

## Effects of Sucrose Concentration on Morphology of Somatic Embryos from Immature Soybean Cotyledons

Chizuko KAGEYAMA,\*\* Takao KOMATSUDA\*\*\* and Kousuke NAKAJIMA\*

\* National Institute of Agrobiological Resources, Tsukuba, Ibaraki 350, Japan

\*\* Shizuoka Prefectural Citrus Experiment Station, Shimizu, Shizuoka 424, Japan

(Received October 27, 1989)

(Accepted January 16, 1990)

Several authors<sup>1,2)</sup> have succeeded in the induction of somatic embryos and regeneration of whole plant by using soybean (*Glycine max* L. Merr.) immature embryos as explants. Lazzeri *et al.*<sup>3,4)</sup> studied the effects of hormones, culture manipulations, nutritional, physical and chemical factors. The efficiency of embryogenesis, however, was not so high. We investigated the effects of sucrose concentration of culture medium and stage of immature embryos on the embryogenesis of soybean.

Plants of soybean variety Masshokutou (Kou 502), which produced a large number of somatic embryos in the previous study,<sup>5)</sup> were grown in a greenhouse under a 10-h photoperiod. Immature seeds were harvested, classified by size in length to 2.0-2.4, 2.5-2.9, 3.0-3.4, 3.5-3.9 and 4.0-4.4 mm, and cultured according to the protocol of Lazzeri *et al.*<sup>6)</sup> with some modifications. Each pair of isolated cotyledons were placed on culture medium in a test tube (120 mm × 25 mm). The medium consisted of MS salts,<sup>7)</sup> B5 vitamins,<sup>8)</sup> sucrose (0.5, 1, 2, 3%) and NAA (10 mg/l). The pH was adjusted at 7.0, and the medium solidified with gelrite (0.22%). Cultures were incubated under continuous dim light at 25°C. At the end of 4 weeks, all the embryo-like, organized structures were counted as somatic embryos, whereby those showing bipolar shape and owning at least one cotyledon and defined hypocotyl were counted as normal somatic embryos, while others such as globular or sticky types without coty-

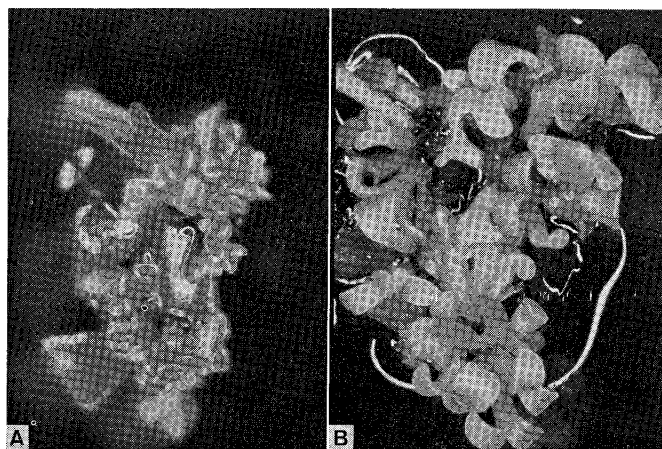


Fig. 1. A : Somatic embryos produced from immature embryos on a medium containing 0.5% sucrose 5 weeks after the initiation of culture.  
B : Somatic embryos developed after 2 weeks of transfer onto a medium containing 1 mg/l NAA and 3% sucrose.

\*\*\* Corresponding author. Off print requests to : T. Komatsuda.

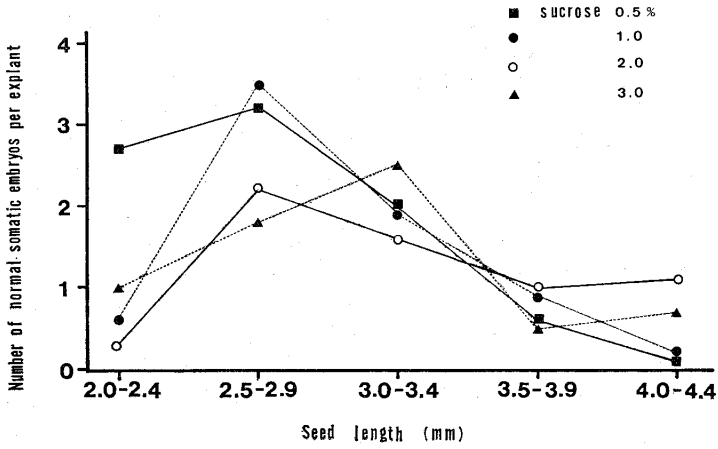


Fig. 2. Effect of sucrose concentration and stage of immature seed on induction of somatic embryogenesis.

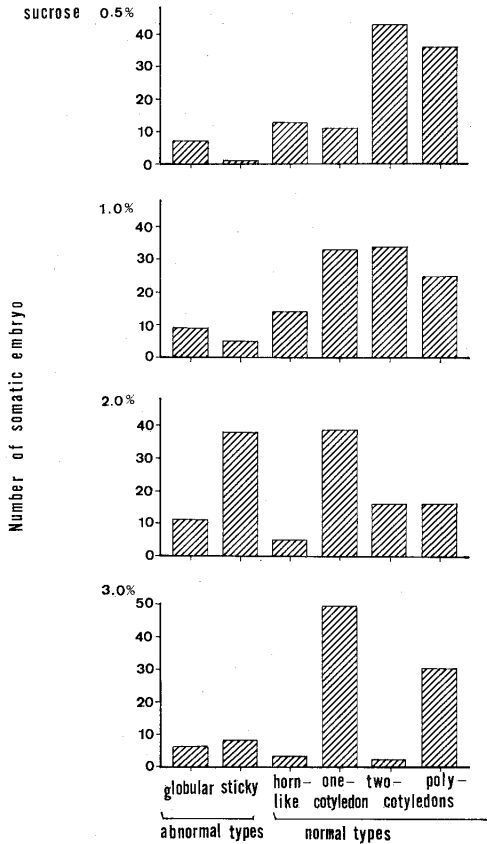


Fig. 3. Effects of sucrose concentration in culture media on the shape of somatic embryos produced. Total number of somatic embryos obtained from 40 explants at 2.5-2.9 and 3.0-3.4 mm in length were presented.

ladons were counted as abnormal ones. Each sucrose concentration within each seed length was represented by twenty explants (pairs of cotyledons).

Green somatic embryos were detected 2 to 3 weeks after the initiation of culture (**Fig. 1, A**). **Figure 2** indicates the effects of sucrose concentration and maturity of seed explants on the production of normal somatic embryos. The combinations of 2.5–2.9 mm long seed with 0.5 or 1.0% sucrose produced 3.2 or 3.5 normal embryos per explant, respectively. The efficiency of normal embryos production was about twice as high as that on the medium containing 3% sucrose previously reported. Lazzeri *et al.*<sup>9)</sup> produced higher numbers of normal embryos per explant on the media containing 1 or 2% sucrose with 6.5 mg/l NAA, and 2% sucrose with 12.5 mg/l NAA. In our experiment, a larger number of normal embryos per cultured explant was provided on the media containing 0.5 or 1% sucrose with 10 mg/l NAA. These results may indicate that the influence of sucrose concentration on the number of normal embryos is dependant upon the genotypes of soybean.

Sucrose concentration also affected the number of cotyledons of somatic embryo. A lower sucrose concentration increased the percentage of two-cotyledon types, and decreased the percentage of one-cotyledon types (**Fig. 3**). The number of embryos of two-cotyledon type in 0.5% sucrose medium were 21 times as many as that in 3% sucrose on 2.5–3.4 mm long seed. On both media containing 0.5 and 1.0% sucrose, most somatic embryos produced were smaller than 2 mm in length and normal shaped (**Fig. 1, A**). After transferred to a maturation medium containing decreased NAA (1 mg/l) and increased sucrose (3%), the small embryos developed into mature size, turned from green to yellow, and stopped enlarging (**Fig. 1, B**). Somatic embryos were continuously produced from the embryogenic cultures on the maturation medium.

We are indebted to Dr. K. Ohyama for his helpful advice on the preparation of this manuscript.

#### References

- 1) Ranch, J. P., L. Oglesby, A. C. Zielinski, 1985. *In Vitro Cell. Dev. Biol.*, **21**: 653–658.
- 2) Barwale, U. B., H. R. Kerns, J. M. Widholm, 1986. *Planta*, **167**: 473–481.
- 3) Lazzeri, P. A., D. F. Hildebrand, G. B. Collins, 1987. *Plant Cell Tissue Organ Cult.*, **10**: 197–208.
- 4) Lazzeri, P. A., D. F. Hildebrand, G. B. Collins, 1987. *Plant Cell Tissue Organ Cult.*, **10**: 209–220.
- 5) Komatsuda, T., K. Ohyama, 1988. *Theor. Appl. Genet.*, **75**: 695–700.
- 6) Lazzeri, P. A., D. F. Hildebrand, G. B. Collins, 1985. *Plant Mol. Biol. Rep.*, **3**: 160–167.
- 7) Murashige, T., F. Skoog, 1962. *Physiol. Plant.*, **15**: 473–497.
- 8) Gamborg, O. L., R. A. Miller, K. Ojima, 1968. *Exp. Cell. Res.*, **50**: 151–158.
- 9) Lazzeri, P. A., D. F. Hildebrand, J. Sunega, E. G. Williams, G. B. Collins, 1988. *Plant Cell. Rep.*, **7**: 517–520.

#### 《和文要約》

ダイズ未熟胚の子葉からの不定胚形成に及ぼすシヨ糖濃度の効果

影山智津子\*, 小松田隆夫, 中島阜介

農業生物資源研究所

\* 静岡県柑橘試験場

培地のシヨ糖濃度がダイズ未熟胚培養からの不定胚形成に及ぼす影響を検討した。最適ステージの未熟胚を使用した場合、試験したシヨ糖濃度 (0.5~3%) のうち、一般的に低濃度ほど子葉と胚軸を持つ正常な形態をした不定胚の形成数が高かった。不定胚の子葉数別に関しては、低濃度ほど2枚の子葉を持った不定胚が多く、高濃度では1枚の子葉を持った不定胚が多く形成された。