Jour. Korean For. Soc. Vol. 96, No. 2, pp. 178~182 (2007)

JOURNAL OF KOREAN FOREST SOCIETY

Effect of Explant Types, Auxin Concentration and Light Condition on *In Vitro* Root Production and Alkaloid Content of *Rauvolfia serpentina* (L.) Benth. ex Kurz

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Abstract : *Rauvolfia serpentina* (L.) Benth. ex Kurz is a medicinal plant and an endangered tropical rainforest plant species. Since the field cultivation that aims to fulfill the industrial needs is never accomplished, tissue culture appears to be the most feasible way to improve the quality and quantity of *R. serpentina*. This experiment used two kinds of explants (roots and shoots) to induce optimal root formation in different combinations of auxin and photoperiod. Each explants exhibited different responses on given treatments. Differentiated root could be produced from explants cultured in IBA 20 mg/L with and without light. The highest number of roots, root length and root weight induced from shoot explants were effective on MS medium containing IBA 20 mg/L and incubated under dark condition, while highest total weight (callus and root) from root explants cultured on MS medium supplemented 10 mg/L IBA and 10 mg/L NAA and incubated under day length (11/13 hr). The root induced from shoot explants produced the highest major alkaloid content. The highest content of ajmaline (2.17 ppm fresh weight) and reserpine (1.30 ppm fresh weight) were observed in shoot explants cultured in MS medium containing combination of IBA 10 mg/L and NAA 10 mg/L and incubated under dark condition, yohimbine (1.47 ppm fresh weight) was in the shoot explants cultured in MS medium containing NAA 20 mg/L and incubated under day length, while serpentine was absent.

Key words **:** *ajmaline, alkaloids, auxin, callus,* Rauvolfia serpentina, *reserpine, yohimbine*

Introduction

Rauvolfia serpentina (L.) Benth. ex Kurz is an endangered tropical rainforest plant species which has been used for medicinal purpose. The root extract has been used as remedy for high blood pressure. Major derivatives of this species have potential to cure diseases such as serpentine could inhibits selectively cancer cell DNA synthesis *in vitro* (Beljanski and Beljanski, 1982; Beljanski and Beljanski, 1984; Beljanski and Beljanski, 1986), reserpine as one of the alternative treatments in therapy-refractory schizophrenia (Lehmann and Ban, 1997) and yohimbine as aphrodisiac that could promote testosterol production in man's body. It was mentioned that among medicinal plants used as aphrodisiac only *R. serpentine* has bi-functions, as anti hypertensive agent and aphrodisiac (Sardianto and Supriyati, 1998).

The major threat to the survival of *R. serpentina* in Indonesia is the over harvesting of its wild populations for roots by the traditional medicine ('Jamu') industry. It has been reported that during from 1984 to 1990, 11,057.34 kg of *R. serpentina* roots were harvested for the Jamu industry. On average, this represents 1,579.62 kg/year and 131.64 kg/month (Hendrian, 1996). Furthermore, in year 2000, the demand was predicted to be approximately 6,898 kg with increment trend of 25.89% per year (Processed Data of BALITTRO, 1990 cited in Sandra and Kemala, 1994). It is widely believed that the demand for *R. serpentina* is increasing and will continue to do so. However, field cultivation that aims to fulfill the industrial needs is never accomplished.

Some alternative cultivation techniques, both by the conventional way as well as by technology inputs such as aeroponic and tissue culture have been tried. The most feasible technique to improve the quantity and

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quality of *R. serpentina* appears to be tissue culture. In this study, the effects of explant types, auxins and light conditions on the root production and alkaloid contents of *R. serpentine* when tissue-cultured and the best treatment that produced the highest yield and best compound of major alkaloid in *R. serpentina* root were determined.

Materials and Methods

1. Plant material and tissue culture

Aseptic shoots were multiplied in MS medium containing cytokinin (BAP 0.5 mg/L) at 24°C±2°C under lamps (16 hours photoperiod) for 2-3 months to obtain enough aseptic shoots. Multiplied shoots were used as explants for growing transformed roots. Roots, which were produced from 7 month cultures, were also used as explants.

The treatments were 2 levels of explant type (shoot; root), 4 levels of auxin (control; IBA 20 mg/L; NAA 20 mg/L; IBA 10 mg/L and NAA 10 mg/L), and 2 levels of photoperiod (day/night: 11/13; 0/24 hours). Non-infected explants were cultured in MS medium containing 0.4% agar, 3% sucrose and various hormone levels. The explants were incubated for 3 months in the given condition. Parameter measurements were done at the end of the culture period.

2. Alkaloid analysis

Fresh roots were analyzed for its chemical composition using HPLC (High Performance Liquid Chromatography) technique. Samples preparation was done as described by Edwards and Strange (1991).

Samples were evaluated by analytical (0.39 cm × 30 cm) reverse phase high pressure liquid chromatography (HPLC) on m-Bondapack C18 (5 mm) columns (Waters Corp.). The analytical reverse phase column was equilibrated with buffer A (0.1% trifluoroacetic acid (TFA, in water) and the samples eluted with a linear gradient of buffer B (0.025% TFA in 66% acetonitrile). The column was eluted at a flow rate of 1.0 mL/min. and the absorbance measured at 214 nm.

3. Experimental design and statistical analysis

The experimental design is a factorial design with 4 levels of auxin (control; IBA 20 mg/L; NAA 20 mg/L; IBA 10 mg/L and NAA 10 mg/L) and 2 levels of photoperiod (day/night: 11/13; 0/24 hours). Each combination of treatments for the experiment was used with 10 replicates.

Statistical analysis was determined by one-way analysis of variance (ANOVA) using SAS for Windows Version 6.12 (SAS Institute Inc., 2001).

Results and Discussion

1. Explant growth responses

1) Root

Figure 1 shows different responses of roots on agar-

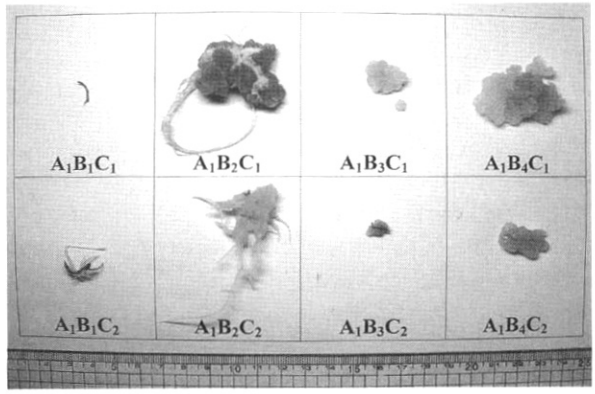

Figure 1. Explants growth after 3 months from in vitro root segments in agar-solidified MS medium containing different auxin concentration and incubated under different light conditions. (A1: root explants; B1: control; B2: IBA 20 mg/L; B3: NAA 20 mg/L; B4: IBA 10 mg/L and NAA 10 mg/L, C1: 11/13 hr day/night and C2: 0/24 hr day/night).

Table 1. Number of root, root length, total weight (callus and root) and root weight from root segment grown in agar-solidified MS medium containing different auxin concentration and incubated under different light conditions.

Treatment	Number of Root	Root Length (cm)	Total Weight (g)	Root Weight (g)
A1B1C1	0.3 ± 0.2 b	0.07 ± 0.05 c	0.005 ± 0.003 c	0.001 ± 0.001 b
A1B2C1	4.3 ± 1.7a	**2.37 ± 0.73 a**	**5.365 ± 1.158 a**	0.164 ± 0.068 a
A1B3C1	0.0 ± 0.0	0.00 ± 0.00 c	0.240 ± 0.116 bc	0.000 ± 0.000 b
A1B4C1	0.0 ± 0.0	0.00 ± 0.00 c	1.123 ± 0.489 bc	0.000 ± 0.000 b
A1B1C2	0.9 ± 0.9	0.10 ± 0.10 c	0.006 ± 0.004 c	0.006 ± 0.004 b
A1B2C2	**5.9 ± 2.4a**	1.04 ± 0.48 b	1.608 ± 0.421 b	**0.228 ± 0.105 a**
A1B3C2	0.0 ± 0.0 b	0.00 ± 0.00 c	0.019 ± 0.010 c	0.000 ± 0.000 b
A1B4C2	0.0 ± 0.0 b	0.00 ± 0.00 c	0.113 ± 0.081 c	0.000 ± 0.000 b

Note: Each value represents mean ± S.E. of 10 replications. Significant differences among different auxins and light conditions at P 0.05 are indicated by a, b and c. (A1: root explants; B1: control; B2: IBA 20 mg/L; B3: NAA 20 mg/L; B4: IBA 10 mg/L and NAA 10 mg/L, C1: 11/13 hr day/night and C2: 0/24 hr day/night).

solidified MS medium containing different auxins and incubated under different light conditions. Callus was produced from all treatments. Callus, which was developed in the early root promotion, showed green color in the light, but it etiolated (white/yellowish) in the dark. Highest average callus weight (5.201 g) was observed when roots cultured on the medium containing IBA 20 mg/L under light condition.

More roots were produced when the roots were cultured on the medium containing IBA 20 mg/L with and without light (A1B2C1 and A1B2C2). Light treatment gave a contradictive effect on the number of root and the root length. Dark-grown root produced more number of roots, while light-grown root showed higher average length of roots. Hamaki (1971) *in* Wattimena *et al.* (1992) mentioned that the best root induction was observed in dark culture. However, root elongation is promoted in the light (Salisbury and Ross, 1991).

Of the treatment cultures, the greatest response in root induction was observed in the combination of IBA 20 mg/L with and without light. Significantly higher total weight (root and callus) (5.365±1.158 g) and root length (2.37±0.73 cm) were observed in the combination of IBA 20 mg/L with light (Table 1).

2) Shoot

Similar to the previous part, which used root as explants, calluses were still produced from all treatments using shoot explants. Different responses of shoot explants on agar-solidified MS medium containing different auxins and incubated under different light conditions are shown in Figure 2.

The greatest response in using shoot for root induction was observed in the combination of IBA 20 mg/L with light condition which produced more root number (11.0 ±3.0), root length (2.60±0.76 cm) and root weight (0.664 ±0.208 g). Highest total weight (4.326±0.846 g) was produced from the explants cultured in MS medium containing IBA 10 mg/L and NAA 10 mg/L under light condition (Table 2). However, this treatment promoted only callus formation.

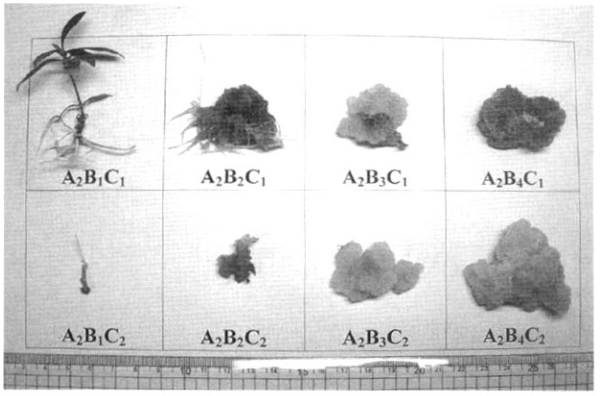

Figure 2. Explants growth after 3 months from *in vitro* shoot segments in agar-solidified MS medium containing different auxin concentration and incubated under different light conditions. (A2: shoot explants; B1: control; B2: IBA 20 mg/L; B3: NAA 20 mg/L; B4: IBA 10 mg/L and NAA 10 mg/L; C1: 11/13 hr day/night and C2: 0/24 hr day/night).

Among the three factors (explant type, auxin and photoperiod), most of the factors and their interactions gave significant differences ($\alpha = 0.05$) in root number, length and weight. However, as a single factor, light condition did not give any significant difference to root number and explant type to all root parameters.

Explants did not exhibit good and uniform responses in producing root compared to the other research which used shoot explants cultured in MS medium containing auxin ten times lower than was used in this experiment. Most explants produced slight to good amount of callus, but failed to differentiate into roots, especially when both explants were cultured in MS medium containing NAA 20 mg/L or NAA 10 mg/L combine with IBA 10 mg/L. Though the differentiation is almost always a result of the manipulation of the levels of auxin and

Table 2. Number of root, root length, total weight (callus and root) and root weight from shoot segment grown in agar-solidified MS medium containing different auxin concentration and incubated under different light conditions.

Treatment	Number of Root	Root Length (cm)	Total Weight (g)	Root Weight (g)
A2B1C1	1.2 ± 0.9 b	0.84 ± 0.46 c	0.022 ± 0.015 c	0.022 ± 0.015 b
A2B2C1	**11.0 ± 3.0a**	**2.60 ± 0.76a**	2.301 ± 0.561 b	**0.664 ± 0.208a**
A2B3C1	0.0 ± 0.0 b	0.00 ± 0.00 c	1.251 ± 0.391 bc	0.000 ± 0.000 b
A2B4C1	0.0 ± 0.0 b	0.00 ± 0.00 c	**4.326 ± 0.846a**	0.000 ± 0.000 b
A2B1C2	0.0 ± 0.0 b	0.00 ± 0.00 c	0.000 ± 0.000 c	0.000 ± 0.000 b
A2B2C2	0.4 ± 0.3 b	0.25 ± 0.17 b	0.277 ± 0.067 c	0.000 ± 0.000 b
A2B3C2	0.0 ± 0.0 b	0.00 ± 0.00 c	1.232 ± 0.504 bc	0.000 ± 0.000 b
A2B4C2	0.0 ± 0.0 b	0.00 ± 0.00 c	1.470 ± 1.089 bc	0.000 ± 0.000 b

Note: Each value represents mean ± S.E. of 10 replications. Significant differences among different auxins and light conditions at P 0.05 are indicated by a, b and c. (A2: shoot explants; B1: control; B2: IBA 20 mg/L; B3: NAA 20 mg/L; B4: IBA 10 mg/L and NAA 10 mg/L, C1: 11/13 hr day/night and C2: 0/24 hr day/night).

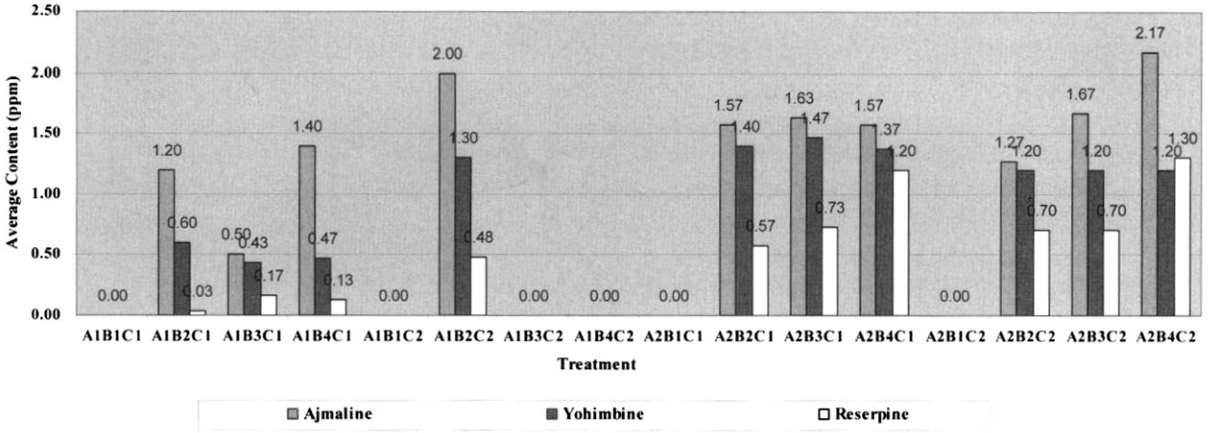

Figure 3. Alkaloid content of root and shoot segment grown in agar-solidified MS medium containing different auxin concentration and incubated under different light conditions. (A1: root explants; A2: shoot explants; B1: control; B2: IBA 20 mg/L; B3: NAA 20 mg/L; B4: IBA 10 mg/L and NAA 10 mg/L; C1: 11/13 hr day/night and C2: 0/24 hr day/night).

cytokinine in the growth medium, it is not always possible to induce differentiation in a callus tissue, either because the correct balance of hormonal and nutritional factors has not been found or because the cells fail to respond to such conditions (Wright and Northcore, 1972). Therefore, the explants were failed to respond either the high concentration or combination of auxin.

The choice of explant is one of the most important factors in obtaining morphogenesis since morphogenetic potential often varies with the original of the explant (Barker, 1969; Mehra and Mehra, 1974). Shoot appeared to be a suitable explant for promoting root in this experiment that gave the higher parameter values such as number of roots (11.0±3.0), root weight (0.664±0.208 g) and root length (2.60±0.76 cm). Also shoots were found to be relatively easier to multiply compared to root.

2. Alkaloid content

In vitro production of secondary metabolite is often associated with the organization and differentiation of plant cells or tissues to a specific organ (Wattimena *et al.*, 1992; Ramachandra Rao and Ravishankar, 2002). The facts that roots contain more alkaloids than other parts have been reported from other researches on *in vivo* and *in vitro* condition of this plant (Roja, *et al.*, 1987; Benjamin *et al.*, 1993). Therefore, root production is still the main objective in applying tissue culture for *R. serpentine*, though the callus in this experiment contained high concentration of major alkaloids.

Roots that were promoted from both root and shoot tips as explant showed the presence of ajmaline, yohimbine and reserpine, but absence of serpentine. In the roots induced from the roots used as explants, the highest content of ajmaline (2.00 ppm or 2.00 mg/g fresh weight), yohimbine (1.30 ppm or 1.30 mg/g fresh weight) and reserpine (0.50 ppm or 0.50 mg/g fresh weight) were observed from the treatment with root explant cultured in MS medium containing IBA 20 mg/L and incubated under dark condition (Figure 3).

On the contrary, in the roots induced from the shoots used as explants, the highest content of each alkaloid was observed in different treatments. The highest content of ajmaline (2.17 ppm or 2.17 mg/g fresh weight) and reserpine (1.30 ppm or 1.30 mg/g fresh weight) were observed from the shoot cultured in MS medium containing combination of IBA 10 mg/L and NAA 10 mg/L and incubated under dark condition. Meanwhile, the highest content of yohimbine (1.47 ppm or 1.47 mg/g fresh weight) was in shoot cultured in MS medium containing NAA 20 mg/L and incubated under light condition.

Previous research (Yahya, 2001) also showed different alkaloid contents depending on the treatments. Highest ajmaline content (0.016 ppm or 0.016 mg/g fresh weight) and reserpine content (14.90 ppm or 14.90 mg/g fresh weight) were observed in the shoot cultured in MS medium containing IBA 2 mg/L and incubated under light condition (14hr day/10hr night), while highest yohimbine content (0.015 ppm or 0.015 mg/g fresh weight) in the shoot cultured in MS medium containing NAA 2 mg/L and incubated under dark condition.

Conclusion

Explants that were used to induce optimal root formation in different combination auxin and light conditions did not give uniform response in producing roots. Callus produced slight to good amount, but failed to differentiate into roots, especially when both explants were cultured in MS medium containing NAA 20 mg/L or NAA 10 mg/L combined with IBA 10 mg/L. Differentiated roots were only induced from explants cultured in IBA

20 mg/L with and without light.

In the roots induced from the root explants, the highest content of ajmaline, yohimbine and reserpine were obtained when it was cultured in MS medium containing IBA 20 mg/L and incubated under dark condition. This treatment also produced high number of roots and root weight.

In the roots induced from the shoot explants, the highest content of each alkaloid was obtained from different treatments. The highest content of ajmaline and reserpine were from the shoot cultured in MS medium supplemented 10 mg/L IBA and 10 mg/L NAA and incubated under dark condition, while the highest yohimbine content was from the shoot cultured in MS medium containing NAA 20 mg/L and incubated under light condition.

There were no specific combinations of explant types, auxins and light conditions that could produce not only high number of roots and root weight but high alkaloid content as well.

Acknowledgement

This research was supported by the ASEAN-Korea Environmental Cooperation Project (AKECOP).

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(Received October 25, 2006; Accepted March 7, 2007)