Short Communication

Screening of Soybean [*Glycine max* (L.) MERRILL] Genotypes for Somatic Embryo Production from Immature Embryo

Takao KOMATSUDA and Su-Wan Ko

National Institute of Agrobiological Resources (NIAR), Kannondai, Tsukuba, 305

Somatic embryo production was screened on 290 *Glycine max* (L.) MERRILL and 5 *Glycine gracilis* genotypes. Among them, we identified a *G. max* genotype which produced an outstanding number of somatic embryos.

KEY WORDS: *Glycine*, soybean, somatic embryogenesis, regeneration, genotype, genetic resources, immature embryo

Introduction

Somatic embryogenesis of soybean occurs from immature embryos and cotyledons of developing seeds (RANCH *et al.* 1985, LAZZERI *et al.* 1985, BARWALE *et al.* 1986). High frequency somatic embryogenesis is desirable for further studies on somaclonal variation, cell selection, and genetic transformation. LAZZERI *et al.* (1987 a,b) showed that auxin type and concentration and sucrose concentration were of major importance, affecting the efficiency of embryogenesis. A previous screening work (KOMATSUDA and OHYAMA 1988) showed that semi-wild soybean, *Glycine gracilis*, generally produced a higher number of somatic embryos than *G. max* genotypes tested. The *G. gracilis*, however, has many undesirable agricultural characters even if it is judged taxonomically to belong to *G. max* now. We therefore surveyed *G. max* genotypes which would give better embryogenic responses than the *G. gracilis* genotypes.

Materials and Methods

290 *Glycine max* genotypes were randomly selected from the entries in the list of Germplasm Seed Storage Center, NIAR, Tsukuba. Five *Glycine gracilis* genotypes were selected following the previous study (KOMATSUDA and OHYAMA 1988). Seeds of all genotypes were kindly provided by the Germplasm Seed Storage Center, and grown in the field. Immature pods were stored at 4°C for 2 days as a cold pretreatment before embryo excision. Immature seeds, the size of half the length of the mature dry seeds, were excised from the pods. After removing the seed coats and embryonic axes, pairs of cotyledons were placed on 10ml of embryogenesis medium, containing MS salts (MURASHIGE and SKOOG 1962), B5 organics (GAMBORG *et al.* 1968), 3% sucrose, 10mg/l naphthaleneacetic acid, and 0.22% Gelrite, at pH 7.0 before autoclaving.

Ten pairs of cotyledons were used for each genotype (exceptions being where some were not counted due to contamination). The culture tubes were incubated under continuous dim light (ca. 50 lux) at 25°C. The numbers of somatic embryos were counted at the end of

Received August 25, 1989.

Fig. 1. Frequency distribution of the number of somatic embryos produced per pair of cotyledons in 295 soybean genotypes.
 ■ *G. gracilis* genotypes

Fig. 2. Seeds of soybean genotypes 'Moshidou Gong 502' (left), 'Brownie DL/64/177' (middle) and 'Tachisuzunari' (right, as a control).

6 weeks. Somatic embryos were developed, germinated, and grown into plants according to KOMATSUDA and OHYAMA (1988).

Results and Discussion

The frequency distribution of the numbers of somatic embryos produced by pairs of cotyledons resembled a Poisson distribution (Fig. 1). Twenty-eight out of 295 genotypes did

not produce any somatic embryos, while 22 genotypes produced more than 2.0 embryos per pair of cotyledons. Somatic embryos were formed directly from cotyledon tissues, and/or indirectly via callus-like brown tissues derived from cotyledons as described previously (KOMATSUDA and OHYAMA 1988).

The highest somatic embryo producer was the 'Brownie DL/64/177', which produced 80 somatic embryos from 10 pairs of cotyledons. Among the somatic embryos, 23 (28%) germinated, and 16 (20%) grew into plantlets. The variety was introduced from South Africa, but any other information about the origin of the variety was not provided. The variety was the sole *G. max* genotype that produced a higher number of somatic embryos than the *G. gracilis* genotype Moshidou Gong 502 (The variety was entered in the list of the Germplasm Seed Storage Center, NIAR, as "MASSHOKUTOU KOU 502"). In Fig. 2 the seeds of the genotypes were compared with that of a standard Japanese variety, "Tachisuzunari".

Literature Cited

- BARWALE, U. B., H. R. KERNS and J. M. WIDHOLM 1986. Plant regeneration from callus cultures of several soybean genotypes via embryogenesis and organogenesis. *Planta* **167**: 473~481.
- GAMBORG, O. L., R. A. MILLER and K. OJIMA 1968. Nutrient requirements of suspension cultures of soybean root cells. *Exp. Cell Res.* **50**: 151~158.
- KOMATSUDA, T. and K. OHYAMA 1988. Genotypes of high competence for somatic embryogenesis and plant regeneration in soybean *Glycine max*. *Theor. Appl. Genet.* **75**: 695~700.
- LAZZERI, P. A., D. F. HILDEBRAND and G. B. COLLINS 1985. A procedure for plant regeneration from immature cotyledon tissue of soybean. *Plant Mol. Biol. Rep.* **3**: 160~167.
- , ——— and ——— 1987 a. Soybean somatic embryogenesis: Effects of hormones and culture manipulations. *Plant Cell Tiss. Organ Cult.* **10**: 197~208.
- , ——— and ——— 1987 b. Soybean somatic embryogenesis: Effects of nutritional, physical and chemical factors. *Plant Cell Tiss. Organ Cult.* **10**: 209~220.
- MURASHIGE, T. and F. SKOOG 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Physiol. Plant.* **15**: 437~497.
- RANCH, J. P., L. OGLESBY and A. C. ZIELINSKI 1985. Plant regeneration from embryo-derived tissue cultures of soybeans. *In Vitro Cell. Dev. Biol.* **21**: 653~658.

栽培ダイズにおける未熟胚からの不定胚形成のスクリーニング

小松田隆夫・柯淑婉

(農業生物資源研究所, つくば市, 〒305)

筆者らは、半野生ダイズ (*Glycine gracilis*) の数系統が、比較的高い不定胚形成能を示す事をすでに報告している。しかし、*G. gracilis* の系統が多く、不良な農業形質もあわせもつことは、育種上の問題点である。そこで、栽培ダイズ (*G. max*) の中から不定胚形成能の高い系統を見出す目的でスクリーニングをおこなった。農業生物資源研究所、植物遺伝資源配布目録から品種を無作為に選択し圃場に播種し、7-9月に着実した栽培ダイズ290品種及び標準としての半野生ダイズ5系統の未熟胚を3% sucrose, 10 mg/l NAAを含むMSB固形培地 (pH 7.0)へ置床した。培養開始6週間後、不定胚形成数を調査した。

供試した品種・系統のうち、不定胚形成を示さないものは28品種、また未熟胚当たり2.0以上の不定胚を形成したものが22品種・系統であった。最高の値を示した品種は、南アフリカから導入されたBrownie DL/64/177であった。この品種は供試した*G. gracilis* 系統の中で最高の値を示した秣食豆 (公502) よりも高い値を示した唯一の品種であった。Brownieは形態的に栽培ダイズの範疇に入ると判断した。