J. Plant Biotechnology(2004) Vol. 6(2). pp. 107~111

Journal of Plant Biotechnology

Effective Acclimation System for *in Vitro* Regenerated Plantlets of Soybean

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Abstract

To establish an efficient acclimation system for regenerated plantlets of soybean, we used various media with hydroponic nutrient solutions before regenerants were transplanted into soil. The hydroponic nutrient solution was essential for the survival of the plantlets. The vermiculite with nutrient solution at pH 5.5 was found to be the best medium with 97-100% survival rate and better growth of regenerants plantlets. Regeneraed grew best in the following order of solutions: Yoshida solution, modified Yoshida solution, Soy I, Soy II, and MS medium. However, Soy I solution (EC 2.9 mS/cm), developed by the Honam Agricultural Research Institute proved to be the most effective for acclimation in terms of the time required for vigorous growth and economical use of chemicals.

Key words: Acclimation, regenerated plantlet, vermiculite, hydroponic system, soybean

Introduction

After *in vitro* plant regeneration through shoot differentiation or somatic embyogenesis, acclimation of the plantlets regenerated is very important to get seeds on a large scale for next generation. However, the current method for acclimation is ineffective and unstable, and no detailed information is available in soybean tissue culture system. Therefore, we carried out the study to increase the survival rate of soybean regenerants using hydroponic nutrient solution.

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Received Nov. 17, 2003; Accepted May. 18, 2004

Materials and Methods

Plant materials

Plantlets were regenerated from immature cotyledons via somatic embryogenesis of soybean cultivars, Pungsannamulkong, Sinpaldalkong #2, Alchankong and Lx 15. The methods for somatic embryogenesis were previously described. Media used were as follows: embryo induction on MSD40 proliferation on MSD20 histodifferentiation and maturation on FNL0S3S3GM and germination on hormone-free MS0 followed by MS0AC (Table 1).

Acclimation

The regenerated plantlets produced new shoot within one week after they were directly transferred to soil, but did not grow any more due to weak root growth (Figure 1A, B). Regenerated plantlets were transplanted to small pots (8 cm×8 cm×7.5 cm) filled with various media (soil, vermiculite, pearlite, horticultural bed soil) without aeration. The pots were placed into 4-liter nutrient solution (Yoshida et al. 1976) at pH 5.0, 5.5, 6.0 and 6.5 in plastic tray (40 cm×32 cm×14 cm), and kept at 27℃ under 23 hr photoperiod with light intensity of about 50 $\mu molm^{-2}sec^{-1}$ for 1 week. After that, plants were moved to a glass house under 16 hr photoperiod to promote flowering. Nutrient solution was refilled to avoid nutrient depletion and also exchanged every three days to maintain pH. The top of each pot was closed with a polyethylene bag to prevent plantlet wilting (Figure 1C) and the bag was gradually opened to harden plantlets (Figure 1D). Survival rate of the regenerants was determined 3 weeks after transplanting.

Table 1. Composition of the media used for soybean somatic embryogenesis.

Medium[a]	Salts	Vitamins	Carbon source	pH	Growth regulator (2,4-D)	Other nutrients
MSD40	MS salts	B_5	3% sucrose	7.0	40 mg/L	-
MSD20	MS salts	B_5	3% sucrose	5.8	20 mg/L	-
FNL0-S3S3GM	FN Lite macro, MS micro	B_5	3% sucrose, 3% sorbitol	5.8	-	30 mM glutamine, 2 mM methionine
MS0	MS salts	B_5	3% sucrose	5.8	-	-
MS0AC	MS salts	B_5	3% sucrose	5.8		1% activated charcoal

[a] Solidifying agent is 0.2% Gelrite. FNL0S3S3GM is liquid medium.

Table 2. Concentration of chemical compounds used in each nutrient solution.

Chemical	Concentration (mg/L)			
	Yoshida	Modified Yoshida	Soy I	Soy II
NH_4NO_3	11.4	-	-	-
$NaH_2PO_4 \cdot 2H_2O$	50.4	-	-	-
$NH_4H_2PO_4$	-	200	200	1,000
KNO_3[†]	89.3	500	500	1,000
$Ca(NO_3)_2$	-	1,000	1,000	-
$CaCl_2$	110.8	-	-	-
$MgSO_4 \cdot 7H_2O$	405	405	500	500
$MnCl_2 \cdot 4H_2O$	1.9	1.9	-	-
H_3BO_3	1.2	1.2	0.6	-
$CuSO_4 \cdot 5H_2O$	0.04	0.04	-	-
$(NH_4)_6 \cdot MO_7O_{24} \cdot 4H_2O$	0.09	0.09	-	-
$ZnSO_4$	0.04	0.04	-	-
$FeSO_4$[†]	9.6	9.6	9.0	-
Citric acid (monohydrate)	14.9	-	-	-

[†] Substitute KNO_3 and $FeSO_4$ for K_2SO_4 and $FeCl_3 \cdot 6H_2O$, respectively by Yoshida et al. (1976).

Nutrient solutions used in the experiment were Yoshida solution, modified Yoshida solution, MS medium, Soy I and Soy II, and they were changed every week (Table 2). After hardening off the regenerants, the rooted plantlets were transferred into soil in pots and grown till maturity.

The experiment employed a completely randomized design with four replications. Plant height, total number of leaves and fresh weight (top and root part) were determined at three weeks after transplanting (DAT). EC and pH values from the nutrient solution were also investigated during a 3-week cultural period.

Results and Discussion

Acclimation of plantlets regenerated *in vitro* is difficult in soybean. Currently, no effective method is available for acclimation in tissue culture of soybean. This experiment was carried out to establish an efficient acclimation system for regenerated plantlets derived from immature cotyledon of soybean by adopting hydroponic solution. Generally, the regenerated plantlets *in vitro* die when directly transferred to soil due not to poor root development and growth. Therefore, we used various media such as vermiculite, pearlite and horticultural bed soil (Figure 1D) for increasing the survival rate of regenerants. Table 3 showed that the hydroponic nutrient solution is essential for acclimation. There was no survival of plant without hydroponic nutrient solution. Among the media, vermiculite with hydroponic nutrient solution is the best with vigorous roots for survival rate (Figure 1E, F). Well-rooted plantlets were transferred to soil set pods with seeds (Figure 1G).

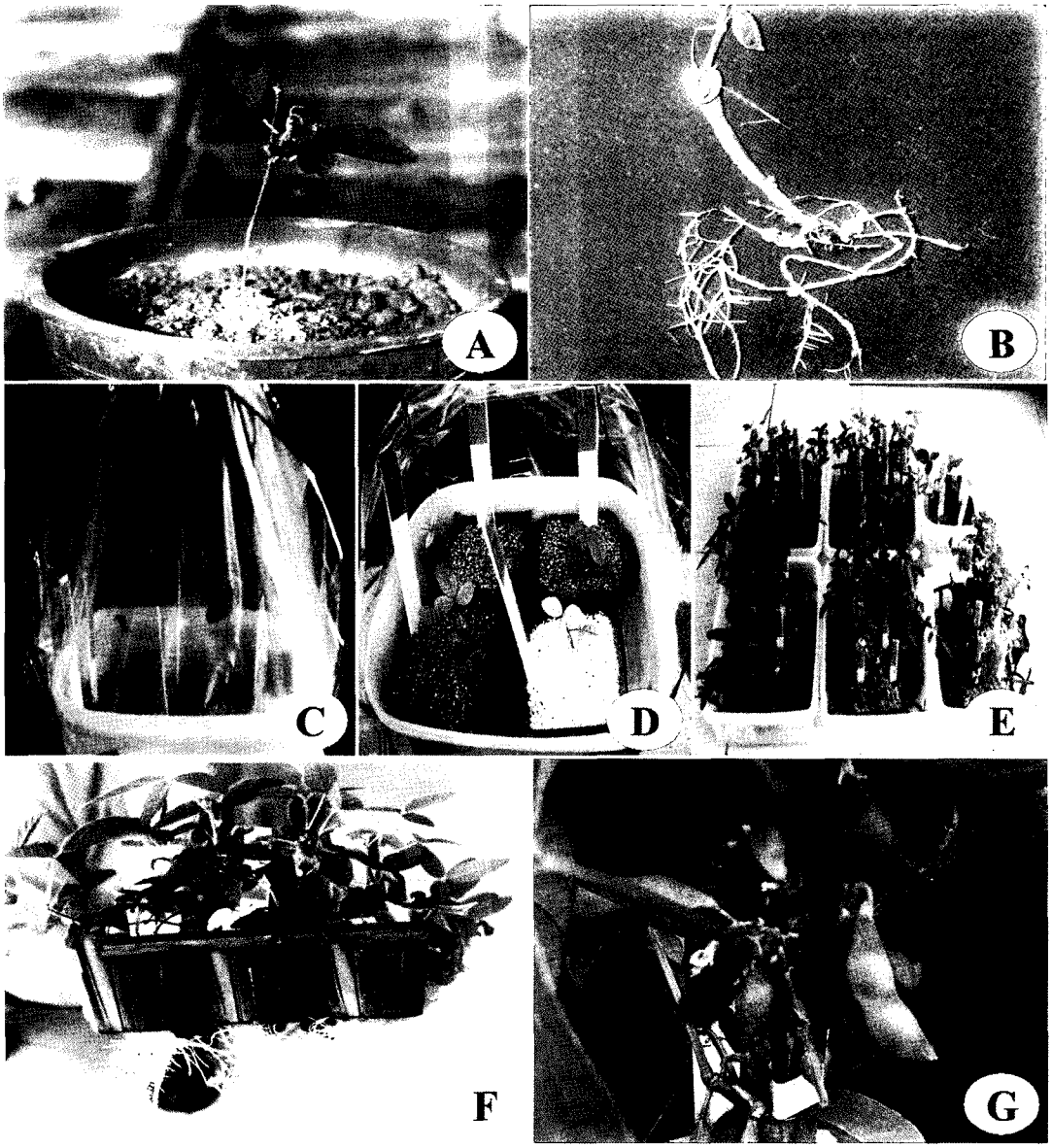

Figure 1. Acclimation of regenerated plantlets derived from soybean somatic embryo using the nutrient solutions. A new shoot was produced when directly transplanted into soil without nutrient solution culture (A), but did not grow any more due to weak root growth (B). The plantlets transferred to pot in a tray covered with polyethylene bag to prevent excessive evaporation for high humidity condition (C) in different media with hydroponic nutrient solutions (D). The best medium for acclimation was achieved in the pots filled with vermiculite with Soy I solution culture (F) as shown vigorous roots (F). Well-rooted plantlets transferred into soil in pots set pods and seeds (G).

Table 3. Survival rate (%) of regenerated plantlet from somatic embryos of soybean in various acclimation media.

Genotype	Media					
	Vermiculite		Pearlite		Horticultural bed soil	
	With[a]	Without	With	Without	With	Without
Lx15	100	0	94	0	88	0
PI 96322	97	0	92	0	93	0

[a]With /without: hydroponic nutrient solution.
- Nutrient : Yoshida solution added to 0.1 mM K_2SiO_3, 1 mM $Ca(NO_3)_2$.

There was no significant difference in the survival rate among Yoshida, Modified Yoshida, and Soy I solution (Table 4). Out of the different nutrient solutions, the growth of shoot and root in regenerated plants was the highest in Yoshida solution. Soy I solution was lower in the fresh weight of root than Yoshida's, but a lot of lateral roots were produced due to fewer ingredients of Soy I. Soy I solution (EC 2.9 mS/cm), developed in this experiment, proved to be the most effective solution for the acclimation of plant regenerated from somatic embryos considering the time and chemicals required, although. However, the growth of plantlets in the solution was a less vigorous than in

Table 4. Agronomic characters at 21 days (seedling stage) after transplanting regenerated soybean (cv. Pungsannamulkong) to various nutrient solutions with vermiculite for acclimation.

Nutrient solution	Plant height (cm)		Fresh weight (mg/plant)		Total number of leaves	Survival rate (%)
	Top	Root	Top	Root		
Yoshida	22.6[a†]	15.2[a]	1,235[a]	1,120[a]	8.4[a]	99[a]
Modified Yoshida	19.8[ab]	15.0[a]	1,132[ab]	1,053[ab]	7.8[a]	98[a]
MS medium	15.4[c]	10.4[c]	539[d]	342[d]	5.7[b]	17[c]
Soy I	21.3[a]	14.1[ab]	1,031[b]	993[b]	7.9[a]	99[a]
Soy II	20.7[ab]	16.0[a]	819[c]	790[c]	6.3[ab]	94[b]

† Mean compared by Duncan's multiple range test at 5% level.

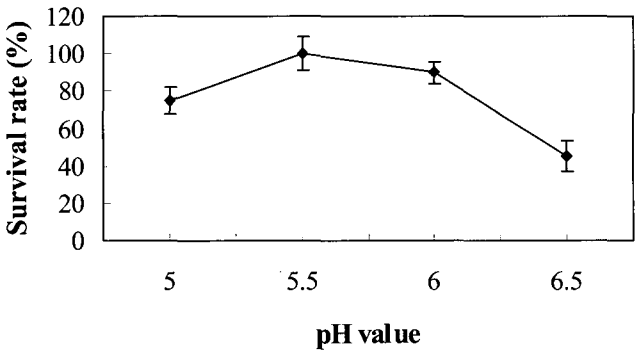

Figure 2. Survival rate (%) of regenerated plantlets by pH conditions in hydroponic culture.

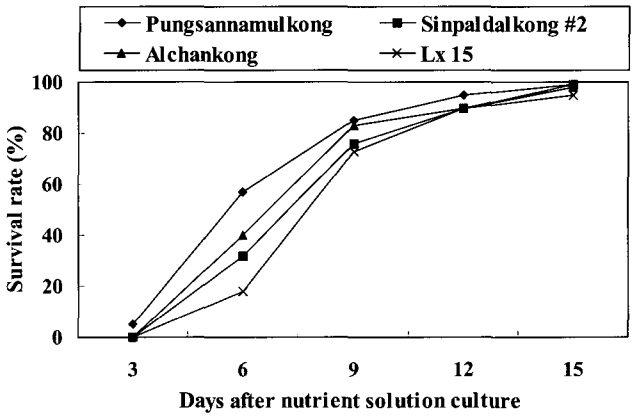

Figure 3. The effect of culture period in nutrient solution on survival rate of regenerated seedlings after transferring to soil. Balanced nutrient solution recommended by Yoshida was used during growing period.

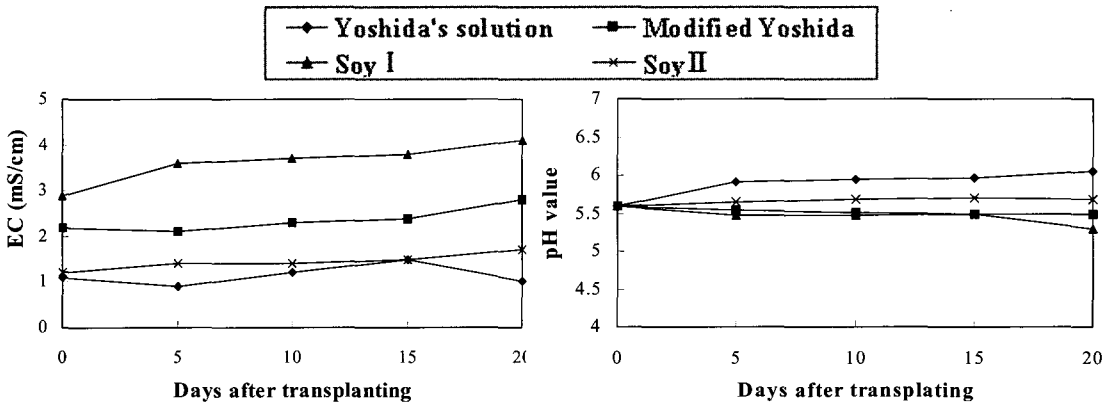

Figure 4. Changes in pH and EC of nutrient solutions during acclimation period.

Yoshida's. Soy I consisted of only six chemicals including sodium nitrate (Table 2). But some soybean plants grow in the nutrient solution gave some symptoms like chlorosis and white leaves. The plants become light green in color due to nitrogen-deficiency. Sometimes, the roots are stunted and too much branched. Therefore, the most important ingredients for plant growth may be nitrogen compounds. The pH increases as the plant absorbs the ammonium ion when nitrate is the sole source of nitrogen, and the high pH causes iron deficiency. Both ammonium and nitrate are present as an effective sources of nitrogen in Soy I solution (Table 2). The pH 5.5 was considered optimum for higher survival rate of seqenerated plantlets in hydroponic culture (Figure 2).

We also studied the effect of culture period in nutrient solution on survival rate of regenerated seedlings after transferring to soil. Analysis of nutrient solution culture regimes revealed that the longer culture (more than 9 days) results in higher survival of plantlets (above 70%), whereas the culture period of 3 days reduced survival of the plantlets (below 10%) (Figure 3). pH and EC of cultural solution varied during growing period (Figure 4).

We described an easy, simple method for acclimation of soybean regenerants *in vitro*. The method is easily applicable to soybean regenerants with good success.

Acknowledgements

This work was supported by a grant from The BioGreen 21 Program, Rural Development Administration, Republic of Korea. S.J. Yun was supported by Research Center for Industrial Development of Biofood Materials in Chonbuk Nat'l Univ., Jeonju, Korea. The Center is designated as a RRC appointed by the KOSEF, Jeonbuk Provincial Government and Chonbuk Nat'l, Univ., Korea.

References

Barwale UB Kerns HR, Widholm JM (1986) Plant regeneration from callus cultures of several soybean genotypes via embryogenesis and organogenesis. Planta 167: 473-481

Dan Y, Reichert NA (1998) Organogenic regeneration of soybean from hypocotyl explants. *In Vitro* Cell Dev Biol-Plant 34: 14-21

Kim J, LaMotte CE, Hack E (1990) Plant regeneration *In Vitro* from primary leaf nodes of soybean (*Glycine max*) seedlings. J Plant Physiol 136: 664-669

Liu W, Moore PJ, Collins GB (1992) Somatic embryogenesis in soybean via somatic embryo cycling. *In Vitro* Cell Dev Biol 28: 53-160

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15: 473-479

Ranch JP, Oglesby L, Zielinski AC (1986) Plant regeneration from tissue cultures of soybean by somatic embryogenesis. In: Vasil IK (ed) Cell culture and somatic cell genetics of plants pp 97-110 Academic Press, New York

Terzi M, Loschiavo F. (1990) Somatic embryogenesis. In: Bhojwani SS (ed) Development in crop science 19: Plant, pp 54-66 tissue culture: applications and limitations. Elsevier, Tokyo

Wright MS, Launis KL, Novitzky R, Duesing JH, Harms CT (1991) A simple method for the recovery of multiple fertile plants from individual somatic embryos of soybean [*Glycine max* (L.) Merrill]. *In Vitro* Cell Dev Biol 27: 153-157

Yeh M-S, Chyuan J-H (1991) *In vitro* culture of immature soybean embryos III. Organogenesis studies on soybean embryogenic axes culture. J Agric Assoc China 40: 77-90