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Short Communication

Changes in lipoxygenase and hydroperoxide decomposition activities in tissue cultures of soybean

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Summary

Glycine max L. tissue cultures were initiated on different media supplemented with appropriate plant growth regulators that specifically induce the formation of callus, root, or shoot primordia. Exogenously applied hormones resulted in important changes in both Lox and HPO decomposition activity. Lox activity was higher in extracts from tissues cultured in medium supplemented with NAA or 2,4-D, while a lowest activity was recorded in extracts from cultures treated with BA. 13-HPOD was cleaved by all tested extracts, while 13-HPOT and 9-HPOD were cleaved exclusively by extracts from tissues cultured in the presence of BA.

Key words: fatty acid degradation pathway – *Glycine max* L. – *in vitro* soybean morphogenesis

Abbreviations: BA benzyladenine. – 2,4-D 2,4-dichlorophenoxyacetic acid. – HPO hydroperoxide. – LA linoleic acid. – LnA linolenic acid. – Lox lipoxygenase. – HPOD HPO of LA. – HPOT HPO of LnA. – NAA naphthalene acetic acid

Introduction

The Lox pathway is a cascade of enzymatic reactions that catalyses the transformation of fatty acids into a wide range of compounds involved in essential physiological processes in plants (Gardner 1995). Depending on the Lox source and on the reaction conditions, variable amounts of 9 and/or 13-HPO are formed (Siedow 1991). The HPOs are the substrates of several enzymes such as HPO lyase and allene oxide synthase (Blee et al. 1998). Spatial and temporal expression studies of Lox genes during plant development demonstrated that various Lox isoenzymes are expressed differentially in a tissue- and organ-specific manner, and in response to different stresses (Fischer et al. 1999). Even though the physiological roles of Lox have been the subject of abundant literature, little information is available concerning the implication of HPO decomposition activities in relation to plant growth and development. HPO lyase activity has been described in various tissues and during germination of soybean seedlings (Sekiya et al. 1986, Olias et al. 1990, Gardner et al. 1991).

The objective of the present study was to evaluate the changes in Lox pathway during morphogenesis. This was

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achieved by the measurement of both Lox and HPO decomposition activities in soybean tissues cultured in different hormonal regimes.

Materials and Methods

Plant material and tissue culture methods

Dry soybean seeds (*Glycine max* (L.) Merill var. Kador) were obtained from Rustica (Toulouse, France). The seeds were surface-sterilized, as previously described (Fauconnier et al. 1993), then incubated for germination in the dark in a hormone-free, solid basal Gamborg medium (B5) (Gamborg et al. 1968). Cotyledon explants from 5 day-old seedlings were transferred onto B5 medium supplemented with 2,4-D (1 mg/L), NAA (2 mg/L), or BA (3 mg/L). The cultures were maintained for three weeks at 25 °C under 16/8 hours photoperiod (80 µE /$m^2 \cdot s$).

Synthesis of fatty acid HPOs

Fatty acid HPOs were synthesized according to Fauconnier et al. (1997). The fatty acid HPOs were purified on C18 microcolumns, and the resulting fractions were further analyzed by HPLC according to Sanz et al. (1993).

Tissue extraction and enzyme activities determination

Fresh plant material was powdered in liquid N_2. The powder (2 g) was stirred for one hour on ice in 16 mL of sodium phosphate buffer (0.1 mol/L, pH 7.0), the resulting suspension was centrifuged (20,000 x g) at 4 °C for 30 min, and the supernatant was used for Lox activity determination according to Surrey (1964). For HPO decomposition activity determination, the powder (2 g) was stirred for one hour on ice in 8 mL of sodium phosphate buffer (50 mmol/L, pH 7) containing EDTA (3 mmol/L), DTT (3 mmol/L), and Triton X-100 (0.5 %) (V/V). The extract was centrifuged (30,000 x g) at 4 °C for 30 min. The resulting supernatant was used for total fatty acid HPO decomposition and for specific HPO lyase activities determination, according to Fauconnier et al. (1997) and Vick (1993), respectively. Protein content in the extracts was determined according to Lowry et al. (1951). Three independent extractions, each consisting of 15 culture flasks, were performed.

Results and Discussion

Callus, adventitious roots, and shoot primordia were induced from 3 week-old soybean cotyledon explants cultured in 2,4-D (1 mg/L), NAA (2 mg/mL), or BA (3 mg/L), respectively. The organogenic cultures, including the remaining original explant, were collected and extracted, and the Lox activity was evaluated at pH 6 and pH 9 using both LnA and LA as substrates. As shown in Figure 1A and B, for both substrates, Lox activity was higher at pH 9.0 than at pH 6.0. This comparative study shows that the highest Lox activity is found in extracts from NAA-cultures, followed by 2,4-D- and BA-cultures.

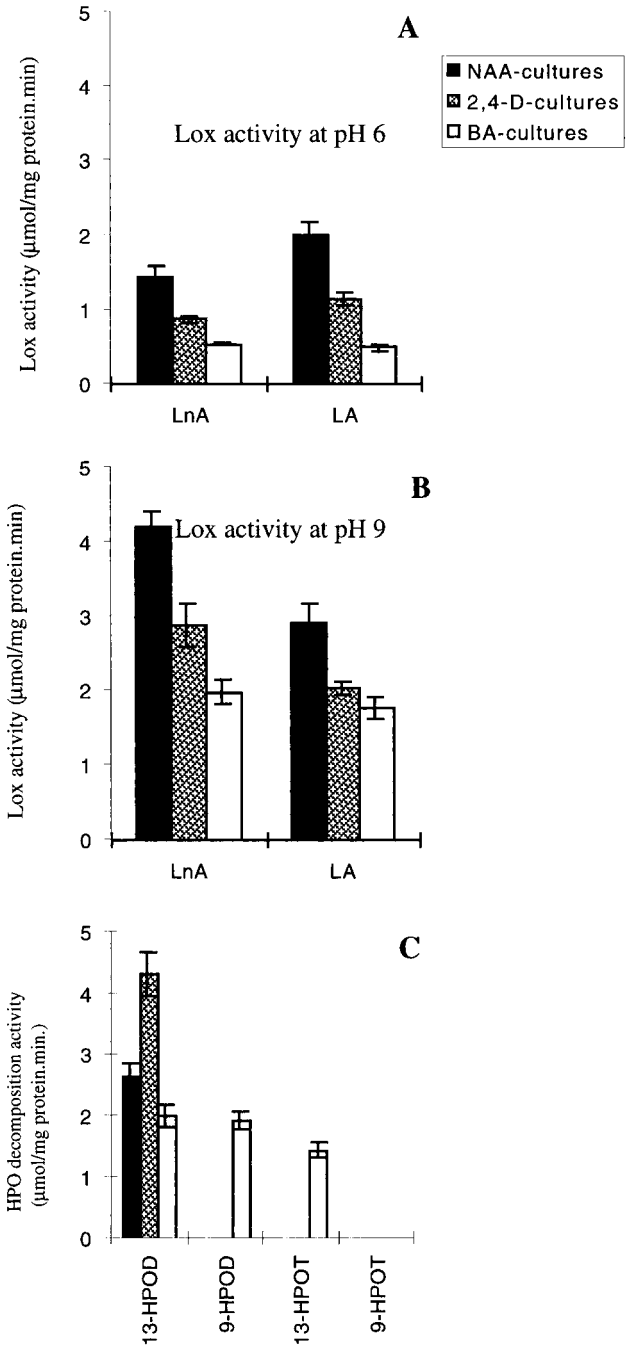

Figure 1. Lox and HPO decomposition activities in soybean tissue culture extracts. **A**: Lox activity at pH 6. **B**: Lox activity at pH 9. **C**: Total HPO decomposition activity.

Three week-old NAA-cultures showed several fast growing adventitious roots at the surface of the original cotyledons. Little is known about the Lox pathway in roots. However, a complex pattern of developmental expression of Lox in tissues that are very active metabolically has been reported for several plant species, including soybean (Eiben and Slusa-

renko 1994). It has also been demonstrated that auxin induces the expression of two Lox genes in cultured, immature zygotic embryo of soybean (Wang et al. 1999 a, b).

Interestingly, Lox activity seems not to be restricted to differentiated stages since it was measured in callus cultures (2,4-D-cultures) (Fig. 1 A, B). This suggests that Lox expression might not be exclusively tissue- and/or organ-specific or related to a particular developmental stage as previously stated (Eiben and Slusarenko 1994).

BA-cultures showed the lowest Lox activity (Fig. 1 A, B). This result is in agreement with the data reported by Grossman and Leshem (1978) showing that cytokinin, when applied to intact *Pisum sativum* plant, considerably lowered endogenous Lox levels. Nevertheless, the biological implications of the cytokinin-mediated lowering of the Lox activity remain to be elucidated.

Total HPO decomposition activity was determined by using 9- and 13-HPOD and -HPOT as substrates. The results illustrated by Figure 1 C show that among the four substrates, only 13-HPOD was cleaved by all the tested soybean tissue culture extracts. The 13-HPOT and 9-HPOD were exclusively cleaved by BA-culture extracts, whereas 9-HPOT is not cleaved by any of the tested tissue extracts. The HPO decomposition pathway in soybean is therefore affected by the hormone regime.

The measured total HPO decomposition activity (Fig. 1 C) is related to the activity of several enzymes present both in the extract and involved in the decomposition of the HPO substrate. In order to better characterize the HPO decomposition activity in the hormone-treated soybean cultures, we specifically measured the HPO lyase activity in these plant extracts using 13-HPOD as substrate. The results show that in BA-cultures, the total HPO decomposition was found to be due to HPO lyase activity, suggesting that C6 aldehyde such as hexanal and the corresponding oxo-acid could be produced in these cultures. Similarly, in 2,4-D-cultures, almost 90 % of the total HPO decomposition is the result of HPO lyase activity. But in the NAA-culture, HPO lyase represents about 50 % of the total HPO decomposition activity. Other HPO decomposition activities could be suspected in NAA-cultures such as allene oxide synthase, HPO-dependent peroxygenase, or epoxygenase that form allene oxydes, epoxides, or diepoxides, respectively (Blee and Schuber 1990).

Evidently, exogenously applied hormones induce dramatic changes in Lox and HPO decomposition activities, suggesting a possible involvement of the fatty acid degradation pathway in morphogenesis maintenance.

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