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Effects of auxins and cytokinins on formation of *Catharanthus roseus* G. Don multiple shoots

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Abstract

The effects of different combinations of plant growth regulators and light intensity on the formation of multiple shoots of *Catharanthus roseus* (L.) were studied. By composing three dimension surfaces and their topo views from experimental data, it was clear that Murashige-Shoog (MS) medium supplemented with 7.0 mg l^{-1} BA and 1.0 mg l^{-1} NAA strongly stimulated the formation of shoots, whereas medium supplemented with 2, 4–D suppressed the formation of shoots or caused shoot dedifferentiated. Light intensities of 550–700 Lux were found to be beneficial to the formation of shoots when MS medium was supplemented with 2 mg l^{-1} 6–BA and 0–1.0mg l^{-1}NAA.

Abbreviations: BA-6 – benzyladenine, NAA – α-naphthalenacetic acid, 2,4–D – 2,4–dichlorophenoxyacetic acid

Introduction

Catharanthus roseus (*C. roseus*) contains various indole alkaloids which are used as anticancer (vinblastine and vincristine) and hypotensive (serpentine and ajmalicine) agents. However, as the dimeric anticancer alkaloids vindoline and vincristine are almost undetectable in cultured cells, attention has been turned to the production of catharanthine and vindoline, which can be used as precursors for the synthesis of vinblastine and vincristine. Unfortunately, vindoline, a major plant alkaloid of *C. roseus*, has not been stable produced in cell culture (Kutney et al. 1980; Stockigt & Soll 1980). It has, however, been reported (Constabel et al. 1982; Krueger et al. 1982) that vindoline can be detected in shoots and leaf organ cultures of *C. roseus*. Subsequent work by Hirata et al. (1987) found that multiple shoot cultures induced from *C. roseus* seedlings produced vindoline and caratharanthine in rather higher levels with leaf tissue containing the highest level of vindoline. Therefore, the medium should be studied to optimize bud formation and subsequent leaf development in order to product high levels of vindoline.

In this paper we examine the effects of auxins, cytokinins and light intensity on shoot formation in *C. roseus*.

Materials and methods

Shoot culture induction

Explants of *C. roseus* (L.) G. Don were taken in spring from the established plant which was outdoor grown in Tianjin Plant Garden. The shoot segments (ca. 1cm) were surface sterilized by brief immersion in 79% ethanol, then in 1% sodium hypochlorite (under reduced pressure) fro 20 min. They were then rinsed three times with sterile water and transplanted onto 50 ml of MS medium solidified with 0.6% agar and supplemented with 0.1mg l^{-1} NAA; 2mg l^{-1} BA; 1mg l^{-1} casein hydroylaste (CH); 30g l^{-1} sucrose; at an initial pH of 5.8 in 150 ml Erlenmeyer flasks. They were cultured at 26±0.5°C under 700 Lux of continuous fluorescent illumination. The frequency of establishment was about 90%. Shoot cultures thus initiated (MSL–90) were maintained on the above medium and

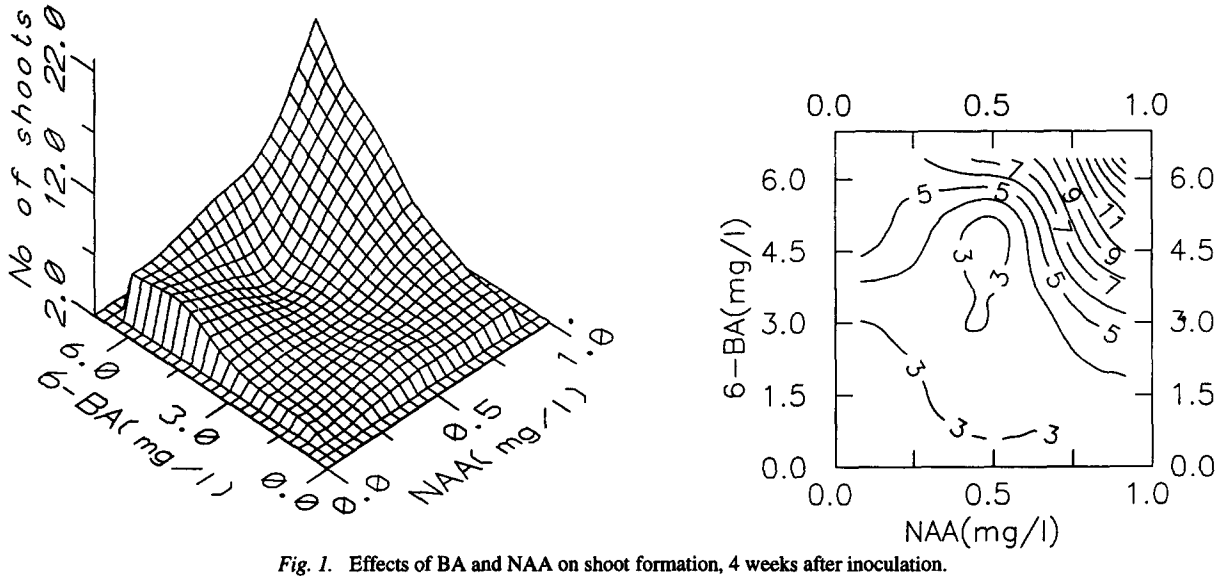

Fig. 1. Effects of BA and NAA on shoot formation, 4 weeks after inoculation.

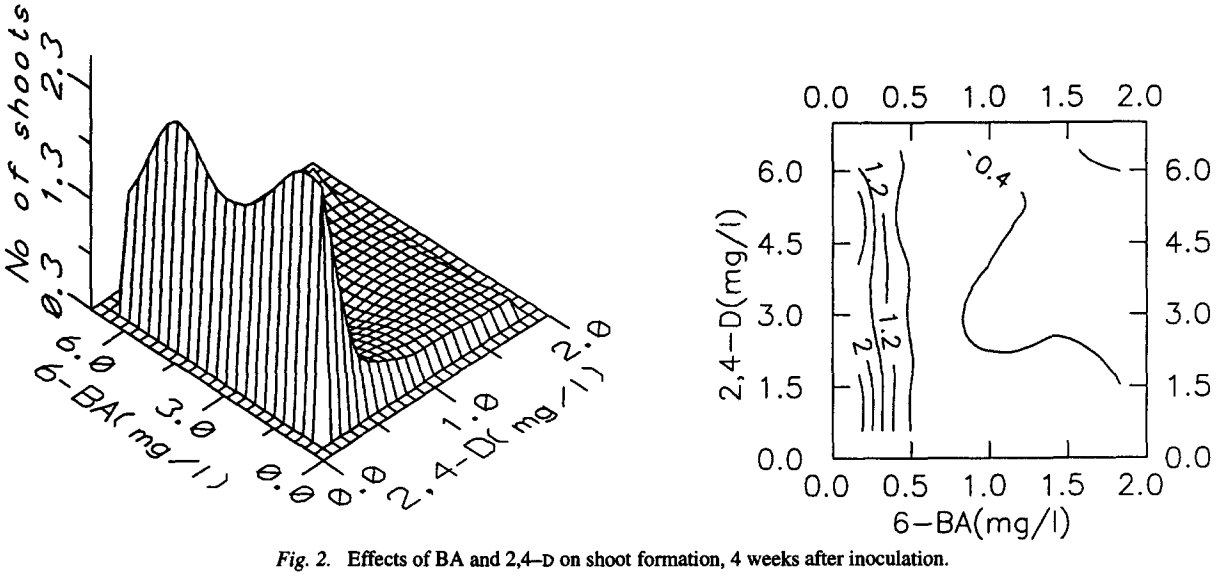

Fig. 2. Effects of BA and 2,4–D on shoot formation, 4 weeks after inoculation.

cultured under same conditions with transfer every 4 weeks.

Experimental design for multiple shoot developing

To examine the effects of BA, NAA and 2,4–D in the medium and light intensity on the growth, shoots of MSL–90 (maintained for about 8 months), were divided into 90 groups. To determine the effects of BA and NAA or 2, 4–D, four shoot pieces were transplanted into flasks with MS medium supplemented with different combination of BA (0.0, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 3.0, 5.0, 7.0 mg l^{-1}), NAA (0.0, 0.5, 1.0 mg l^{-1}) or 2,4–D (0.0, 0.5, 1.0, 2.0 mg l^{-1}) and were cultured under 700 Lux continuous illumination. To determine the effects of light intensity, four shoot pieces were transplanted into flasks with MS medium supplemented with BA 2.0 mg l^{-1} and different combination of NAA (0.0, 0.5, 1.0, 2.0 mg l^{-1}) and cultured under different light intensities (300, 500, 700, 1000, 1800 Lux). After four weeks' culture, the number of shoots derived from each piece were counted (values represent the mean of three flasks).

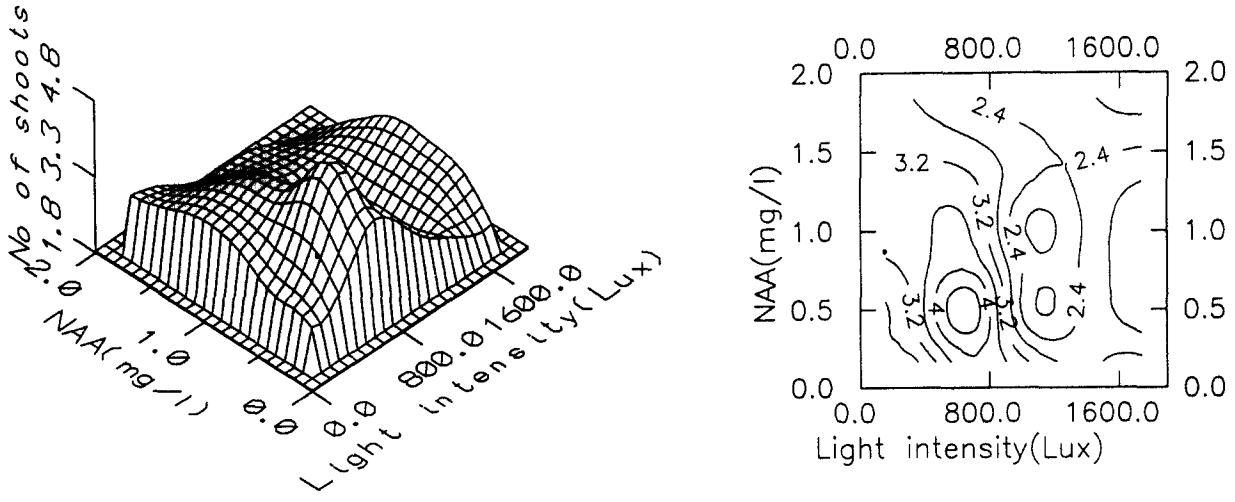

Fig. 3. Effects of NAA and light intensity on shoot formation, 4 weeks after inoculation

Results and discussion

Effects of BA and NAA on shoot multiplication

Figure 1 shows a three dimensional graph of how the number of shoots per initial piece varied with BA and NAA and its corresponding topo view. The standard error of the means of the three replicate flasks was found to be typically ± 8%. It was found that with increasing BA and NAA concentrations, the growth index increased rapidly. In particular, the medium that contains BA (7.0 mg l^{-1}) and NAA (1.0 mg l^{-1}) stimulated the formation of shoots. In the NAA free medium, the number of shoots were also found to increase with BA concentrations of up to 5.0 mg l^{-1}. This result is consistent with that of Hirata et al. (1987) who reported that the number of shoots induced form *C. roseus* (L.) G. Don seedlings increased with increasing BA concentration, but the shoots were not clearly distinguished from unorganized tissue at concentrations higher than 5 mg l^{-1}. They also found that with a combination of NAA and BA, multiple shoots were not induced, but whole seedlings formed callus. This may be due to the difference between inducing from seedlings and from established shoot cultures. Using Murashige–Skoog Revised Tobacco medium with 5 ppm BA, Krueger et al. (1982) induced leaf organ cultures from seeds of *C. roseus* (Dwarf Periwinkle) through callus and maintained it for over 18 months without any root or any defifferented callus material. However, this result is somewhat different from that of Endo et al. (1982) who reported that with *C. roseus* (L.) G. Don var. *Little delicata* in B5 medium, 3 μM (0.66 mg l^{-1}) BA was preferable for both weight increase and formation of new shoot primorda. It was suggested that for other cultivars and varieties of *C. roseus* the repeating experiments should be done if one wants to use those results. Since our results were obtained after many experimental runs, it can be pointed out that if *C. roseus* (L.) G. Don were used in any future experiments, these results are applicable.

Effects of BA and 2,4–D on shoot multiplication

Figure 2 clearly shows that when the 2,4–D concentration is increased form 0 to 0.5 mg l^{-1}, the formation of shoots is suppressed, and that when the 2,4–D concentration is greater than 0.5 mg l^{-1}, shoots dedifferentiate into unorganized tissue. This effect of 2,4–D on shoot cultures is contradictory to the report by Anderson (1982) who successfully grew leaf organ cultures of *Cinchona legeriana* on MS medium containing 1.0 mg l^{-1} 2,4–D and 0.1 mg l^{-1} mg l^{-1} kinetin. This may be due to the differences between BA and kinetin, or to difference in species.

Effects of light intensity and NAA on shoot multiplication

Figure 3 shows a three dimensional graph of how the growth index varied with NAA and light intensity and its corresponding topo view. It can be seen that the formation of shoots varies with NAA concentration and with light intensity. When the light intensity is in the

range of 550–700 lux, it stimulates the formation of shoots. Until now, we have found only a few reports on the effects of light intensity on formation of shoots. Endo et al. (1987) reported that shoot cultures of *C. roseus* could be maintained up to the second subculture in the dark, but after that, the growth of shoots stopped. However, they did not examine the effect of light intensity on the formation of shoots.

Acknowledgements

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