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Transgenic barley plants overexpressing a 13-lipoxygenase to modify oxylipin signature

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Abstract

Three chimeric gene constructs were designed comprising the full length cDNA of a lipoxygenase (LOX) from barley (*LOX2:Hv:1*) including its chloroplast targeting sequence (cTP) under control of either (1) CaMV35S- or (2) polyubiquitin-1-promoter, whereas the third plasmid contains 35S promoter and the cDNA without cTP. Transgenic barley plants overexpressing *LOX2:Hv:1* were generated by biolistics of scutella from immature embryos. Transformation frequency for 35S::*LOX* with or without cTP was in a range known for barley particle bombardment, whereas for Ubi::*cTP-LOX* no transgenic plants were detected. In general, a high number of green plantlets selected on bialaphos became yellow and finally died either in vitro or after potting. All transgenic plants obtained were phenotypically indistinguishable from wild type plants and all of them set seeds. The corresponding protein (LOX-100) in transgenic T0 and T1 plants accumulated constitutively to similar levels as in the jasmonic acid methyl ester (JAME)-treated wild type plants. Moreover, LOX-100 was clearly detectable immunocytochemically within the chloroplasts of untreated T0 plants containing the LOX-100-cDNA with the chloroplast target sequence. In contrast, an exclusive localization of LOX-100 in the cytoplasm was detectable when the target sequence was removed. In comparison to sorbitol-treated wild type leaves, analysis of oxylipin profiles in T2 progenies showed higher levels of jasmonic acid (JA) for those lines that displayed elevated levels of LOX-100 in the chloroplasts and for those lines that harboured LOX-100 in the cytoplasm, respectively. The studies demonstrate for the first time the constitutive overexpression of a cDNA coding for a 13-LOX in a monocotyledonous species and indicate a link between the occurrence of LOX-100 and senescence.

Keywords: *Hordeum vulgare* L.; Poaceae; Lipoxygenase; Oxylipin levels; Transgenic barley plants

1. Introduction

In the last decade numerous data accumulated that oxygenated fatty acids and metabolites derived therefrom, collectively called oxylipins, play an important role in the regulation of environmentally induced and developmental-specific processes during plant life (Weber, 2002). Most of these oxylipins are generated via the lipoxygenase pathway.

Abbreviations: cTP, chloroplast targeting peptide; 35S, cauliflower mosaic virus 35S promoter; 13-HPOT, (13*S*,9*Z*,11*E*,15Z)-13-hydroperoxy-9,11,15-octadeca-trienoic acid; JA, jasmonic acid; JAME, jasmonic acid methyl ester; α-LeA, α-linolenic acid; LOX, lipoxygenase; MS, Murashige and Skoog; OPDA, 12-oxo-phytodienoic acid; PAT, phosphinothricin *N*-acetyltransferase; PPT, phosphinothricin; Ubi-1, polyubiquitin-1 promoter.

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Lipoxygenases (LOXs, linoleate:oxygen oxidoreductases, EC 1.13.11.12), ubiquitous in higher eukaryotes, are non-heme iron containing dioxygenases which catalyse the stereospecific addition of molecular oxygen to polyunsaturated fatty acids either at carbon atom C-9 or at carbon atom C-13 of C-18 fatty acids leading to the formation of unsaturated fatty acid hydroperoxides. Based on this positional specificity LOX isoenzymes are grouped into linoleate 9-LOX and 13-LOX types. An additional classification of plant LOXs is based on comparison of their primary structure. Enzymes with a high sequence similarity (>75%) are termed type 1-LOXs while those enzymes showing only a moderate overall sequence similarity (~35%) but carrying a putative chloroplast transit peptide have been classified as type 2-LOXs (Rosahl, 1996).

In plants, α-linolenic acid (α-LeA) or linoleic acid as the main substrates are converted via LOX into hydroperoxy polyunsaturated fatty acids, which are the substrates for at least seven different pathways (Feussner and Wasternack, 2002). Most of these LOX-generated substances from the different reactions are involved in developmental processes and defence responses as supported by the following data: (1) Jasmonic acid (JA), a plant hormone derived from the LOX pathway, acts as an important signalling compound on pollen development, root growth and defence responses to herbivore and pathogen attack as demonstrated by JA-biosynthesis and JA-insensitive mutants as well as transgenic approaches (Wasternack and Hause, 2002; Howe, 2004; Pozo et al., 2004; Devoto and Turner, 2005), (2) C_6 volatiles are able to induce defence related genes (Paré and Tumlinson, 1997; Bate and Rothstein, 1998; Koch et al., 1999), (3) aldehydes derived by the action of a hydroperoxide lyase revealed antimicrobial activity (Croft et al., 1993), (4) divinyl ethers such as colnelenic acid accumulate after pathogen attack and exhibit antimicrobial activity (Weber et al., 1999), and (5) transgenic plants with overexpression of a LOX gene or by antisense expression of LOX sequences are affected in defence responses (Bell et al., 1995; Rancé et al., 1998; Mène-Saffrané et al., 2003). Based on these effects LOXs are thought to be a key regulator of a complex signalling network.

In barley leaves, JA treatment leads to the formation of JA-induced proteins of different molecular masses (Lehmann et al., 1995). Among them are three LOX forms with the molecular masses of 92, 98 and 100 kDa (LOX-92, LOX-98, LOX-100). They were identified as 13-LOXs located within the chloroplasts (Feussner et al., 1995). The cDNA of the most abundant form LOX-100 was isolated and designated as *LOX2:Hv:1* (Vörös et al., 1998). The protein accumulates in JA-treated primary leaves, and its gene expression is induced by salicylate treatment but not by pathogens (Hause et al., 1999). In addition to *LOX2:Hv:1*, two full length cDNAs (*LOX2:Hv:2, LOX2:Hv:3*) were isolated from primary leaves of barley seedlings. Both of them encode 13-LOXs, and immunogold labelling revealed preferential localization of the proteins in the stroma of plastids (Bachmann et al., 2002). Metabolic profiling revealed that 13-LOX-derived products are specifically directed into the reductase branch upon salicylate treatment (Weichert et al., 1999), whereas JAME treatment caused a selective induction of LOX and hydroperoxide lyase activity resulting in the endogenous occurrence of volatile leaf aldehydes (Kohlmann et al., 1999). Further analyses revealed that the three *13-LOXs* are differentially expressed during treatment with jasmonate, salicylate, glucose or sorbitol (Bachmann et al., 2002) suggesting varying activity of the three enzymes under different stress conditions.

As a first step to study the physiological function of *LOX2:Hv:1*, we have used a transgenic approach for *Hordeum vulgare* L. cv. 'Salome'. We transformed the cDNA encoding LOX-100 with and without the chloroplast targeting signal in a homologous approach via biolistics using scutella from immature embryos. Based on that, activity and quantity of the enzyme should be modified in the cytoplasm and the chloroplasts. Enzymatic activity of LOX-100 in different compartments should lead to different profiles of oxylipins due to the localization of the other enzymes of the LOX pathway. Furthermore, a possible influence of LOX-100-derived products on plant growth and development was expected. This is the first report for monocots on stable overexpression of a 13-LOX either in chloroplasts or cytoplasm.

2. Results

2.1. Generation of transgenic barley plants

The designed constructs p35S::*cTP-LOX*Ubi::*bar* (L1), pUbi::*cTP-LOX*35S::*pat* (L2) and p35S::*LOX*Ubi::*bar* (L3) (Fig. 1) were found to be functional as revealed by transient assays using direct gene transfer into mesophyll protoplasts from in vitro grown seedlings of cv. Salome. For the selectable *bar* or *pat* gene, a strong activity could be detected in the phosphinothricin *N*-acetyltransferase (PAT)-assay two days after transformation independently from the promoter used, whereas immunoblot analysis revealed a weak accumulation of LOX-100 in transient assays (data not shown).

Immature scutella of the spring type cv. Salome used as starting explants were transformed through particle bombardment with the three different LOX constructs and selected on bialaphos-containing medium. Putative transgenic plants were regenerated via somatic embryogenesis from the bombarded scutella with all three constructs, and subsequently selected (Table 1). The regeneration frequency is calculated as number of plants regenerated per scutella used for selection. For the constructs L1 and L3 comprising *LOX2:Hv:1* under control of the 35S promoter, a regeneration frequency of 4.2% and 5.3%, respectively, was found. In contrast, plasmid L2 containing the Ubi-1 promoter in front of the LOX-100-encoding cDNA yielded only one tenth of the regeneration frequency observed for L1 and L3. A high number of green and well-rooted