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Effects of Auxins and Cytokinins on Organogenesis of Soybean *Glycine max* L.

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Key words: first leaf, IAA, cytokinins, organogenesis, soybean seedling

Abstract

To select the section with shoot formation ability, the calli and shoot formation from three sections (first leaf including cotyledonary node, hypocotyl and cotyledon explants) of 5-days-seedlings of soybean were induced on MS medium supplemented with 1.0 mg/L BAP, 3% sucrose, and 0.3% gelrite for one month. The first leaf section exhibited the highest shoot formation rate (51%), followed the hypocotyl section (10%) and the cotyledon section (0%). The shoot formation rates and shoot number of the four excised sections (whole first leaf, a half of the first leaf, a third of the first leaf and only node) of the first leaf were also investigated on the same medium. A half of the first leaf explant and the third of the first leaf explant had higher shoot formation rates (76-80%) and numbers (3-4 / explants) than those in other two explants. Effects of six cytokinins (kinetin, zeatin, BAP, 2iP, PBA, and TDZ) on shoot formation were determined, using the half of the first leaf explants. Zeatin (1.0 mg/L) exhibited the highest in shoot formation rate (94%) and numbers (8 / explant). In addition, the combined effects of three cytokinins (zeatin, BAP, and TDZ; 0.5, 1.0, 2.0 mg/L, respectively) and an auxin (IAA; 0.0, 0.5, 1.0, 2.0 mg/L) were determined. The combination (1:1, v/v) of zeatin (1.0 mg/L) and IAA (1.0 mg/L) exhibited the highest in shoot formation rate (96%) and numbers (16 / explant), twice more than zeatin (1.0 mg/L) alone. The shoot cuttings were transferred and cultivated on the rooting media supplemented with only auxin, IBA at various concentrations. The highest root formation (8 / shoot) was achieved on the medium supplemented with 1.5 mg/L. After 4 weeks of cultivation, the plantlets with an extensive root system were transplanted in pots with a soil mixture of vermiculite and fine sand. Transferred to field, about 75% of the plantlets survived.

Introduction

The way in which a callus forms a new plant *in vitro* is variable. Frequently, with relatively high cytokinin-to-auxin ratios, only a shoot system first develops; then adventitious roots are formed spontaneously from the stems while still in the callus. This formation of shoots or of shoots and adventitious roots by the callus is called organogenesis. However, calli become embryogenic and forms an embryo that develops into a root and shoot; this is called embryogenesis.

Soybean is regenerated both via organogenesis (Barwale et al., 1986; Kim et al., 1990; Wright et al., 1986) and via embryogenesis (Barwale et al., 1986; Ranch et al., 1985; Bailey et al., 1993; Ghazi et al., 1986; Kim et al., 2000; Rajasekaran and Pellow, 1997). The embryogenesis has been achieved by culturing the cotyledonary explant of the seedling, while the organogenesis has been achieved by mainly using the primary leaf node explant of the seedling. It means that the regeneration ability depends on the varieties and explants.

In the present study, for the regeneration of soybean via organogenesis, the explant was selected and the cytokinin-to-auxin ratio was determined with terms of the shoot formation rate and the number.

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Materials and Methods

Materials

The seeds of soybean [*Glycine max* (L.) Merrill cv. Alchankong] were harvested on October 1999 at a field of Rural Development Administration, National Honam Agricultural Experiment Station and stored at room temperature until use. The seeds were sterilized with 70% ethanol for 1 min and with 2% sodium hyphochlorite for 20 min, and then washed 4 times with sterilized distilled water. They were allowed to germinate on two layers of Advantec Toyo (No. 2) filter paper in 100 mL beakers containing 10 mL of distilled water that had been sterilized for 15 min at 121℃. They were maintained at 25±1℃ in darkness for 5 days.

Selection of explants and culture conditions

The first leaf with the cotyledonary node (0.2-0.3 cm in length), hypocotyl (0.2-0.3 cm in length), and cotyledon (0.3-0.4 cm in length) regions was excised from the 5-days-seedlings (Figure 1). They were cultured on MS medium (pH, 5.8) (Murashige and Skoog, 1962) supplemented with 1.0 mg/L BAP, 3% sucrose and 0.3% gelrite at 25±1℃ under a 16/8h (light/dark) photo-period with an intensity of 80 μE $m^{-2}s^{-1}$ provided by white fluorescent lamps for one month. Among the regions from the 5-days-seedlings, the first leaf-cotyledonary node regions exhibited the highest shoot formation rate and number (Figure 2). So, the first leaf region was again excised into the whole first leaf, a half of the first leaf, a third of the first leaf, and the only cotyledonary node explants. They were cultured on the same medium under the above condition for one month. The half of the first leaf explant and the third of the first leaf explant had higher shoot formation rate and number than other two explants (Figure 3). The half of the first leaf explants was selected for this experiment. The data were analyzed with a t-test at 5% or 1% level.

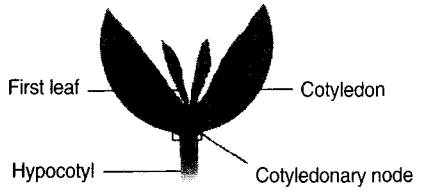

Figure 1. A diagram of the excised sections from the 5-days-old seedling of soybean.

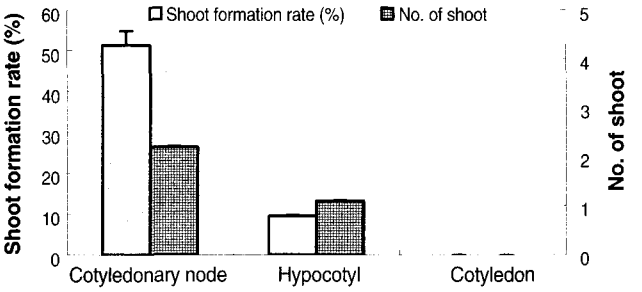

Figure 2. A comparison of shoot formation ability of the excised portions. The explants were cultured on MS medium supplemented with an auxin, 1.0 mg/L of IBA for one month. Values represent means± SE for n=24.

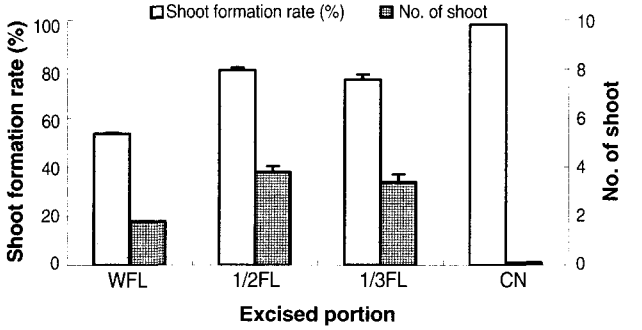

Figure 3. A comparison of shoot formation ability of excised portions for the first leaf with cotyledonary node. The explants(WFL; the whole first leaf, 1/2FL; the half of the first leaf, 1/3FL; the third of the first leaf, CN; the cotyledonary node) were cultured on the medium supplemented with 1.0 mg/L of IBA for one month. Values represent means±SE for n=24.

To investigate the effects of cytokinins on the shoot formation of the half of the first leaf explants, six cytokinins, kinetin, zeatin, BAP, 2iP, PBA, and TDZ (0.25, 0.5, 1.0, 1.5, 2.0 mg/L) were supplemented respectively into the medium. In addition, to investigate the combined effects of three cytokinins and an auxin on shoot formation, zeatin, BAP, TDZ (0.5, 1.0, 2.0 mg/L) and IAA (0.0, 0.5, 1.0, 2.0 mg/L) were supplemented respectively into the medium. The half of the first leaf explants were inoculated on the media and cultured under the above condition. After 6 weeks of culture, the effects were determined in terms of shoot formation rate (shoot formation/inoculation number ×100%) and the number per explant. The data were analyzed with LSD (least significant difference) test at 5% level.

Rooting and acclimatization

For rapid rooting of multiple shoots, an auxin, IBA (0.0, 0.5, 1.0, 1.5, 2.0 mg/L) was, respectively, supplement-

ed into the MS medium. The shoot cuttings were transferred onto the media in a bottle and cultivated for 4 weeks (Figure 7C). The effect of IBA on the root formation was investigated in terms of the rate or number. After 4 weeks of cultivation, the plantlets with an extensive root system were transplanted in pots with a soil mixture (1:1, v/v) of vermiculite and fine (0.2-0.02 in mm) sand. The plantlets regenerated were transplanted to field and the acclimatization of the plantlets was investigated in a term of survivability in the field.

Results and Discussion

Selection of explants for shoot formation ability

The first leaf with cotyledonary node, hypocotyl, and cotyledon regions was excised from the seedlings. They were cultured on MS medium supplemented with IBA (1.0 mg/L). The calli and adventitious shoots were formed (Figure 7A). There were great differences in the shoot formation rate (%) and the number, although the calli occurred from all the excised regions (Figure 2). The decreasing order of shoot formation rate was the first leaf (50%), hypocotyl (10%), and cotyledon (0%) ($P<0.01$). The numbers of shoot formed were 2 for the first leaf, 1 for the hypocotyl, and 0 for the cotyledon ($P<0.05$). Therefore, the first leaf with cotyledonary node was selected.

The organogenic regenerations of soybean were conducted mainly using the cotyledonary node explants (Graybosch et al., 1987; Gulati and Jaiwal, 1994; Mante et al., 1989; Thom et al., 1996). However, Kim et al. (1990) had used the primary leaf node explants, like in this experiment, while Dan and Reicher (1998) had used the hypocotyl explants and Mante et al. (1989) had used the mature cotyledon explants.

In the present study, these results indicate that the endogenous IAA content of the first leaf is more than the other regions, i.e., IAA is in vivo synthesized in the meristem of shoot apex and more differently distributed into leaf regions with a polarity.

On the other hand, the first leaf including the cotyledonary node was excised into four explants, named as the whole first leaf (WFL), the half of the first leaf (1/2 FL), the third of the first leaf (1/3 FL), and the only cotyledonary node (CN). They were cultured on the same medium under the above condition for one month. The 1/2 FL and 1/3 FL explants had higher shoot formation rates (76-80%) and number (3-4 / explant) than those in other two explants ($P<0.01$) (Figure 3). These results suggest that shoot formation ability of the explants relates to the in vivo level of the endogenous auxin (IAA) or cytokinin (zeatin). Thus, the contents are regarded to be measured further.

Effects of cytokinins on shoot formation of the first leaf explant

To investigate the effects of cytokinins alone, the 1/2 FL was cultured on the media supplemented with various concentrations from 0.25 to 2.00 mg/L of six cytokinins (zeatin, kinetin, BAP, 2iP, PBA, TDZ) for one month. The shoot formation and the numbers were investigated (Figure 4). Among the cytokinins, only endogenous zeatin exhibited the highest shoot formation rate (94%) and the number (8 / explant). The optimum concentration was 1.0-1.5 mg/L. The effect of zeatin on shoot formation rate was similar to that of kinetin, but the effect of exogenous zeatin on the number was higher than that of exogenous kinetin. Other exogenous cytokinins (BAP, 2iP, PBA, TDZ) were less effective on both the shoot formation rate and the numbers ($P<0.05$).

In other reports, BAP was the most effective in soybean (Wright et al., 1986) and 2iP was the most effective in mung bean, resulting in shoot formation rather than shoot elongation (Gulati and Jaiwal, 1992; 1994).

These results suggest that not only the shoot formation ability of the explant relates to the content of the endogenous auxin (IAA) or cytokinin (zeatin), but also, although the mechanism on them is not clear, the differences maybe due to the chemical and/or structural differences.

Effects of the combinations of cytokinin and IAA on shoot formation of the first leaf explant

To obtain the optimum cytokinin-to-auxin ratio on the shoot formation, the first leaf-the 1/2 FL explants were cultured on the media supplemented with combinations of an auxin (IAA) and three cytokinins (zeatin, BAP, and TDZ) for six weeks (Figure 7B). The combination of IAA and zeatin exhibited a higher shoot formation rate and a higher numbers than other two cytokinin combinations (Figure 5). Especially, the combination (1:1) of IAA (1.0 mg/L) and zeatin (1.0 mg/L) exhibited the highest shoot formation rate (96%) and the highest numbers (17/ explant), twice more than zeatin (1.0 mg/L) alone. The only IAA supplement inhibited the shoot formation. It was reported that the combination with an auxin, AdS (1.0 mg/L) and 2iP (0.5 mg/L) increased the shoot formation in horsegram (Varisai Mohamed et al., 1999). These results suggest strongly that both auxin and cytokinin coordinately regulated the shoot formation.

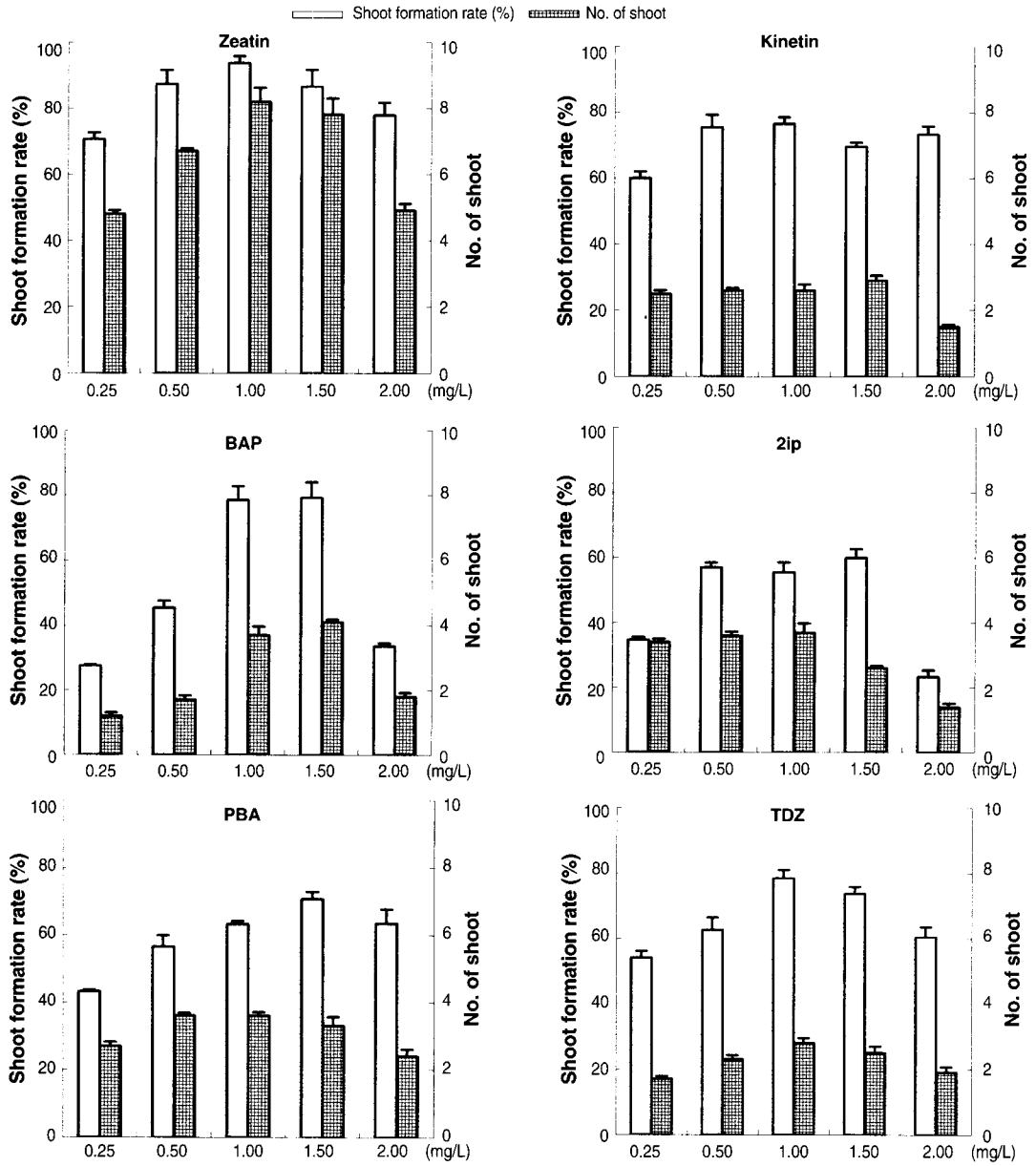

Figure 4. The effects of cytokinins on shoot formation of the first leaf (with cotyledonary node) explant. Values represent means±SE for n=20.

Rooting and acclimatization

Although the adventitious roots will be formed spontaneously from the stem while still in the callus, to induce the rapid rooting from the shoot cuttings, they were transferred and cultivated on the media supplemented only with an IBA at various concentrations, well known as a rooting auxin for one month. The highest root formation (8 / shoot) was achieved at the medium supplemented with 1.5 mg/L of IBA (Figure 6, Figure 7C). The same effects were reported in pigeon pea (Sivaprakash et al., 1994) and in horsegram (Varisai Mohamed et al., 1999).

After one month, the plantlets with an extensive root system were transplanted in pots with a soil mixture of vermiculite and fine sand. Transferred to a bottle (Figure 7D) and a pot (Figure 7E), 75% of plantlets survived in the field.

In conclusion, the first leaf, the 1/2 FL explant had high shoot formation ability. The endogenous auxin and cytokinin was more effective on the shoot formation than synthetic ones. The formation rate and the number greatly was promoted at a cytokinin-to-auxin ratio of 1:1, suggesting that, in other species, the optimum ratio for rapid multiplication of the shoot must be determined. The

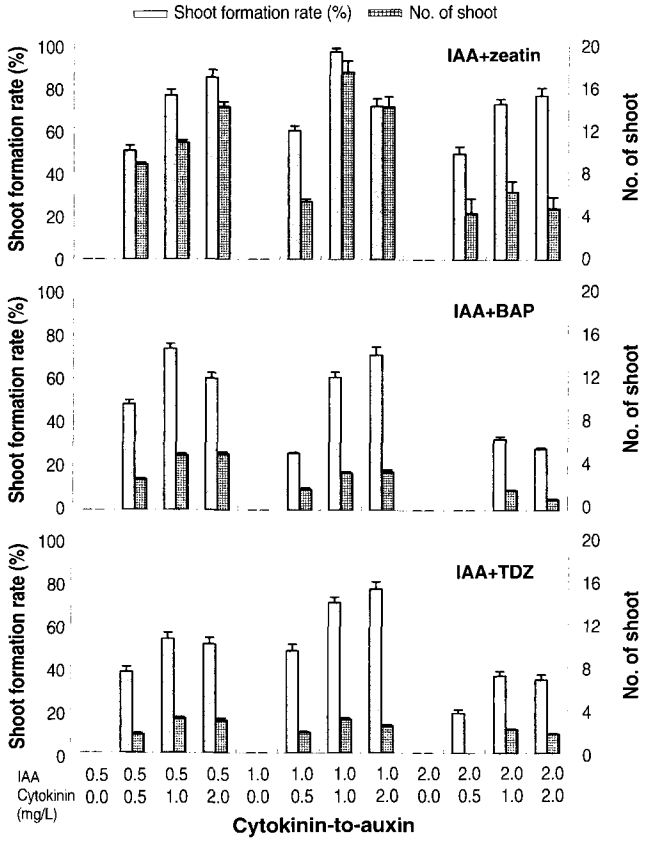

Figure 5. The effects of combinations of an auxin and cytokinins on shoot formation of the half of the leaf explants. Values represent means±SE for n=12.

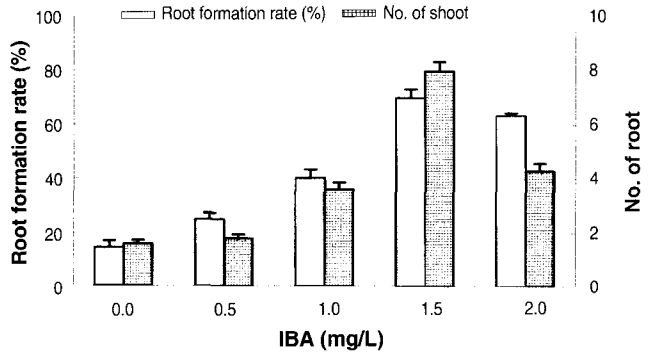

Figure 6. The effects of IBA on the adventitious root formation from the shoot cuttings. Values represent means± SE for n=16.

rooting from the shoot cutting was promoted by adding of a rooting auxin, IBA into the medium, but the acclimatization of the plantlets for field remains to be solved.

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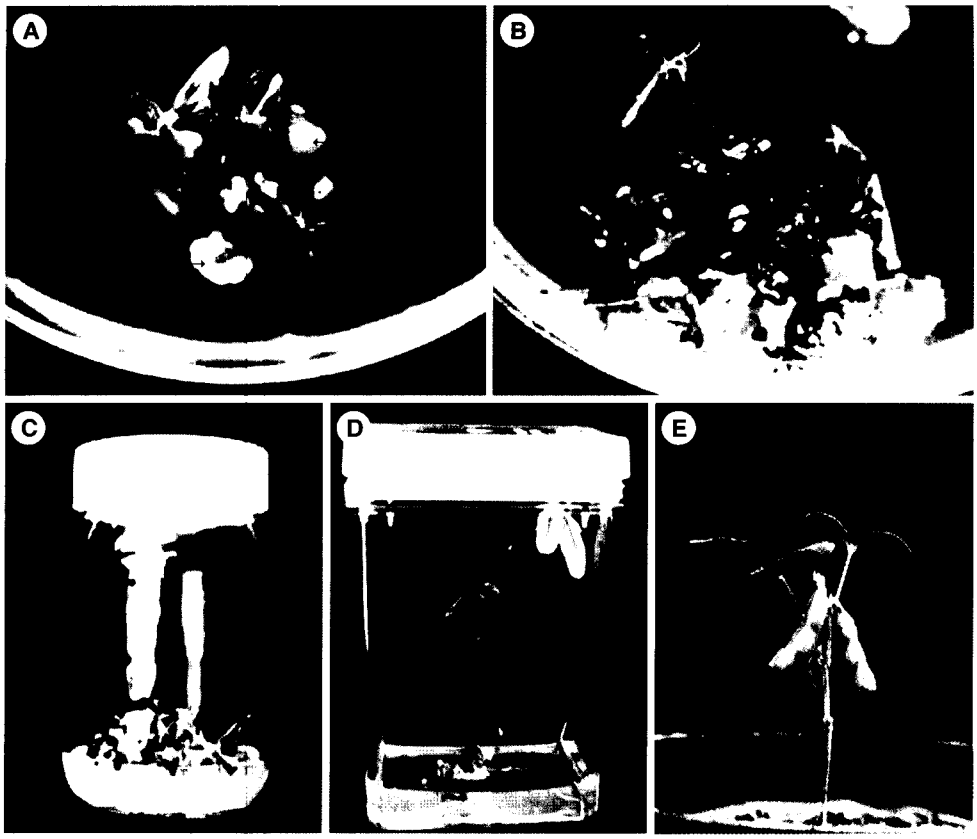

Figure 7. Summary for plantlet regeneration system via organogenesis. A; Formation of calli and multiple shoots from the first leaf (with cotyledonary node) explant on the medium supplemented with a cytokinin, 1.0 mg/L of BAP during one month, B; Formation of calli and multiple shoots of the first leaf-the half of first leaf explant on the medium supplemented with an auxin, 1.0 mg/L of IAA and a cytokinin, 1.0 mg/L of zeatin during one month, C; Development of root system from the shoot cuttings on the medium supplemented with a rooting auxin, 1.5 mg/L of IBA, D; Acclimatization of the plantlet in a bottle, E; Acclimatization of the plantlet in a pot in the field.

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