissues with increased developmental potency. In certain cases, the proper transdifferentiation of cells cannot take place (due to extensive wounding, continuous presence of exogenous hormones, overexpression of cell fate regulators or cell division factors etc.) and the cells overproliferate and form an ill differentiated cell mass that contains cells with various degrees of proliferation and/or developmental potencies. These heterogeneous tissues of different origin are termed in general as “callus tissues” that can be classified into several types. In summary, dedifferentiated cells as such do not exist and callus is not a homogenous mass of “dedifferentiated” cells.

There are many pathways allowing the regeneration of whole plants from a variety of explants/cells that do not rely on cellular totipotency. Plant regeneration via sequential organogenesis is one example of such pathways. By definition, direct autonomous embryo development initiated in a single cell can only be considered as a proof of cellular totipotency. Even somatic embryos can form via various developmental pathways not all of which include a single totipotent cell. Moreover, in all cases of plant regeneration developmental potency needs to be induced (or released) in somatic cells. Therefore, the statement that “(all) plant cells are totipotent” is erroneous. Based on our present knowledge we can only state that certain plant cells under appropriate conditions might (re)gain pluri- or totipotency.

Summary

Due to unexpected difficulties (lower efficiency and reproducibility than expected), surprising findings (mixed parallel regeneration of shoots and somatic embryos), and more thorough theoretical considerations (totipotent cell is not a prerequisite of somatic embryogenesis) our primary goal,

revealing the role of chromatin organization in the reactivation of totipotency in somatic plant cells, failed. However, during the thorough optimization of the regeneration system, we realized important aspects of hormonal regulation: 1) removal of the exogenous cytokinin in time result in a regeneration pathway switch from shoot regeneration to somatic embryogenesis; 2) shoot derived auxin prevents the regeneration of shoots/somatic embryos from roots; 3) blocking the shoot-to-root auxin transport mimics the effect of wounding (cutting off the root). These findings can help to define conditions that allow/promote plant regeneration from the roots of other plant species as well. As a side project, the role of the nucleosome assembly protein, NRP, in the coordination of environmental signals (day length, in our study) with plant growth (biomass), development (ageing and flowering time) and age-related pathogen tolerance. This knowledge can help us to better understand the plant's responses to overall environmental changes including that of the climate. We reviewed the role of chromatin remodeling in plant regeneration in order to promote further research in this direction. In addition, we re-evaluated several terms and "dogmas" of in vitro plant cell and tissue culture. The clarification of the terminology is important to avoid further misunderstanding and to overcome potential "terminology-raised" barriers in plant regeneration research.

Output

Papers

Published

1. Barna B, Gémes K, Domoki M, Bernula D, Ferenc G, Bálint B, Nagy I, Fehér A. Arabidopsis NAP-related proteins (NRPs) contribute to the coordination of plant growth, developmental rate, and age-related pathogen resistance under short days. Plant Sci 2018;267:124–34. Available from: https://www.sciencedirect.com/science/article/pii/S0168945217306179

IF: 3.437 Cit: 0

2. Fehér A. Somatic embryogenesis - Stress-induced remodeling of plant cell fate. Biochim Biophys Acta - Gene Regul Mech 2015;1849(4):385–402. (invited review)
Available from: http://dx.doi.org/10.1016/j.bbagrm.2014.07.005
IF: 6.332 Cit: 66

Under review

1. Dóra Bernula, Péter Benkő, Nikolett Kaszler, Ildikó Domonkos, Ildikó Valkai, Réka Szőllősi, Györgyi Ferenc, Ferhan Ayaydin, Attila Fehér, Katalin Gémes (2019) Timely removal of exogenous cytokinin and the prevention of auxin transport from the shoot to the root affect the regeneration potential of Arabidopsis roots. Plant Science (under review) (current IF: 3.712)
Link to the submitted MS: https://app.box.com/s/jcfcjgniwzn03dbzjfdbjc7mjkem6ur6

2. Fehér A (2019) Callus, Dedifferentiation, Totipotency, Somatic Embryogenesis: What these Terms Mean in the Era of Molecular Plant Biology? Frontiers in Plant Science (under review) (current IF: 3.677)
Link to the submitted MS: https://app.box.com/s/juy8d5mdxd6ix0pguhmxjijyqvgrttyy

Book Chapter

Feher A, Bernula D, Gemes K: The Many Ways of Somatic Embryo Initiation, In: Loyola-Vargas VM, Ochoa-Alejo N (szerk.) Somatic Embryogenesis: Fundamental Aspects and Applications. Dordrecht: Springer, 2016. pp. 23-37. (ISBN:978-3-319-33704-3) (invited chapter)

Lectures

Attila Fehér: The many ways of embryo development from somatic cells. VISCEA Conference - "Plants in Vitro: Theory and Practice" Vienna, February 8 – 9, 2016 (invited lecture)

Fehér Attila: Totipotency of plant cells - a review, A MAGYAR NÖVÉNYBIOLÓGIAI TÁRSASÁG XI. KONGRESSZUSA Szeged, 2014. augusztus 27-től 29-ig, 2014

Theses

BSc

Benkő Péter: Szomatikus embriógenezis Arabidopsis-ban (Somatic embryogenesis in Arabidopsis) SZTE, TTIK, 2015

MSc

Benkő Péter: Az auxin szomatikus embriógenezisben betöltött szerepének vizsgálata indukált Arabidopsis gyökéren (The role of auxin in somatic embryogenesis from Arabidopsis roots) SZTE, TTIK, 2017

Kaszler Nikolett, A poliaminok szerepe Arabidopsis thaliana in vitro regenerációs rendszerben (Role of polyamines during in vitro regeneration of Arabidopsis thaliana) SZTE, TTIK, 2018.

Student's scientific competition (TDK/OTDK) lectures

Benkő Péter: Az auxin gátolja a szomatikus embriógenezis folyamatát indukált Arabidopsis gyökéren. SZTE TTIK, Biológia TDK 2016, őszi forduló

Benkő Péter: Az auxin gátolja a szomatikus embriógenezis folyamatát indukált Arabidopsis gyökéren. OTDK, Molekuláris Biológia szekció, 2017.

Kaszler Nikolett: A poliaminok hatása *Arabidopsis* növények *in vitro* regenerációs folyamataiban. SZTE TTIK, Biológia TDK 2018, őszi forduló. (1st price)

Posters

Dóra Bernula, Attila Feher, Katalin Gemes: In vitro somatic embryogenesis on Arabidopsis root explants. VISCEA Conference - “Plants in Vitro: Theory and Practice” Vienna, February 8 – 9, 2016

Dóra Bernula: In vitro somatic embryogenesis on Arabidopsis root explants. FIBOK - “Fiatal Biotechnológusok II. Országos Konferenciája, 2016” Gödöllő, 2016. március 21-22.

Bernula D., Gémes K., Benkő P., Domonkos I., Ferenc Gy., Ayaydin F., Fehér A.: Az auxin gátolja a szomatikus embriógenezis folyamatát indukált Arabidopsis gyökéren, In: Györgyey János (szerk.) A Magyar Növénybiológiai Társaság XII.Kongresszusa. Szeged, Magyarország, 2017.08.30-. 2017.09.01. ISBN:978-963-12-9736-2, 2017

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Péter Benkő, Dóra Bernula, Ildikó Domonkos, Attila Fehér, Katalin Gémes: Inhibitory effect of auxin on plant regeneration from cytokinin-induced Arabidopsis roots. Straub-napok, Programfüzet, MTA SZBK, Szeged, 2018

Nikolett Kaszler, Dóra Bernula, Ágnes Szepesi, Attila Fehér, Katalin Gémes: Role of polyamines during plant regeneration of Arabidospsis thaliana. Straub-napok, Programfüzet, MTA SZBK, Szeged, 2018

Succesful grant application

Katalin Gémes NKFIH FK128997 “A növények regenerációs képességének javítása a poliaminok metabolizmusának módosításán keresztül.” 2018. Dec.1. – 2022. Nov. 30.

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Fehér A, Bernula D, Katalin G (2016) The Many Ways of Somatic Embryo Initiation. In: Loyola-Vargas VM, Ochoa-Alejo N (eds) Somatic Embryogenesis: Fundamental Aspects and Applications. Springer International Publishing, Cham, pp 23–37

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