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Isoenzyme expression during root and shoot formation in *Solanum nigrum*

A.M. HASSANEIN*, A.M. AHMED**, A.I.I. ABED-EL-HAFEZ* and D.M. SOLTAN*

*Botany Department, Faculty of Science, South Valley University, 82524 Sohag, Egypt**
*Botany Department, Faculty of Science, Assuit University, 71516 Assuit, Egypt***

Abstract

In *Solanum nigrum*, the initiation of root primordia occurred directly on stem explants or microshoots cultured on half strength MS medium supplemented with 1 mg dm^{-3} indolebutyric acid (IBA), indole-3-acetic acid, or naphthalene acetic acid, IBA being most effective. Initiation of shoot primordia occurred on B5 medium supplemented with 0.5 mg dm^{-3} benzylaminopurine (BAP) or kinetin; BAP was more active. During shoot formation, the increased content and the expression of new isoenzymes of peroxidase (POX), esterase and malate dehydrogenase was found. On the other side, expression of new indophenol peroxidase isoenzyme was detected during root formation. Organogenesis of *S. nigrum* has no effect on glutamate oxaloacetate transaminase and glutamate dehydrogenase content. Differential expression of POX could be detected between basal and upper side of explants.

Additional key words: auxins, cytokinins, esterase, peroxidase, glutamate oxaloacetate transaminase, glutamate dehydrogenase, malate dehydrogenase, indophenol peroxidase.

Introduction

During differentiation of cultured plant tissues under the influence of phytohormones, marked molecular, biochemical and physiological variations were reported. For example, repetitive DNA was strongly reduced during linear growth phase of carrot callus, whereas abundant DNA amplification occurred at the time of adventitious root

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Abbreviations: BAP - benzylaminopurine; 2,4-D - 2,4-dichlorophenoxyacetic acid; EST - esterase, GDH - glutamate dehydrogenase; GOT - glutamate oxaloacetate transaminase; IAA - indole-3-acetic acid; IBA - indolebutyric acid; IPOX - indophenol peroxidase; KIN - kinetin; MDH - malate dehydrogenase; MS medium - Murashige and Skoog's medium; NAA - naphthalene acetic acid; POX - peroxidase.

Fax: (+ 002) 093 601159, e-mail: science@sohag.jwnet.eun.eg

formation (Schäfer *et al.* 1978, Dührssen *et al.* 1984). Under auxin treatments microshoots of apple showed starch grains in cells of the vascular bundles and primary rays before root primordia formation (Jásik and De-Klerk 1997).

While adventitious roots and shoots are mostly formed on stem segments, the regeneration of adventitious shoots from roots is usually difficult. In addition, when adventitious shoots or roots were induced from the same organ, they were initiated from different tissues (*e.g.* De Klerk *et al.* 1997). Auxins induced root formation from microshoots, stem internodes and calli, and their formation pattern was influenced by auxin type (Hu and Wang 1984, Endress 1994). On the other side, the adventitious bud formation was induced from these plant tissues with BAP and KIN.

Isoenzymes have been reported as sensitive molecular markers in plant tissue culture (*e.g.* Siminis *et al.* 1993, Calderòn *et al.* 1995, Melo *et al.* 1997, Scandalios and Sörenson 1997). Preliminary experiments showed that *Solanum nigrum* has a high ability to form roots and shoots on media containing different auxins and cytokinins. Therefore, the aim of this work was to induce root and shoot development in *S. nigrum* and to study the associated isoenzyme expression.

Materials and methods

Plants: The experiments were carried out using established shoot culture of *Solanum nigrum* L. (see Gasquez and Barralis 1979, Hassanein 1993) on B5 medium (Gamborg *et al.* 1968) supplemented with 0.5 mg dm^{-3} BAP. For adventitious root formation, microshoots or stem internodal sections were cultured on half strength MS basal medium (Murashige and Skoog 1962) supplemented with 1 mg dm^{-3} of IBA, IAA or NAA. On the other side, induction of adventitious bud formation from stem internodal or microshoots segments was carried out using B5 medium, to which 0.5 mg dm^{-3} BAP or KIN was added. Sixty stem explants were cultured in 3 Petri dishes (9 cm width) considered as a replicate. The number of shoots and roots was determined after four weeks.

To investigate if the changes in isoenzyme expression takes place in whole cutting or only in the tissues participating in the regeneration, the cultured stem internodal segments were divided into two parts, the lower side of the explant (1 mm) which showed enlargement and root or shoot formation, and the remainder of the explant. At day 8 on root or shoot induction medium the proteins in the two parts were extracted and subjected to isoenzyme analysis.

Callus induction: The regenerative callus was formed via protoplast culture according to Binding and Mordhorst (1984). Apical tips including about three young leaves were collected, cut into small segments (1 - 3 mm) and incubated in the enzyme solution (3 % *Rohament CP*, cellulase and pectinase, *Röhm 80029*) for 14 h. The protoplasts were passed through steel sieve (pore 40 μm) to remove undigested cell clumps. Protoplasts were centrifuged at 100 *g* for 5 min. The protoplasts were plated as described by Binding *et al.* (1988). Medium V-KM (Binding and Nehls 1977) containing 0.56 mg dm^{-3} BAP, 1 mg dm^{-3} NAA and 0.1 mg dm^{-3} 2,4-D and the

organic nutrients of the 8P medium of Kao and Michayluk (1975) was used for protoplast culture. After two weeks the protoplasts were transferred to B5 agar (0.8 %) medium containing the hormonal part of V-KM (BTM medium). After further two weeks the established calli were transferred for induction of shoots or roots using the previously described media. It is worthy to mention that callus could be also formed from stem segment using BTM medium. After four weeks on specific medium the number of calli showing plant regeneration was determined.

Isoenzyme analysis: One gram of the plant materials was ground on ice in a mortar in 1 cm^3 of extraction buffer that consisted of 0.1 M Tris-HCl, pH 7.0, and contained 0.2 M cysteine. The homogenate was centrifuged at 15 000 *g* at 4 °C for 15 min. Supernatants were collected for immediate electrophoresis (*BIO RAD* electrophoresis system, *Protean II xi 2-D Cell*) in 7.5 % polyacrylamide slab gels. Gels were run at 18 mA for 8 h at 8 °C in 0.025 M Tris + 0.192 M glycine buffer (pH 8.9). Six isoenzymes were stained: esterase (Wetter and Dyck 1984, Brewer 1970), peroxidase (Siegel and Galston 1967), malate dehydrogenase, glutamate oxaloacetate transaminase, indophenol peroxidase and glutamate dehydrogenase (Brewer 1970).

Results and discussion

In *Solanum nigrum*, shoot and root formation proceeded with slight enlargement at the base of stem or microshoots segments cultured in vertical position on shoot or root induction medium (Figs. 1,2). This enlargement was not detected when roots were formed on half strength MS medium without auxin (Fig. 1). IBA was the most suitable auxin for root formation and development. The same number of roots were induced by IAA and IBA (about 11 per explant) but the growth of roots induced by

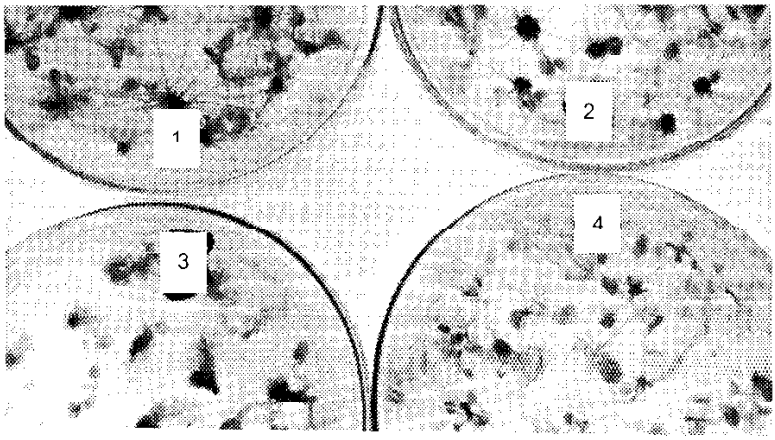


Fig. 1. Root formation from the base of microshoot cuttings cultured on half strength MS medium supplemented with 1 mg dm^{-3} IBA (*1*), IAA (*2*), NAA (*3*), or without phytohormone (*4*).

IAA was slower than those induced by IBA (Fig. 1). NAA induced short-hairy roots (Fig. 1). On the other hand, both BAP and KIN induced shoot formation but the number of shoots formed on medium with BAP (16 per explant) was higher than with KIN (8 per explant).

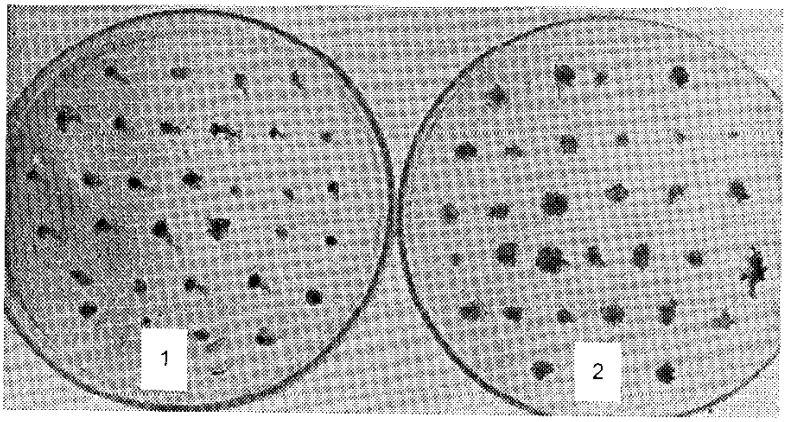


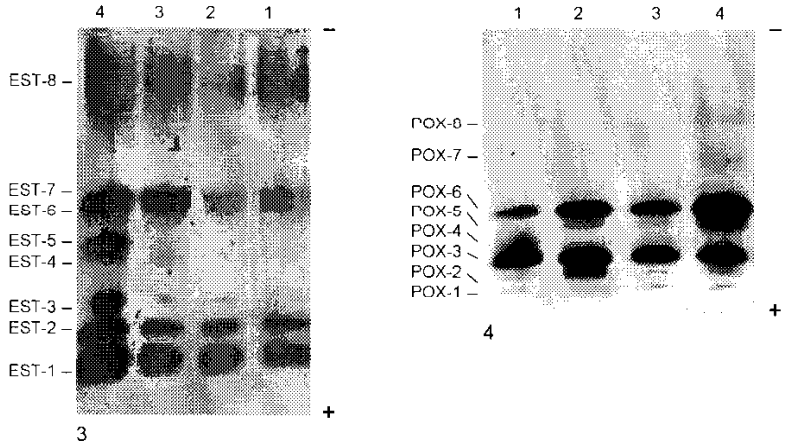
Fig. 2. Shoot formation from the base of stem internode cuttings cultured on B5 medium supplemented with 0.5 mg dm^{-3} KIN (*1*) or BAP (*2*)

The shoot regeneration from callus obtained via protoplast culture (60 %) on shoot induction medium was higher than from callus obtained from stem segment (12 %), root formation was similar. Therefore, calli produced via protoplast culture were used to study the isoenzyme expression during organogenesis under the influence of different phytohormones.

Expression patterns of isoenzyme bands displaying esterase (EST) activity (Fig. 3) showed great similarities between stem segments cultured on media containing 1 mg dm^{-3} of IBA, IAA and NAA. Faint bands corresponding to EST-3 and EST-4 were decreased with increasing the tendency to root organogenesis. They were hardly detected under the influence of IBA, the most suitable auxin for root formation. On BAP containing medium, *S. nigrum* stem segments showed similar isoenzyme but in high staining intensity. In addition, new esterase isoenzyme band (EST-5) was expressed only under the influence of BAP. Chawla (1991) reported that with the development of shoots or roots new isoenzymes of esterase, peroxidase and acid phosphatase appeared but none of them could be related to the process of induction of morphogenesis.

The pattern of peroxidase expression in stem segments (data not shown) was similar to that in callus during root and shoot initiation (Fig. 4). Differences in staining intensity of some POX bands were detected between calli induced to from roots under the influence of different auxins. POX-5 and POX-6 could be detected in high staining intensity under shoot formation. In addition, the expression of POX-7 and POX-8 was observed. Increase the staining intensity of POX bands gives indication about increase of the activity of these isoenzymes as was reported

previously (Khavkin and Zabrodina 1994, Hassanein 1998). Comparison between the upper and lower sides of explant showed (data not shown) great similarities between upper side of explants, if they were cultured on BAP or IBA containing medium, or without phytohormones. On the other hand, lower side of explants showed differential expression when they were cultured on BAP or IBA containing medium (Fig. 4)



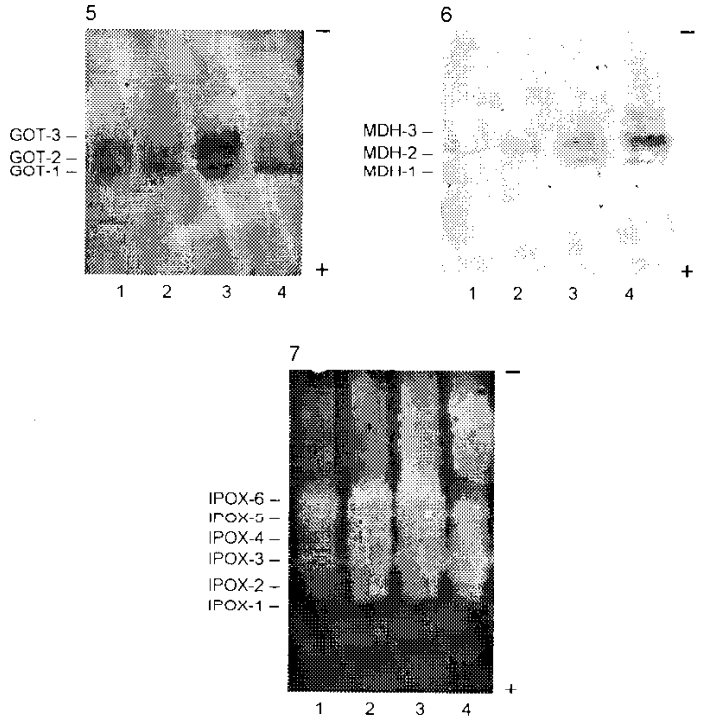
Figs. 3 and 4. Native gel electrophoresis of EST isoenzymes (Fig. 3, *left*) and POX isoenzymes (Fig. 4, *right*) during shoot and root formation of stem cuttings (esterase) or calli (peroxidase) cultured on B5 medium supplemented with 0.5 mg dm^{-3} BAP (*lane 4*) and half strength MS medium supplemented with 1 mg dm^{-3} of each NAA (*lane 3*), IAA (*lane 2*) and IBA (*lane 1*).

The GOT (Fig. 5) and GDH (data not shown) isoenzyme profiles (two bands of GOT and one band of GDH) detected during root development induced by different auxins did not differ from those during shoot development. The only exception, it is the expression of GOT 3 when the stem segments were cultured on NAA containing medium.

As in case of peroxidase and estrase, the number and staining intensity of MDH bands of stem segments under suitable condition for shoot formation was higher than under conditions for root formation on IBA containing medium (Fig. 6). Only one band in low staining intensity (MDH-2) was expressed during root formation.

The number of IPOX bands was low in stem segments induced to form shoots in comparison to those induced to form roots. IPOX-6 was detected when IBA, IAA or NAA was used to induce root formation. The IPOX pattern of expression did not depend on the type of auxin used. Elstner and Osswald (1994) reported that *de novo* synthesized phenols and indophenol oxidase play an important role as antioxidants and regulators of enzyme activities. These processes may be enhanced also during

root formation of *S. nigrum*. The capacity of phenolic compounds to promote root formation is known and depends on plant species (*e.g.* Hu and Wang 1984).



Figs. 5, 6 and 7. Native gel electrophoresis of GOT isoenzymes (Fig. 5, *left*), MDH isoenzymes (Fig. 6, *right*), and IPOX isoenzymes (Fig. 7, *bottom*) during shoot and root formation of cuttings cultured on B5 medium supplemented with 0.5 mg dm^{-3} BAP (*lane 4*) and half strength MS medium supplemented with 1 mg dm^{-3} of each NAA (*lane 3*), IAA (*lane 2*) and IBA (*lane 1*).

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