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Genetic transformation of barley to modify expression of a 13-lipoxygenase

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ABSTRACT Immature scutella of barley were transformed with cDNA coding for a 13-lipoxygenase of barley (LOX-100) via particle bombardment. Regenerated plants were tested by PAT-assay, Western-analysis and PCR-screening. Immunocytochemical assay of T0 plants showed expression of the LOX cDNA both in the chloroplasts and in the cytosol, depending on the presence of the chloroplast signal peptide sequences in the cDNA. A few transgenic plants containing higher amounts of LOX-derived products have been found. These are the candidates for further analysis concerning pathogen resistance. **Acta Biol Szeged 49(1-2):33-34 (2005)**

KEY WORDS

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Plant defense mechanisms against biotic and abiotic stresses enclose a complex system of constitutive as well as inducible reactions. Oxygenated fatty acids, called oxylipins, generated via the lipoxygenase pathway, play an important role in the regulation of environmentally induced and developmental-specific processes.

Lipoxygenases (LOXs, linoleate:oxygen oxidoreductases, EC 1.13.11.12) catalyze the formation of hydroperoxy derivatives of polyunsaturated fatty acids and thus the first step in the synthesis of fatty acid metabolism in plants. Metabolites of the LOX-pathway have been identified as compounds with antimicrobial activity, growth regulators, flavours and odours as well as signal molecules (Rosahl 1996; Feussner and Wasternack 2002). Based on these effects and on the correlation between increases in LOX content and the onset of specific processes, LOX has been proposed to be involved in the plant response to wound stress.

For barley, a cDNA coding for a LOX-100, a polypeptid of 100 kDa located within the chloroplasts, was isolated (Vörös et al. 1998). The protein is induced upon treatment with jasmonic acid methyl ester (JAME) and is strongly involved in the endogenous increase of volatile leaf aldehydes exhibiting direct antimicrobial activity. On the basis of these data, our primary aim was to study the lipoxygenase-dependent signal transduction pathway in a homologous system. Here we report the transformation of barley with the cDNA of LOX-100 controlled by different target sequences. The effects of different promoters on transformation efficiency and localization of LOX cDNA depending on the presence of chloroplast signal peptide sequences in the transgenic plants are discussed. Our results are among the first in studies on the possible influence of LOX-100 on signal transduction and its involvement in pathogen response.

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Materials and Methods

cDNAs

LOX-100 cDNA (LOX2:Hv:1) of 3073 bp was isolated from barley leaves treated with JAME (Vörös et al. 1998). LOX cDNA lacking chloroplast-signal-peptide sequences (LOX-cpsignal) was prepared in two main cloning steps:

1. N-terminal sequences including signal peptide were removed from the LOX cDNA using the internal BamHI restriction site. The fragment was cloned in pQE31 vector and showed little activity on expression.

2. Post-signal-peptide N-terminal sequences (244-477 bp) were synthesized by PCR and ligated to the fragment cloned in the previous step. The new clone showed significantly higher activity.

Plasmid vectors

pUbiLOX contains LOX cDNA under the control of the Ubi-1 promoter and the pat gene driven by the 35S promoter. The preparation of the basic vector construct was described elsewhere (Monostori et al. 2003).

p35SLOX contains the LOX cDNA under the control of the 35S promoter and the bar gene driven by the Ubi-1 promoter. The LOX cDNA was placed into the pRT104 plasmid, followed by the insertion of the 35S::LOX::polyA fragment from this construct into the pAHC20 plasmid.

p35SLOX-cpsignal is basically identical with p35SLOX with the difference of lacking the chloroplast signal peptide sequences of LOX. On vector preparation, the first 1-106

Table 1. Western-blot and PCR analysis of T0 plants.

Construct	Number of plants				
	Western-blot tested/+	bar or pat PCR tested/+	1. lox PCR tested/+	2. lox PCR tested/+	ratio bar+/lox+
Salome					
p35SLOX	37/33	38/23	38/3	3/3	23/6 (26.0%)
pUbiLOX	6/3	6/0	6/0	0/0	0/0
p35S LOX-cpsignal	94/66	94/45	94/12	12/11	45/12 (26.7%)
Golden Promise					
p35SLOXUbibar	3/3	3/2	3/0	0/0	2/0
p35SLOX-cpsignal	15/14	15/14	15/4	4/4	14/4 (28.6%)

bp fragment of the LOX cDNA - containing non-coding sequences and the ATG start codon upstream the signal-peptide - was amplified by PCR and ligated upstream the LOX-cpsignal construct described above. This enhanced LOX-cpsignal construct cloned in pUC18 was used to replace the chloroplast-signal-containing N-terminal sequences of LOX in the p35SLOX plasmid vector.

Stable transformation and *in vitro* selection

Scutella were isolated from immature embryos (1.8-2.5 mm) of spring barley cultivars 'Golden Promise' and 'Salome' and cultured on modified MS medium containing 2 mgL^{-1} 2,4-D. Gene-transfer via particle bombardment using PDS-1000/He particle gun and selection on Bialaphos-containing media were performed as described (Sharma et al. 2005).

Analysis of regenerated plants

Putative transgenic (T0) plants were analyzed biochemically using PAT-assay and Western-analysis. After PCR-screening with primers for the selectable genes the presence of LOX cDNA was checked also with PCR in two steps: first primers for a 335 bp lox fragment were used (1. lox PCR), then all PCR LOX positive plants were tested with a second primer pair for a 1600 bp fragment (35S promoter + LOX; 2. lox PCR). Localization of LOX-100 was detected by immunocytochemical analysis in cross sections of leaf segments. Products of LOX metabolism were analysed by HPLC and GC/MS (Vörös et al. 1998).

Results and Discussion

Transgenic barley plants expressing both the full-length LOX2:Hv:1 and the LOX-cpsignal cDNAs, respectively, have been produced. The regeneration rate on Bialaphos-containing media was 0.06–5.35%, however the number of surviving plants subjected to biochemical and PCR analysis was significantly lower (data not shown). Different promoters had a significant impact on the success of transformation. Introduction of constructs including the LOX cDNA under the control of the Ubi-1, known as a strong promoter in monocots, did not result in any LOX positive plants (Table 1). This suggests that strong expression of LOX2:Hv:1 negatively influences plant development. Immunocytochemical assays in leaf segments of T0 plants revealed that the expression of the LOX cDNAs under the 35S-promoter was possible both in the chloroplasts and in the cytosol, depending on the presence of the chloroplast signal peptide sequences in the construct. Co-transformation rate between the bar gene and the LOX cDNAs was 26.0-28.6% (Table 1). This is significantly lower than the usual 85-100% in transgenic barley plants (Lemaux et al. 1999).

HPLC and GC/MS analysis from leaf material of T0 and wildtype plants showed that (1) due to the high variation of enzyme activity in control plants, the number of them has to be increased, and (2) the putative over-expressing plants have to be additionally stressed since the 13-LOX depends on its substrate. Transgenic plants exhibiting higher concentrations of LOX-derived products can be used for further analysis concerning pathogen resistance.

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