In Vitro Organogenesis of Soybean and Agronomic Characterization of Obtained Plants

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ABSTRACT

Organogenesis of soybean through shoot bud induction was done using cotyledonary nodes of soybean cultivars Sicinang and Sriyono as sources of explants. A Murashige-Skoog (MS) medium supplemented with thidiazuron increasing from 0.005 to 0.05 mg/l plus NAA or 2,4-D 0.01 mg/l was used in the study. The shoots obtained were subcultured and the original cotyledonary nodes were cut off after subculturing the shoots. Growths and productions of the F_1 and F_2 *in vitro* derived plants were recorded. The results indicated that increasing concentration of thidiazuron from 0.005 to 0.05 mg/l and the presence of either NAA or 2,4-D 0.01 mg/l in the medium were beneficial to shoot regeneration. During the induction period, the average number of shoots obtained were 5.8 from cultivar Sicinang and 5.2 from cultivar Sriyono. The shoots were then cut off and subcultured into a fresh medium containing 0.01 mg/l thidiazuron. In the first subcultures, the numbers of shoots ranged from 2.8-6.2 shoots per explants, while the cotyledonary node produced 21-25 shoots. After the third subcultures, the cotyledons dried out and no further buds were formed. Plants derived from *in vitro* cultures of both subcultures I and II were shorter than the original seed derived plants. The seed derived plants were also mature earlier and more tolerant to shading of up to 70% shading. The plants obtained from *in vitro* subculture seem to be more suitable to be grown intercropped with other plants.

Key words: *In vitro*, organogenesis, *Glycine max*

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is an important crop for food and industrial raw material. Since 1996, the government of Indonesia has been importing soybean from other countries, due to various constraints to soybean production in the country. An alternative way to fulfill the demand of soybean is to expand the soybean growing area. However, there were constraints to expanding the soybean growing area. One of the constraints is soybean plants grow poorly in yellow-red podzolic type of soil, the dominant type of soil in Indonesia. This type of soil is highly acidic and has high contents of Aluminum (Al) and Manganese (Mn) that are toxic to the plant. This soil condition is found mostly in Sumatera, Kalimantan, Sulawesi, Maluku, and Papua islands prospected for expansion of soybean production.

Recently, researchers have started with soybean-breeding program to overcome the soil acidity problem through conventional and non-conventional approaches. One of the non-conventional techniques is *in vitro* selection, although the results have not been satisfying. Therefore, efforts need to be done to improve the technique by induction of adventive and axillary shoots from Indonesian soybean varieties with juvenile explants.

The technique includes the use of some plant growth regulators (PGR) to induce shoots initiation from juvenile explants. The plant growth regulators commonly used for this purpose are BAP and thidiazuron. The PGR are a group of cytokinins that have strong effect on induction and increase of cell division (Davies, 1995). Indication of the successful of this research is the formation of plantlets in large quantities, so that they can be used in selection.

MATERIALS AND METHODS

In Vitro Experiment

Soybean seeds of cvs. Sriyono and Sicinang, obtained from previous research (Sopandie *et al.*, 1996), were surface sterilized with detergent and washed with flowing tap-water for 15 minutes. The seeds were then soaked in sterile distilled water for 12 hours followed with surface sterilization by soaking in 1% sodium hypochlorite (NaClO) solution and 0.1% mercuric chloride ($HgCl_2$) solution in a laminar airflow. The sterilized seeds were planted in a half strength of MS medium supplemented with 0.005-0.05 mg/l thidiazuron and 0.01 mg/l NAA or 0.01 mg/l 2,4-D. Fourteen days after germination, roots of the seedlings were removed and the remaining upper plant parts were subcultured in a germination medium (subculture I). Three weeks after subculturing, when several new shoots have grown on the cotyledonary node, the original explants were cut off and transferred into a fresh medium to promote further multiplication. The regenerated shoots were then excised and also transferred to fresh media for subculturing (subculture II). Subculture III preparation was done similar to that of subculture II. The medium used for Subculture I, II, and III was a half strength MS medium supplemented with 0.05 mg/l thidiazuron and 0.01 mg/l NAA. To induce root formation, shoots from subcultured I, II, and III were transplanted to a full-strength MS medium with no addition of PGR. Finally, all the plantlets were acclimatized in the glasshouse.

Glasshouse Experiment

Soybean plantlets obtained from the previous trial were acclimatized on a coconut fiber medium under 70% shading. The plants were divided into groups according to date of acclimatization. Acclimatized plants (F_1) of each group of were harvested separately. All the F_1 seeds were replanted in the following season to obtain F_2 seeds. Parameters observed were plant height, number of nodes, internode length, date of anthesis, time to harvest, number of pod per plant, percentage of empty pods, total pod weight per plant, and plant dry weight were recorded from every generations.

RESULTS AND DISCUSSION

Effect of Thidiazuron Concentration and Auxine Type on Shoot Multiplication

Addition of thidiazuron and NAA as supplements into the half strength MS medium induced shoot bud formation on cotyledonary node explants of soybean cvs. Sriyono and Sicinang. All explants cultured developed shoot buds, although not all of them survived and produced shoots. The highest survive shoot-bud percentages produced by cvs. Sriyono and Sicinang on the medium supplemented with 0.05 mg/l thidiazuron and 0.01 mg/l 2,4-D were 82.6 and 76.9%, respectively. The lowest percentage of shoot-bud survival were found on the medium containing 0.005 mg/l thidiazuron and 0.01 mg/l 2,4-D for both cultivars (Table 1). Addition of 0.05 mg/l thidiazuron combined with either NAA or 2,4-D, increased survival of shoot bud percentages.

Thidiazuron at 0.05 mg/l concentration in the culture medium induced the highest average of shoot-bud multiplication (Figure 1) on cv. Sriyono (5.85 shoots per explants) and Sicinang (5.2 shoots per explants). The average shoot-bud multiplication of both cultivars on the culture medium containing 0.05 mg/l thidiazuron was significantly different from that in the medium containing 0.01 mg/l thidiazuron. There was no interaction between thidiazuron concentration and type of auxine uséd in the medium.

Results of this experiment indicated that the best combination of PGR concentration as a supplement for soybean culture media was 0.05 mg/l thidiazuron + 0.01 mg/l NAA. This combination induced production of the highest surviving shoots and average number of shoots per explants. In this experiment, cv. Sriyono performed better than cv. Sicinang. Based on the results, the combination of thidiazuron 0.05 mg/l and NAA 0.01 mg/l were used as supplements for subculture II and III media. There was a reduction in the average number of shoots per explants when the regenerated shoots were subcultured. The highest average number of shoots per explants was 6.2 with 75.7% survive shoots per explants that was regenerated from subculture I of cv. Sriyono. The lowest number of shoots per explants in this cultivar was 2.7 with 78.4% shoots survived from regeneration of subculture III. On the other hand, on cv. Sicinang, the

Table 1. Shoot-bud generations of cotyledonary nodes of soybean cultivars Sriyono and Sicinang cultured in MS media supplemented with combinations of plant growth regulators (PGR)

Combination of PGR concentration (mg/l)		Sriyono		Sicinang	
		Shoot-bud survival (%)	Number of bud per explants	Shoot-bud survival (%)	Number of bud per explants
NAA	Thidiazuron				
0.01	0.005	66.70	2.1	51.70	2.3
0.01	0.01	62.42	3.0	67.10	3.0
0.01	0.05	78.28	6.2	75.70	5.8
2,4-D	TDZ				
0.01	0.005	54.50	3.1	48.80	3.0
0.01	0.01	73.90	4.6	66.80	3.3
0.01	0.05	82.56	5.5	76.90	4.6

highest average number of shoot per explants was 5.8 with 68.3% survive shoots per explants that was regenerated from the first subculture. The lowest number of shoots per explants in this cultivar was 1.5 with 90.0% survive shoots from regeneration of subculture III. On both cultivars, results of regeneration from subculture II were not different from subculture III (Table 2).

Growth and Yield of Post Acclimatization of Two Subculture Regenerates

Plantlets of cvs. Sriyono and Sicinang obtained from subculture I (R1), subculture II (R2), and subculture III (R3) were acclimatized in the glasshouse. Plants grown from all R1 and R2 plantlets of cvs. Sriyono and Sicinang grew well and produced good pods, but those from the R3 plantlets were not survived. Morphologically, the acclimatized R1 and R2 plants of cultivar Sriyono were different from the control (seeded plants). Plant heights of the R1 and R2 plants from either group I or group II, were surprisingly far shorter than those of the seeded plants. The height of the R plants were only about one fifth of the control plants under 70% shading. Due to low number of nodes, the internode lengths of the R plants were also shorter than that of the control plants. The R plants reached generative stage of plant growth earlier than the control plants. Anthesis days of the R1 plants were about a week earlier than that of the controls. On the other hand, days to anthesis on the R2 plants and time to harvest on both R1 and R2 plants were also

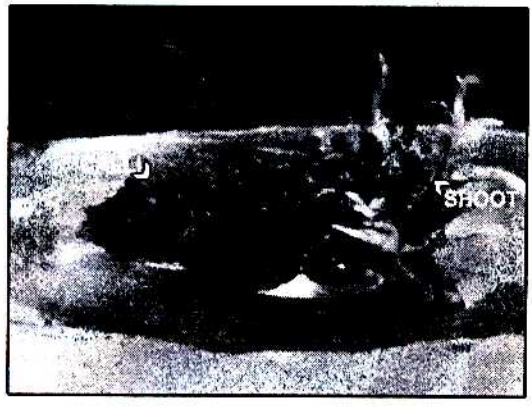


Figure 1. Shoot multiplication of soybean cultivar Sriyono regenerated in subculture II

Table 2. Shoot multiplication on soybean cultivars Sriyono and Sicinang during subculture

Regeneration	Sriyono		Sicinang	
	Survive shoots (%)	No. of shoots per explants	Survive shoots (%)	No. of shoots per explants
Regeneration I	75.7	6.2	68.3	5.8
Regeneration II	76.3	2.8	73.4	2.7
Regeneration III	78.4	2.7	90.0	1.5

earlier than the controls. Yields of the plants that were represented by number of pods, pod weight, or seed weight, of the R plants were higher than those of the seeded plants under 70% shading. All variables observed, except for days to anthesis of the R1 plants were not different from that of the R2 plants (Table 3). On both cultivars, the average plant heights of the R plants were much shorter than those of the seeded plants. Morphologically, the R plants were more compacts than that of the seeded plants under 70% shading. The number of nodes of R1 Group I plants was not different from that of the control, although the other R plants had less number of nodes. As a result of the difference on plant height and number of nodes, internode length of R plants were also shorter than that of the seeded plant under 70% shading. With respect to days to anthesis and time to harvest, the R plants were earlier than seeded plants. Yield of R2 group I plant, in term of number of pod, pod weight, and seed weight, were less than that of the control. Other R plants, however, yielded higher than that of the control (Table 4).

The growth of R plants from cvs. Sriyono and Sicinang were not etiolated under shaded conditions. Until 70% plant shading, leaf colors of both cultivars were still dark green and the plants produced no empty pods.

Table 3. Morphological characteristics and yields of post acclimatized two-subculture regenerate plants of cultivar Sriyono in the glasshouse

Variables	Seeded plants		R1		R2	
	Group I	Group II	Group I	Group II	Group I	Group II
Plant height (cm)	100.8±7.61	100.6±9.57	21.7±9.47	16.5±7.00	15.0±4.94	16.67±3.21
Number of nodes	8.2±1.50	9.5±1.29	7.6±1.67	5.25±0.98	6.0±1.41	6.0±1.00
internode length (cm)	12.29±1.83	11.28±0.91	2.85±0.94	3.1±1.04	2.62±0.17	2.62±0.34
Days to anthesis	37.5±1.15	38±1.82	31.16±2.77	31±2.21	22.5±3.53	23±2.00
Time to harvest (days)	99±8.63	97.5±6.02	83.66±4.08	79.5±2.64	81.5±2.12	82.3±2.08
Number of pods	9.5±7.88	5.5±1.29	12.6±3.53	18±4.83	10±5.65	9.66±1.52
Empty pods (%)	0	0	0	0	0	0
Pod weight (g)	2.48±1.83	1.37±0.53	3.8±2.60	4.78±2.13	4.63±3.43	4.34±1.43
Seed weight (g)	0.95±1.30	0.84±0.36	1.66±0.83	1.87±0.34	1.51±1.19	1.43±0.51

Table 4. Morphological characteristics and yields of post acclimatized two-subculture regenerate plants of cultivar Sicinang in the glasshouse

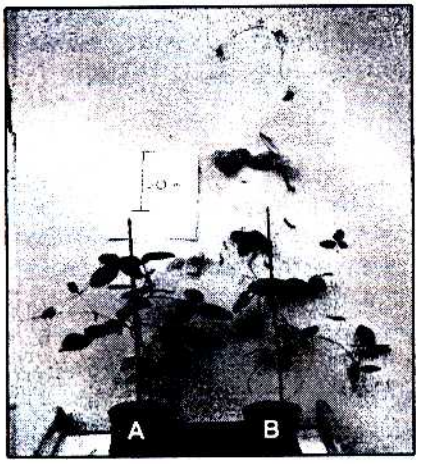
Variables	Seeded plants		R1		R2	
	Group I	Group II	Group I	Group II	Group I	Group II
Plant height (cm)	105.8±9.55	103.8±7.72	50.5±3.43	35.0±9.29	13	34.1±1.78
Number of nodes	8.4±1.81	10.2±1.30	8.0±1.41	5.8±1.30	4	6.67±0.57
internode length (cm)	12.6±2.25	10.23±0.62	6.3±0.57	5.5±2.10	3.2	5.25±1.64
Days to anthesis	37.5±1.14	38.4±2.07	30.0±1.41	31.0±2.34	21	25.0±2.64
Time to harvest (days)	100.5±7.63	95.8±5.40	81.5±0.70	80.4±2.48	82	81.0±2.00
Number of pods	10.0±1.58	5.0±1.58	19.51±0.6	16.0±5.24	4	9.66±3.00
Empty pods (%)	0	0	0	0	0	0
Pod weight (g)	2.81±1.34	1.29±0.43	5.49±4.42	4.43±1.98	1.44	4.34±1.49
Seed weight (g)	0.78±0.54	0.84±0.32	1.62±0.69	1.53±0.42	0.33	1.06±0.49

Morphological changes in the R plants, as indicated by short plant heights, short internode length, and early days to anthesis and early harvest age were probably due to the genetic or epigenetic changes. It is known that tissue culture may cause genetic variability through somaclonal variability (Karp, 1995). In this experiment, 2,4-D was probably the hormone responsible for the genetic changes (mutation). It was proven that this hormone could induce somaclonal variation under *in vitro* culture. This hormone affected certain types of cells (Ghosh and Gadgil, 1979), cell division (Gould, 1984), and intensity of unorganized cell growth (Karp, 1992).

The primary factors that cause variability in regeneration of tissue culture are the extent of meristem development, source of explants, plant genotype, and composition of the medium (Karp, 1995). Genetic changes can be as a result of caryotypic changes, nuclear mutation or cytoplasmic genes, translocation, deletion, inversion, chromosome changes, and non-conventional mutation (Pesche and Phillips, 1992).

Growth and Yield of the First Generation (F_1) of Post Acclimatized R1 and R2 Plants

In the previous experiment, the seeds from both the R and seeded plants were harvested and replanted as the first generation (F_1) plants in the glasshouse under 70% plant shading. The result indicated that both cultivars showed similar response to the glasshouse condition. The F_1 of the R1 plants from cvs. Sriyono and Sicinang were morphologically shorter (Figure 2), reached generative stage earlier, and yielded higher than the seeded plants. On the other hand, the F_1 of the R2 plants were not significantly different morphologically from the seeded plants, although the F_1 plants reached generative stage earlier and yielded higher than the control (Table 5)



Notes: A = first generation (F1 of R1), B = seeded plant (control)

Figure 2. Vegetative growth of cultivar Sicinang under 70% shading

Table 5. Morphological characters and yields of the first generation (F_1) of post acclimatized R1 and R2 plants of soybean cultivars Sriyono and Sicinang in the glasshouse

Variables	Sriyono			Sicinang		
	Seeded plants	R1	R2	Seeded plants	R1	R2
Plant height (cm)	160.5±4.25	91.0±7.61	131.8±8.42	148.3±1.50	88.0±5.48	167.8±0.09
Number of nodes	11.25±2.06	9.5±1.50	11.75±0.50	12.25±0.72	9.25±2.22	10.75±1.50
Internode length (cm)	11.60±0.40	9.53±1.83	11.22±0.72	12.11±1.73	6.51±0.83	12.97±3.60
Anthesis (days)	37.5±1.29	34.0±1.15	33.5±1.00	38.5±4.50	33.5±0.57	34.0±0.57
Time to harvest (days)	100.5±4.03	80.8±8.65	88.0±1.54	114.3±2.21	86.8±7.68	85.5±5.19
Number of pods	5.75±0.95	11.25±7.88	17.75±6.70	5.25±0.29	15.7±5.18	9.75±4.19
Empty pods (%)	0	0	7.25	6.25	11.7	10.5
Pod weight (g)	1.77±0.47	2.70±1.82	3.7±0.85	0.89±0.29	3.56±1.16	2.08±1.02
Seed weight (g)	1.11±0.26	1.77±1.30	2.47±0.65	0.55±0.21	2.16±0.6	1.04±0.36

Characters of the First Generation (F_1) from R1 and Seeded Plants

The results indicated that the F_1 plants from R1 generation from either cultivar Sriyono or Sicinang were tolerant to shading, because they were able to grow well under shading condition up to 70% with light intensity 102-119 cal/cm^2/day. Under such condition, plant height, time to harvest, and pod numbers of Sriyono were 91 cm, 80.75 days, and 11.25 pods, while of Sicinang were 88 cm, 86.75 days, and 15.70 pods, respectively. According to Asadi *et al.* (1997), plant height, time to harvest, number of pods per plant, and seed yield of the shaded tolerant genotypes were usually ranged from 63-87 cm, 81-85 days, 9-30 pods, and 0.93-1.17 t/ha, respectively, under shading condition. Based on criteria mentioned, the F_1 of R1 plants from soybean cultivars Sriyono and Sicinang were classified as tolerant to shading.

The presence of morphological changes in the first generation (F_1) indicated that there had been genetic changes on the *in vitro* regenerated plants. The F_1 of R1 plants were more tolerant to shading than their seeded parents.

CONCLUSION

Thidiazuron had beneficial effect to the *in vitro* shoot multiplication. The best medium to induce intensive shoot multiplication is a half strength MS medium supplemented with a combination of 0.05 mg/l thidiazuron and 0.01 mg/l NAA. The cotyledonary node is an organ that has a morphogenetic ability and able to produce new plants differently from their origin. The extent of shoot multiplication in subculture decreased shoot survivals of both cultivars Sriyono and Sicinang. The R1 and R2 plants of both cultivars had morphological characters and yields different from the seeded plants. These plants were also tolerant to shading of up to 70% with light intensity 102-119 cal/cm^2/day. The tolerance to shading in the R1 plants of both cultivars was inherited by the F_1 generation.

REFERENCES

Asadi, D.M. Arsyad, H. Zahara, dan Darmijati. 1997. Pemuliaan kedelai untuk toleran naungan dan tumpangsari. Buletin AgroBio 1(2):15-20.

Davies, P.J. 1995. The plant hormone: Their nature, occurrence, and function. *In* Davies, P.J. (*Ed.*). Plant Hormone: Physiology, Biochemistry, and Molecular Biology. 2nd edition. Kluwer Academic Publ., Netherlands. p. 1-5.

Ghosh, A. and V.N. Gadgil. 1979. Shift ploidy level of callus tissue: A function of growth substances. India J. Biol. 17:562-564.

Gould, A.R. 1984. Control of the cell cycle on cultured plants. Critical Rev. Plant Sci. 1:315-344.

Karp, A. 1992. The role of growth in somaclonal variation. British Soc. Plant Growth Regulation Bull. 2:1-9.

Karp, A. 1995. Somaclonal variation as a tool for crop improvement. Euphytica 85:295-302.

Pesche, V.M. and R.L. Phillips. 1992. Genetic variation of somaclonal variation on plants. Advances in Genetics 30:41-75.

Sopandie, D., M. Jusuf, Supijatno, dan Hamim. 1996. Fisiologi genetik adaptasi kedelai terhadap cekaman kekeringan dan pH rendah dengan Al tinggi. Laporan Akhir RUT I. 107 hlm.