Plant Cell Reports (1989) 8:230-233

Plant Cell Reports
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The influence of auxins, light and cell differentiation on solasodine production by *Solanum eleagnifolium* Cav. calli

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Received December 7, 1988/Revised version received May 26, 1989 - Communicated by J. M. Widholm

Abstract. The effect of auxins, light and cellular production of Solanum eleagnifolium Cav. calli were studied. 2,4-dichlorophenoxyacetic acid (4.5 µM) was the plant growth regulator used for calli initiation and this produced the highest solasodine concentration. The solasodine concentration in darkness was significantly lower than that achieved under a photoperiod of 16 h. Differentiated tissue obtained by adequate hormonal balance (several ratios of 3-indolebutyric acid to 6-benzylaminopurine) produced higher yields of solasodine than non-differentiated tissue. 3-indolebutyric acid (2.5 µM) and 6-benzylaminopurine (8.8 µM) increased the productivity of solasodine by 100%.

Abbreviations. BAP, 6-benzylaminopurine; KIN, Kinetin; 2,4-D, 2,4-dichlorophenoxyacetic acid, IAA, 3-indoleacetic acid, NAA, 1-naphtaleneacetic acid; IBA, 3-indolebutyric acid; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; DW, dry weight;

$$\text{GI, growth index} = \frac{\text{Final DW} - \text{Initial DW}}{\text{Initial DW}}$$

Key words: Solasodine - Solanum eleagnifolium - Calli

Introduction

Solasodine, the most important alternative source for steroidal drug production after diosgenin, is present in hundreds of species of the genus Solanum but only a few of them are considered to be important for commercial production (Mann 1978).

Solanum eleagnifolium Cav. var. leprosum, a native specie from Argentina, contains solasodine, which is present as the glycoside solamargine. This compound is mainly present in fruits of the whole plant (Nigra et al. 1985). Calli from different explants (hypocotyl, root, cotyledon, leaf and fruits) have the same ability of producing solasodine (Nigra et al. 1987). In general, light affects production and accumulation of secondary metabolites in plant cell cultures. In the case of solasodine production the effect of light is rather controversial since Chandler and Dodds (1983) reported an inhibitory effect on solasodine production by light. Conversely, Bhatt et al. (1983) found increased amounts of solasodine in S.nigrum calli under a 16 h photoperiod.

On the other hand, the production of secondary metabolites in plant cell cultures depends on growth and, in some cases on cellular differentiation, which according to Misawa (1985), is an important factor for obtaining high yields of secondary metabolites. It is not yet clear to what extent secondary metabolism depends on the development of specific structures in plant cell cultures, and it is unknown whether these two processes are genetically and/or physiologically linked (Misawa 1985)

The aim of this paper was to study the effect of auxins on the initiation of calli and also the influence of light, auxins and morphological differentiation on solasodine production by calli of S.eleagnifolium Cav. var. leprosum, a species which has not been previously studied in this respect.

Materials and Methods

Explants were obtained from seedling (hypocotyls) of S.eleagnifolium Cav. as previously described (Nigra et al. 1987). For calli initiation, the explants were cultured in modified mineral nutrient medium (Murashige and Skoog 1962) with the addition of myo-inositol (100 mg l^{-1}), sucrose (30 g l^{-1}), RT vitamin complex (Khanna and Staba 1968). The pH was adjusted to 5.6-5.8 and the medium solidified with 8 g l^{-1} of agar. This medium will be referred to as MSRT medium. The plant growth regulator used depended on the type of experiment considered.

For calli initiation studies, explants from hypocotyls were cultured in five different media, which consisted of the MSRT medium, supplemented with one of the following auxins: IAA (5.7 µM), 2,4-D (4.5 µM), NAA (5.4 µM), IBA (4.9 µM) or 2,4,5-T (3.9 µM). The cultures were incubated at 24 ± 2°C and exposed to a 16 h photoperiod using fluorescent lamps at an irradiance of approximately 1.8 W.m^{-2}.

The effect of auxins was studied with calli initiated on MSRT medium plus 2,4-D (4.5 µM). After three subcultures in the same medium, they were transferred to four different media, each containing the MSRT medium plus one of the following auxins: 2,4-D, IBA, NAA, IAA at 4.5 µM concentration, using the same culture conditions described above. For studying the influence of light, calli from hypocotyls were started and subcultured three times during 25-27 days in MSRT medium plus

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2,4-D (4.5 µM). Afterwards, the calli were divided into two sets. One of them was exposed to a 16 h photoperiod and the other one was kept under constant darkness. Both sets of calli were subcultured at 24 ± 2°C for 25-27 days. For organogenesis assays, hypocotyl calli initiated in MSRT medium plus 2,4-D were transferred to MSRT medium supplemented with BAP plus IBA or KIN plus 2,4-D. The BAP:IBA and KIN:2,4-D concentrations added are shown in Table I. The cultures were incubated for a period of 30 days at 24 ± 2°C with a photoperiod of 16 h. An additional experiment was conducted in order to analyze the effect of prolonged subcultures (six months) in the presence of 2,4-D (4.5 µM) on the organogenesis promoted by IBA and BAP (Table I).

Table I. Culture media with different relationships between IBA:BAP and KIN:2,4-D added as plant growth regulators to MSRT medium

BAP (µM) \ IBA (µM)	4.9	2.5	1.2
8.8	RiB_1	RiB_2	RiB_3
4.4	RiB_4	RiB_5	RiB_6

KIN \ 2,4-D	4.5	2.2
9.3	RK_1	RK_3
4.6	RK_2	RK_4

The estimations of growth and solasodine concentrations were carried out as described previously (Nigra et al. 1987).
Ten replicates were used in all determinations and variance analysis was conducted in each test.

Results and Discussion

Effects of Auxins on Initiation of Calli
Figure 1 shows the effects of different auxins on the initiation of calli from hypocotyl explants. After a week of culture the explants observed in the medium containing 2,4-D presented tissular hyperplasia. The same material after four weeks produced friable calli, in accordance with observations already reported (Nigra et al. 1987). When the auxins used were NAA or 2,4,5-T, calli formation and abundant roots were observed after eight weeks. Calli separated from the roots and transferred to the same medium, proliferated profusely.
Regeneration of whole plants with no calli formation was observed after eight weeks in the case of IAA. When IBA was the auxin employed, no calli were produced, but non-proliferating buds occurred. These results suggest that 2,4-D is the plant growth regulator to use for calli initiation and NAA and 2,4,5-T are the auxins to be preferred as inducers of root culture in S.eleagnifolium Cav.
Although untransformed root cultures may be exploited for secondary metabolite production, their establishment and maintenance is difficult in some species. In this respect transformed roots induced by Agrobacterium rhizogenes are stable and grow faster than untransformed ones (Flores et al. 1987), even without decreasing metabolite production.

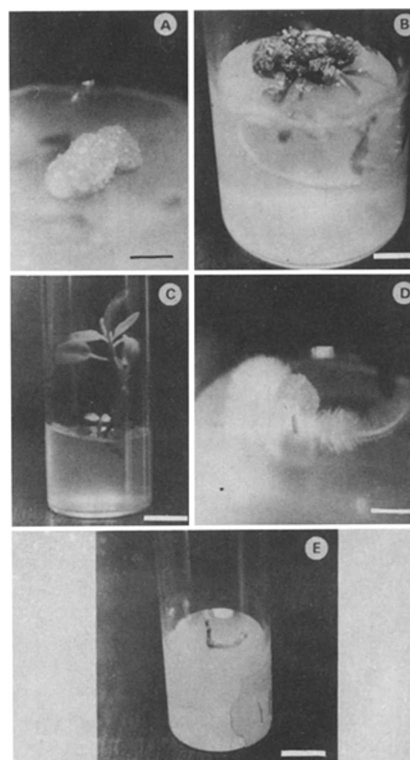

Fig.1. Effect of different auxins on calli initiation of S.eleagnifolium Cav. cultivated on MSRT medium supplemented with A) 2,4-D 4.5 µM (bar:0.28 cm); B) 2,4,5-T 3.9 µM (bar: 0.35 cm); C) IAA 5.7 µM (bar: 0.6 cm); D) NAA 5.4 µM (bar: 0.20 cm); E) IBA 5.4 µM (bar: 0.6 cm). Culture time: 26 days

Effect of Auxins on Growth and Solasodine Production
Figure 2 shows the effect of different auxins on growth and solasodine production in S.eleagnifolium Cav.calli, started with 2,4-D MSRT medium through five succesive subcultures.
The highest values, both in growth and solasodine production, were obtained using 2,4-D. With this auxin, solasodine concentration obtained was about 1.4 mg g^{-1}DW while with the other auxins it was about 0.5-0.8 mg g^{-1}DW. The relatively high content obtained with IBA, IAA and NAA, in the first and second subcultures may be a residual effect of 2,4-D used in calli initiation and maintenance. From the third subculture no significative differences, both in growth and on solasodine production were obtained between cultures grown on media with IAA, IBA and NAA.
These results differ from S.laciniatum which produces the same level of solasodine in tissue cultures with 2,4-D, NAA and IAA (Hosoda et al. 1979). Concerning the influence of 2,4-D, this auxin has generally been found to be less suitable for triggering secondary metabolism in plant cell cultures than either IAA of NAA (Mantell and Smith 1983). In this respect, and according to Misawa (1985), 2,4-D stimulates the dedifferentiation of cells and consequently diminishes the level of secondary metabolites. However, this is not always the case, since production of L-dopa by Macuma pruriense s, ubiquinone 10 by Nicotiana tabacum and diosgenin by Dioscorea deltoidea were stimulated by high levels of 2,4-D respectively (Brain 1976; Ikeda et al. 1976; Misawa 1985).

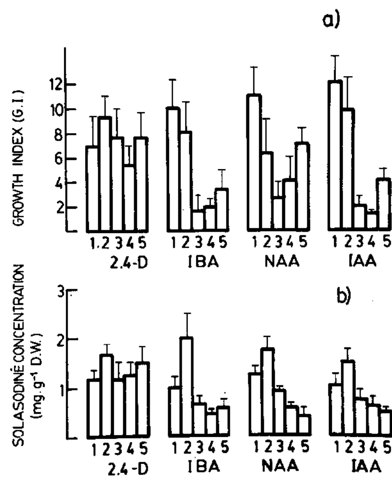

Fig.2. Effect of different auxins (4.5 μM) on a) growth index and b) solasodine production in calli cultures on MSRT medium. Culture time was 26 days. Bars represent standard deviation. 1, 2, 3, 4, 5: subculture numbers.

Influence of Light on Growth and Solasodine Concentration

Figure 3 shows the effect of light on growth index and solasodine concentration of S.eleagnifolium Cav., calli. From the second subculture the solasodine concentration was significantly lower ($P \leq 0.01$) in darkness than in calli obtained under light conditions where it was about 1.15-1.5 $mg.g^{-1}$ DW. These results are in agreement with those reported by Bhatt et al. (1983) who found increased amounts of solasodine in S.nigrum calli maintained under a 16 h photoperiod, but differ from those reported by Chandler and Dodds (1983), who found an inhibitory effect of light on solasodine accumulation by S.laciniatum calli. The increases of solasodine production under light could be attributed to a promotion effect of photosynthesizing chloroplasts as has been reported by Cooner (1987) in the case of S.laciniatum.

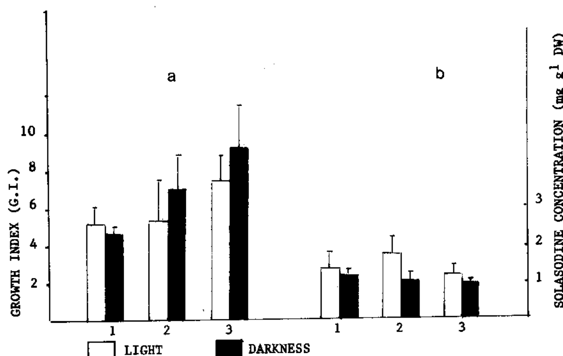

Fig.3. Effect of light on a) growth index and b) solasodine concentration in S.eleagnifolium calli cultivated on MSRT medium plus 2,4-D (4.5 μM). Culture time was 26 days. Bars indicate standard deviation. 1, 2, 3: subculture numbers

Effect of Organogenesis on Solasodine Production

The combinations of 2,4-D and KIN used were unable to promote organogenesis. In the case of S.carolinense the combinations of 2,4-D:KIN were able to promote cyto-differentiation but the relation was critical. The highest proportion of tracheary elements (15% of the total number of cells in culture) was produced when cultures were grown on medium containing a high ratio of 2,4-D:KIN (20:1) (Reynolds 1987).

On the other hand, BAP in combination with IBA produced organogenetic changes whose characterization was as follows (Figure 4): formation of vascular nodules with incipient organogenesis on RiB_5, RiB_6 media (B and C); formation of buds in RiB_1, RiB_2 and RiB_4 media (A); internode elongation and foliar expansion in some buds in RiB_2 medium (D).

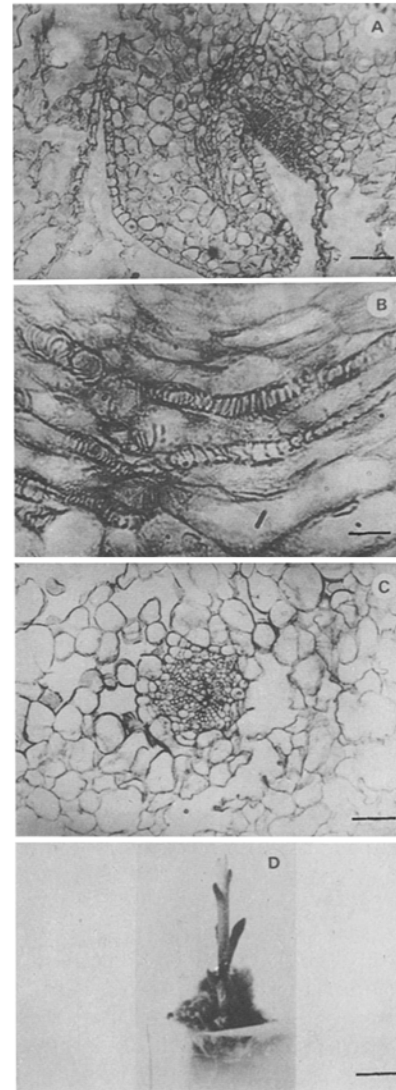

Fig.4. Organogenesis in calli of S.eleagnifolium after 30 days in culture. A) Bud and foliar primordium (bar: 140 μm); B-C) vascular nodules (B bar: 35 μm, C bar: 140 μm); D) internode elongation and foliar expansion in bud (bar: 0.35 cm)

Besides, experiments made with the same combinations of BAP:IBA but using calli maintained in medium MSRT with 2,4-D during six months, showed no organogenetic changes. This behaviour can be attributed to the irreversible dedifferentiation effect induced by 2,4-D during prolonged subcultures (Chandler and Dodds 1983).

Table II. Solasodine production in differentiated and non differentiated tissue of S.eleagnifolium Cav.

MEDIUM	PLANT CELL MATERIAL	SOLASODINE mg g^{-1}DW
RiB_1	non differentiated calli	0.66 ± 0.12
	buds	1.00 ± 0.16
RiB_2	non differentiated calli	0.86 ± 0.04
	buds	1.68 ± 0.12
RiB_4	non differentiated calli	0.96 ± 0.25
	buds	1.31 ± 0.07
RiB_5	non differentiated calli	0.47 ± 0.16
	calli with vascular nodes	1.02 ± 0.85
RiB_6	non differentiated calli	0.79 ± 0.06
	calli with vascular nodes	1.27 ± 0.47

Table 2 shows the solasodine productivity in nondifferentiated and differentiated tissues. The high solasodine productivity obtained in differentiated tissues is in agreement with the results reported by Chandler and Dodds (1983); Misawa (1985); Emke and Eilert (1986), for secondary metabolite production in other plant species. The beneficial effect of cellular differentiation can be due to an increased capacity of vacuolar cell accumulation of metabolites of the differentiated tissues (Aitchison es al. 1977).

The solasodine concentrations found in this study are comparable to those obtained from calli of other species of Solanum such as S.laciniatum (1.24 mg g^{-1}DW) (Chandler and Dodds 1983) and S.nigrum (1.40 mg g^{-1}DW) (Bhatt et al. 1983). Concerning the yields of solasodine in S.eleagnifolium the values obtained in this work (1.4-1.5 mg g^{-1}DW) are higher than those reported by Khanna et al. (1976) who found 0.32 mg g^{-1}DW but lower than those previously reported by us (Nigra et al. 1987). In our case the difference may be attributed to variation between cell lines.

The yields of solasodine obtained in our cultures (1.5 mg g^{-1}DW) are lower than those reported in fruits (30-50 mg g^{-1}DW) of plants of the same species but higher than the concentration found in leaves and stems (0.3 mg g^{-1}DW) (Nigra et al. 1985).

In S.laciniatum it has been reported (Mann 1978) that the whole plant contained up to 3% of solasodine (dry weight basis) whereas calli contained either no of low levels of solasodine (Vãgũjfalvoi et al. 1971; Hosoda and Yatazawa 1979).

We can conclude that the factors studied in this work such as growth regulators, light, darkness and cellular differentiation can alter the level of solasodine in S.eleagnifolium calli, but the maximum concentration attained still remains lower than that observed in fruits.

In order to improve solasodine production from plant cell cultures further research related to media composition including elicitors, is required.

In adition studies related to produce strains with higher productivity are also needed.

Acknowledgements. We thank Dr. O.H. Caso for his valuable discussions. This work was supported by the Secretaria de Ciencia y Tecnología (SECyT) and the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), República Argentina.

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