55

Polypeptide Profiles from Somatic and Zygotic Embryos of Asparagus (*Asparagus officinalis* L.)

Takeo Saito*, Masashi Hirai and Shigeo Nishimura**

National Research Institute of Vegetables, Ornamental Plants and Tea, Ano, Mie 514-23, Japan
* Present address: *Kurume Branch National Research Institute of Vegetables, Ornamental Plants and Tea*, Kurume, Fukuoka 839, Japan
** Present address: *Plant–Biotechnology Institute, Ibaraki Agricultural Center*, Ago, Iwama, Nishiibaraki, Ibaraki 319-02, Japan

Somatic embryogenesis offers several advantages for clonal propagation of plants[1]. Although there are numerous reports about somatic embryogenesis in many plant species[2,3], the low rate of the germination of somatic embryos is still a major problem in propagation in many cases. The potential of somatic embryos to grow into vigorous plants can be predicted by determination of their biochemical, physiological and morphological similarity to normal zygotic embryos[4]. Understanding the difference between zygotic and somatic embryos could help to overcome difficulties encountered in maturation of somatic embryos. Etienne *et al.*(1993) examined endogenous IAA and ABA contents in somatic and zygotic embryos in *Hevea brasiliensis*[5]. They suggested that the very low IAA and ABA contents and the lack of variation of these contents during the development of somatic embryos are related to the abnormal development of the somatic embryos into plants. In another study a positive correlation was observed between the somatic embryo's total protein content and the vigor of seedlings from somatic embryos in alfalfa[6]. The storage proteins of the somatic embryos were similar to those of the zygotic embryo based on molecular masses and isoelectric points in *Picea abies*[7,8]. Comparison of storage protein profiles between zygotic and somatic embryos of *Picea glauca/engelmanii* complex revealed striking similarities in the regulation of storage protein gene expression during the early and middle stages of maturation, but differences during the later stages of maturation[4].

High frequency somatic embryogenesis in asparagus (*Asparagus officinalis* L.) can be achieved from suspension cultures by elevating the concentration of gelrite in the regeneration medium in combination with an aseptic ventilative filter as a capping material of the culture vessel[9]. However, only 10% of the somatic embryos germinated normally and the rest of the embryos stopped growing and formed only roots, or re-callused. The poor germinability may come from abnormal development during somatic embryogenesis. Therefore, we compared polypeptide profiles of somatic and zygotic embryos by SDS–PAGE to understand the development of somatic embryo of asparagus.

Field grown plants of asparagus cv. 'Welcome'(Sakata seed corporation, Yokohama, Japan) were used as source of explants. The lateral buds of sterilized young spears were excised into 0.5 to 4 mm long segments and inoculated on LS medium[10] containing 10 μM 2, 4-dichlorophenoxy-acetic acid(2, 4-D), 2% sucrose and 0.8% agar. Induced embryogenic calli were maintained in

suspension culture, and then somatic embryos were obtained on LS hormone-free medium with 1% gelrite as previously described protocol[9]. An aseptic ventilative filter, Milli Wrap (Millipore Inc.) was used as a capping material of a culture vessel.

Immature zygotic embryos approx. 1 (ZE-1), 2 (ZE-2) and 3 (ZE-3) mm long and mature embryos were dissected from the seeds of field grown asparagus. ZE-1 was transparent and surrounded by translucent endosperm; ZE-2 was translucent and surrounded milky endosperm while ZE-3 was white and surrounded by dough ripe endosperm.

Embryogenic calli were sampled at 7 days after the subculturing and somatic embryos at 4 (SE-4), 7 (SE-7) and 10 (SE-10) weeks after transfer to the regeneration medium. Stems, crowns and roots were sampled from two week-old seedlings derived from zygotic embryo and somatic counterpart germinated aseptically on half-strength LS medium with 2% sucrose and 0.8% agar.

One embryo was homogenized on ice with 400 μl of the homogenization buffer containing 62 mM Tris-HCl (pH 6.8), 2.3% sodium dodecyl sulfate (SDS), 5% 2-mercaptoethanol, 10% (v/v) glycerol, 0.005% bromophenol blue and sea sand in a 1.5 ml microtube. Homogenates were heated in boiling water for 2 min., and centrifuged at 15,000$\times$g for 20 min. at 4℃. Sucrose was added to the supernatants and they were recentrifuged at the same condition. Soluble proteins of the embryogenic callus and the seedlings were extracted by the same method.

The supernatant (6 to 20 μl) were loaded on 1.0 mm thick polyacrylamide gel containing 0.1% SDS. The stacking and separating gels were 4.5% and 15% acrylamide, respectively. The electrode buffer solution was composed of 25 mM Tris-HCl (pH 8.3), 192 mM glycine and 0.1% SDS. The gels were run for 4 hs at constant current (15 mA), then stained with silver-staining kit (Wako, Osaka, Japan). We replicated all experiments at least 3 times using different samples and confirmed that polypeptide profiles show the same pattern.

Somatic embryos of asparagus develop from globular into club- or banana-shaped structures after transfer to the regeneration medium[9]. The somatic embryos had already reached club- or banana-shaped structure 4 weeks after the regeneration treatment (SE-4) and the structure was not changed 7 or 10 weeks after the treatment. Zygotic embryos were also club-shaped in structure and very similar in appearance to somatic embryos (**Fig. 1**).

Fig. 2 shows the polypeptide profiles of the somatic and zygotic embryos and the embryogenic callus. the polypeptides can be grouped into 3 categories as follows: I; polypeptides commonly present in all samples, II; polypeptides present in embryogenic calli and somatic embryos of all stages but absent or faint in zygotic embryos, and III; polypeptides absent or faint in embryogenic
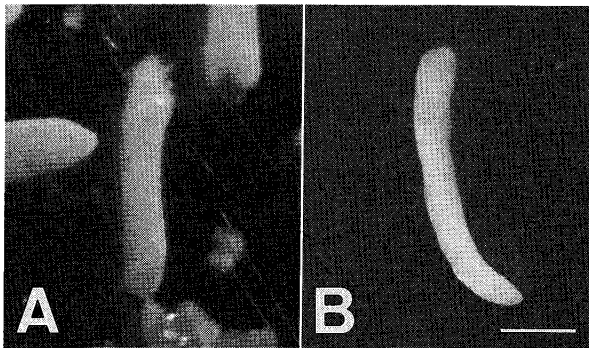

Fig. 1 Somatic embryos 4 weeks after transferring to the regeneration treatment (A) and mature zygotic embryos (B).
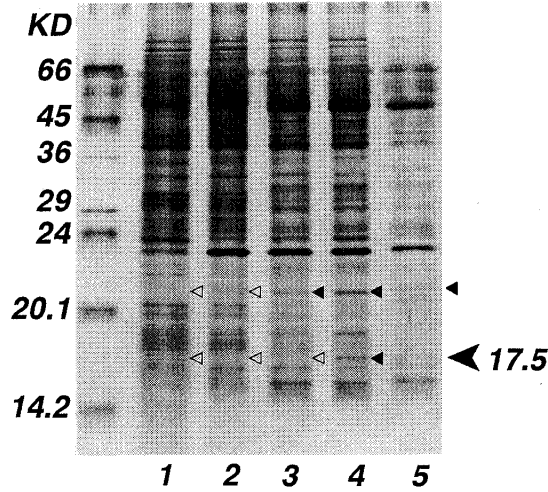Bar equals 1 mm.

Fig. 2 SDS-polyacrylamide gel electrophoresis of the protein extracted from embryogenic callus (lane 1), somatic embryo 4 (SE-4), 7 (SE-7) and 10 (SE-10) weeks after transferring to the regeneration treatment (lane 2, 3 and 4, respectively) and mature zygotic embryo (lane 5).

Arrows indicate the 21 and 17.5 kD polypeptides. Open arrows mark the position of the polypeptides where they are not detectable. Molecular masses of protein standards $(Mr \times 10^{-3})$ are indicated on the left.

calli and younger somatic embryo but present in mature zygotic embryos and older somatic embryo. The majority of polypeptides belonged to Category I, which were thought to be essential for the fundamental metabolism in cells and/or cell propagation regardless of embryo-differentiation.
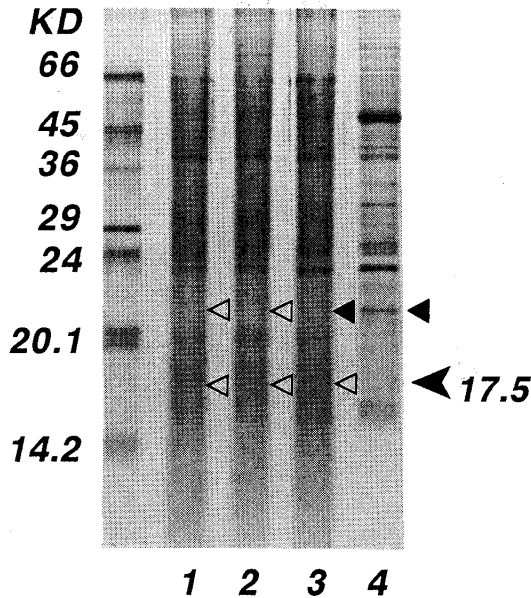

Fig. 3 SDS-polyacrylamide gel electrophoresis of protein extracted from immature zygotic embryos approx. 1 (ZE-1), 2 (ZE-2) and 3 (ZE-3) mm long (lane 1, 2 and 3, respectively) and mature zygotic embryo (lane 4).

Arrows indicate the 21 and 17.5 kD polypeptides. Open arrows mark the position of the polypeptides where they are not detectable. Molecular masses of protein standards $(Mr \times 10^{-3})$ are indicated to the left.
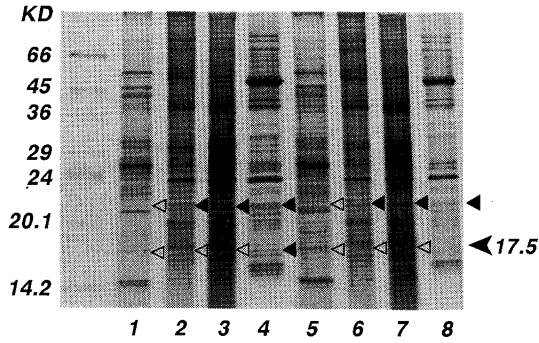
Fig. 4 SDS–polyacrylamide gel electrophoresis of protein extracted from stem, crown and root of seedling derived from somatic embryo(lane 1, 2 and 3, respectively), ones derived from zygotic embryos(lane 5, 6 and 7, respectively)and from mature zygotic embryo(lane 4 and 8).

Arrows indicate the 21 and 17. 5 kD polypeptides. Open arrows mark the position of the polypeptides where they are not detectable. Molecular masses of protein standards $(Mr \times 10^{-3})$ are indicated to the left.

The polypeptides from 18 to 20 kD, and others of about 35 kD belonged to Category II.   The 17. 5 kD and 21 kD polypeptides were classified into Category III.   The 17. 5 kD polypeptide was thought to be associated with maturation of embyo, since it was stably present in SE-10 and mature zygotic embryo, but absent or faint in ZE-1, 2, 3(**Fig. 3**), root, crown and stem of asparagus seedlings(**Fig. 4**).   On the other hand, the 21 kD polypeptide was not mature embryo-specific since it was present in ZE-3(**Fig. 3**), crown and root(**Fig. 4**).

Although the somatic embryos are morphologically similar to the zygotic embryos, the former in the early stage(SE-4)lack the polypeptides in Category III, and had the polypeptides in Category II.   It is, therefore, suggested that they were physiologically different from the mature zygotic counterparts.   This could be one of the reasons why the majority of the somatic embryos of asparagus induced by the previously described protocol[9] are not able to germinate into plants.

On the other hand, the polypeptide profiles of the somatic embryos in the later stage(SE-10) became similar to those of mature zygotic embryos(**Fig. 2**), suggesting the metabolic change from immature to mature state.

We used here a ventilative vessel to induce somatic embryos.   Ventilation through Milli Wrap (Millipore Inc.)lowered the water content of the embryos, and the desiccation is beneficial for the development of asparagus embryos[9].   Desiccation may play a regulatory role in the maturation of somatic embryos.   The effects of desiccation on the germination of somatic embryos into plants have been reported in *Brassica napus*[11] and conifers[12].   Desiccation probably triggers the developmental process necessary for the germination of the embryos[13].

The 17. 5 kD polypeptide described in this report may be associated with maturation or preparation for germination of asparagus embryos since it was only found in the older somatic embryo(SE-10)and mature zygotic embryos(**Fig. 2, 3**).   The polypeptide could be used as a marker for suitable conditions for maturation of the somatic embryos in asparagus.

References

1) Ammirato, P. V., 1983. In "Handbook of Plant Cell Culture, Vol. 1" (eds. by Evans, D. A., W. A. Sharp, P. V. Ammirato, Y. Yamada), p. 82-123, Macmillian Publishing Co., New York.

2) Williams, E. G., G. Maheswaran, 1986. Ann. Bot., **57**: 443-462.

3) Ammirato, P. V., 1989. Int. Asoc. Plant Tissue Cult. Newsletter, **52**: 2-16.

4) Flinn, B. S., D. R. Roberts, C. H. Newton, D. R. Cyr, F. B. Webster, E. P. Taylor, 1993. Physiol. Plant., **89**: 719-730.

5) Etienne, H., B. Sotta, P. Montoro, E. Miginiac, M. P. Carron, 1993. Plant Sci., **92**: 111-119.

6) Lecouteux, C. G., F. M. Lai, B. D. McKersie, 1993. Plant Sci., **94**: 207-213.

7) Hakman, I., P. Stabel, T. Eriksson, 1990. Physiol. Plant., **80**: 441-445.

8) Hakman, I., 1993. Physiol. Plant., **87**: 148-159.

9) Saito, T., S. Nishizawa, S. Nishimura, 1991. Plant Cell Rep., **10**: 230-234.

10) Linsmaier, E. M., F. Skoog, 1965. Physiol. Plant., **18**: 100-127.

11) Senaratna, T., L. Kott, W. D. Beversdorf, B. D. McKersie, 1991. Plant Cell Rep., **10**: 342-344.

12) Roberts, D. R., W. R. Lazaroff, F. B. Webster, 1991. J. Plant Physiol. **138**: 1-6.

13) Kermode, A. R., J. D. Bewley, 1985. J. Exp. Bot., **36**: 1906-1915.

《和文要約》

アスパラガス (*Asparagus officinalis* L.) の体細胞胚と種子胚における
タンパク質泳動パターンの比較

齋藤猛雄*・平井正志・西村繁夫**

野菜・茶業試験場野菜育種部
* 現：野菜・茶業試験場久留米支場
** 現：茨城県農業総合センター生物工学研究所

アスパラガスの体細胞胚から抽出したタンパク質のSDS電気泳動パターンは，再分化処理後の期間が長いほど完熟種子胚の場合と類似したパターンを示した．また17.5 kDのポリペプチドは種子の成熟に関連し，体細胞胚の成熟条件を検索する上でのマーカーになると思われた．