SOMATIC EMBRYOGENESIS IN IN VITRO CULTURE OF *PLANTAGO ASIATICA* L.

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ABSTRACT

Early stages of direct somatic embryogenesis in *Plantago asiatica* were observed by light microscopy. In calli of *Plantago asiatica* two phenomena occurred simultaneously: somatic organogenesis and somatic embryogenesis.

KEY WORDS: *Plantago asiatica*, indirect somatic embryogenesis, somatic organogenesis.

INTRODUCTION

Asiatic plantain (Fig. 1) – *Plantago asiatica* L. (Plantaginaceae) grows in the Far East countries (Yamazaki 1993). It has been used for medicinal purposes since ancient times (Cheng et al. 1992; Komoda et al. 1989; WHO 1999). Recently it has also been used in the food industry (Kanamaru 1994) and in production of cosmetic (Ito 1996).

So far Asiatic plantain multiplication has been obtained only through indirect somatic organogenesis (Tu Yisheng 1996) and via the micropropagation of shoot-tips (Makowczyńska and Andrzejewska-Golec 1998).

There are no reports on the induction of somatic embryogenesis in this plant.

MATERIAL AND METHODS

Seeds of *Plantago asiatica* L. (Botanical Garden Faculty of Science, Univ. of Tokyo) were surface sterilised by immersion in 2.0% sodium hypochlorate for 10 min., then rinsed 3 times in sterile water. Then, they were germinated in aseptic conditions on Murashige and Skoog medium-MS (Murashige and Skoog 1962) supplemented with 0.2 mg/dm³ kinetin (K) and 1 mg/dm³ gibberellic acid (GA3) and 0.7% Difco Bacto Agar. The cultures were placed in the dark and after germination they were transferred into light.

The source of explants (hypocotyls, roots, cotyledons) were 4 week-old seedlings. For embryogenic callus initiation, these explants were placed on the MS medium with 0.5 or 1 mg/dm³ 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.2 mg/dm³ of 6-benzylaminopurine (BAP) – passage 0.

The resulting calli after 6 weeks of passage 0 were transferred onto agar medium MS with 0.5 mg/dm³ 2,4-D and 0.2 mg/dm³ BAP or 0.2 mg/dm³ α -naphthaleneacetic acid (NAA) and 0.2

mg/dm³ BAP or only 0.5 mg/dm³ BAP – 6 weeks of passage 1. The calli obtained from the first passage were grown on MS medium supplemented with 0.5 mg/dm³ abscisic acid (ABA) for prevention of aberrations in somatic embryogenesis (Wróblewski 1994) and 1 mg/dm³ BAP – passages 2-5.

Histological organisation of the obtained calli was investigated by light microscopy.

RESULTS

Passage 0 (Figs 2-5)

The best result for callus initiation was obtained with the root as explant. Resulting calli were light green-yellow with granu-

Fig. 1. *Plantago asiatica* L. – plants regenerated by the micropropagation of shoot-tips (Makowczyńska and Andrzejewska, unpublished). $\times 0.1$.

Figs 2-17. *Plantago asiatica* L. in *in vitro* culture on MS medium with growth regulators. Passages: 0-5.

Figs 2-5. Passage 0. Fig. 2. Callus from hypocotyl. Figs 3,5. Callus from cotyledon. Fig. 4. Callus from root. Figs 2, 3, 5. 0.5 mg/dm³ 2,4-D and 0.2 mg/dm³ BAP; Fig. 4. 1 mg/dm³ 2,4-D and 0.2 mg/dm³ BAP.

Fig. 2. Bicellular stages (arrows). $\times 600$.

Fig. 3. Proembryoids (arrows). $\times 500$.

Fig. 4. Somatic embryos: globular stage embryos (1), a polycotyledonary torpedo stage (2). $\times 50$.

Fig. 5. A meristematic center. $\times 600$.

lar surface with compact and soft friable fragments. In the calli tissue of various origin: from hypocotyls, roots and cotyledons, proembryogenic complexes, proembryos and embryos (connected with the callus by suspensor or without suspensor) were observed. Somatic embryos occurred as globular, rare heart and torpedo stages. There was no synchronism in the developmental stages. Aberrant somatic embryos were also observed – among others, polycotyledonary somatic embryos.

Histological study showed also meristematic centres and tracheary elements with spiral wall thickenings. Somatic organogenesis has not been observed.

Passage 1 (Figs 6-7)

Calli from this passage had friable consistence and nonhomogenous colour (it consists of light yellow, light brown and milk-white fragments). Its surface was granular. In calli of various

Figs 6-7. Passage 1. Calus from hypocotyl. 0.2 mg/dm^3 2,4-D and 0.2 mg/dm^3 BAP.

Fig. 6. Rhizogenesis of callus. Bar 1 cm.

Fig. 7. Somatic embryos: globular stage (1), heart stage (2), aberrant torpedo stage (3). $\times 100$.

Figs 8-11. Passage 2. Fig 8. Callus from hypocotyl. Figs 9-11. Callus from root. 0.5 mg/dm^3 ABA and 1 mg/dm^3 BAP.

Fig. 8. Globular and heart embryo stages in group. $\times 75$.

Fig. 9. Caulogenesis of a somatic embryo. $\times 75$.

Fig. 10. Caulogenesis of callus. $\times 200$.

Fig. 11. Rhizogenesis of callus. $\times 200$.

Figs 12-14. Passage 4. Figs 12, 13. Callus from root. Fig. 14. Callus from cotyledo. 0.5 mg/dm^3 ABA and 1 mg/dm^3 BAP.

Fig. 12. Microshoots on callus. $\times 50$.

Fig. 13. Tracheary elements in callus (arrow). $\times 400$.

Fig. 14. Globular stage (arrow). $\times 300$.

origin histological study showed globular, heart and rare torpedo stage embryos; often with aberrations.

The rooting efficiency and rare caulogenesis were observed in the culture supplemented with BAP and NAA or only BAP.

Resulting microshoots observed by light microscopy had well-shaped chloroplasts, stomata and headed hairs typical for *Plantaginaceae* family (with a single cell stalk and two-cell head). Meristematic centres with tracheary elements single or in groups were observed in the calli tissue, too.

Passage 2 (Figs 8-11)

Resulting calli from passage 2 had many green fragments. Its surface was granular and its consistence was friable. Globular, heart and torpedo-shaped embryos were observed by microscopy. Aberrations of embryos (polycotyledonary embryos, repetitive embryos) were observed quite often. Sometimes in embryos shoot or root apices developed.

Figs 15-17. Passage 5. Callus from root. 0.5 mg/dm^3 ABA and 1 mg/dm^3 BAP.

Fig. 15. Caulogenesis of callus. Bar 1 cm.

Fig. 16. Stoma on microshoot. $\times 600$.

Fig. 17. A headed hair on microshoot. $\times 750$.

Somatic organogenesis (high rooting efficiency and rare caulogenesis) has been also observed.

Passage 3

Calli from this passage had green, white, light yellow and brown fragments. Caulogenesis and intensive rhizogenesis were observed. Calli from passage 3 were not histologically investigated.

Passage 4 (Figs 12-14), passage 5 (Figs 15-17)

Calli from passage 4 had many green fragments. It was characterized by intensive caulogenesis. Calli from passage 5 had green and brown fragments (necrosis).

Light microscopy observations showed many microshoots connected with callus tracheary elements. The microshoots had chloroplasts, stomata and many headed hairs (see: passage 1). By microscopy globular and rare heart stages were observed.

DISCUSSION

Somatic embryogenesis via callus in the culture of *Plantago asiatica* is similar to that in the culture *Plantago ovata* (Chowdhury et al. 1996) and many other species (Faure et al. 1996; Kononowicz 1990; Ludova and Ostrolucka 1998; Magnusson and Bornman 1985; Mikuła et al. 1996; Wróblewski 1994). The earliest stage observed by us in vitro culture of Asiatic plantain was bicellular stage, which is rarely described (Faure et al. 1996; Schumann et al. 1995). Proembryos and various stages of somatic embryos could be observed at the same time, showing that there is no synchronism in somatic embryogenesis. It has been observed by other authors, too (Faure et al. 1996; Schumann et al. 1995). Somatic embryos observed by us showed large variation in size at the same stage. It was described by other authors (Burza et al. 1994). The abnormalities of stages occurring in somatic embryogenesis of Asiatic plantain were similar to the abnormalities in somatic embryogenesis of various plants (Kononowicz 1990; Mikuła et al. 1996; Schumann et al. 1995).

In calli of *Plantago asiatica* two phenomena occurred simultaneously: somatic organogenesis and somatic embryogenesis. We could observe simultaneous processes of calli tissue dedifferentiation (proembryogenic masses, proembryoids) and its re-differentiation (vascularization, rhizo- and caulogenesis).

We are going to induce the formation of normal mature somatic embryos and regeneration of *P. asiatica* whole plants from these embryos.

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LITERATURE CITED

- BURZA W., WOŹNIAK B., TARKOWSKA J.A., MALEPSZY S. 1994. Cytological analysis of somatic embryogenesis in cucumber (*Cucumis sativus* L.). II Natural fluorescence and direct somatic embryogenesis from protoplasts. Acta. Soc. Bot. Pol. 63: 265-268.
- CHOWDHURY A.R., KUNDU S., RAYCHAUDHURI S.S. 1996. Regeneration of *Plantago ovata* Forsk. through somatic embryogenesis. Cytobios 85: 255-261.
- FAURE O., AARROUF J., NOUGAREDE A. 1996. Ontogenesis, differentiation and precocious germination in anther-derived somatic embryos of grapevine (*Vitis vinifera* L.): proembryogenesis. Ann. Bot. 78: 23-28.
- ITO M. 1996. Hair tonic and growth stimulant compositions containing testosterone 5 α -reductase inhibitors extracted from plants. Jpn Kokai Tokyo Koho JP 08 301 1730 A2 19, Nov 1996 Heisei 9 pp. (Japan). Appl. 95-132896, 1 May 1995; C.A. 126: 108633, 1997.
- KANAMARU MATSUMI, 1994. Health pet food. Jpn. Kokai Tokyo Koho IP 08, 191, 668[96,191, 668]30 Jul 1996. IPA Appl 94/310,004, 18 Nov 1994; 7 pp (Japan); C.A. 125: 194148, 1996.
- KOMODA Y., CHUJO H., ISHIHARA S., UCHIDA M. 1989. HPLC quantitative analysis of plantaginins in shazenso (*Plantago asiatica* L.) extracts and isolation of plantamajoside. Rep. Inst. Med. & Dent. Eng. 23: 81-85.
- KONONOWICZ H. 1990. Embryogeneza roślin *in vitro*. Biotechnologia 2-3: 52-59.
- LUDOVA A., OSTROLUCKA M.G. 1998. Morphogenic processes in callus tissue cultures and de novo regeneration of plants in *Actinidia chinensis* Planch. Acta Soc. Bot. Pol. 67: 217-222.
- MAGNUSSON I., BORNMAN C.H. 1985. Anatomical observations on somatic embryogenesis from scutellar tissues of immature zygotic embryos of *Triticum aestivum*. Physiol. Plant. 63: 137-145.
- MAKOWCZYŃSKA J., ANDRZEJEWSKA-GOLEC E. 1998. Mikropropagacja *Plantago asiatica* metodą hodowli wierzchołków pędów. In: Botanika polska u progu XXI wieku. Materiały sympozjum i obrad sekcji 51 Zjazdu Pol. Tow. Bot. Miądlukowska J. (ed.). PTB, Odd. Gdański, Kat. Ekologii Roślin i Ochrony Przyrody Uniwersytetu Gdańskiego. Gdańsk. p. 306.
- MIKUŁA A., WESOŁOWSKA M., KAPUSTA J., SKRZYPCZAK L., RYBCZYŃSKI J.J. 1996. Cytomorphological studies of somatic embryogenesis of *Gentiana tibetica* (King) and *G. cruciata* (L.). Acta Soc. Bot. Pol. 65: 47-51.
- MURASHIGE T., SKOOG F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.
- SCHUMANN G., RYSCHKA U., SCHULZE J., KLOCKE E. 1995. Anatomy of somatic embryogenesis. In: Biotechnology in Agriculture and Forestry. Somatic Embryogenesis and Synthetic Seed I. Bajaj Y.P.S. (ed.). Springer-Verlag, Berlin-Heidelberg. Vol. 30. pp. 71-84.
- TU YISHENG. 1996. Tissue culture of asiatic plantain (*Plantago asiatica*). Zhongcaoyao 27: 296; CA 125: 190764, 1996.
- WHO 1999. Semen Plantaginis. In: WHO monographs on selected medicinal plants. Geneva, Vol. 1. pp. 202-212.
- WRÓBLEWSKI T.A. 1994. Proces somatycznej embriogenezy – charakterystyka szczegółowa. Post. Biol. Kom. 21: 11-32.
- YAMAZAKI T. 1993. Plantaginaceae. In: Flora of Japan. Iwatsuki K. et al. (eds). Kodansha LTD, Tokyo. Vol. 3. pp. 184-186.

SOMATYCZNA EMBRIOGENEZA W HODOWLI IN VITRO
PLANTAGO ASIATICA L.

STRESZCZENIE

Wczesne etapy pośredniej somatycznej embriogenezy badano pod mikroskopem. W tkance kalusowej *Plantago asiatica* występują równocześnie somatyczna organogeneza i somatyczna embriogeneza.

SŁOWA KLUCZOWE: *Plantago asiatica*, pośrednia somatyczna embriogeneza, somatyczna organogeneza.