Transport of Potato Lipoxygenase into the Vacuole

Larsen, Mia Kruse Guldstrand; Welinder, Karen Gjesing; Jørgensen, Malene

Publication date:
2009

Document Version
Publisher's PDF, also known as Version of record

Transport of potato lipoxygenase into the vacuole

Mia K. G. Larsen, Malene Jørgensen and Karen G. Welinder
Section of Biotechnology, Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University; mail: kgw@bio.aau.dk, mj@bio.aau.dk or miala05@bio.aau.dk

Introduction

Lipoxygenase (lox) is a non-heme iron dioxygenase, which makes up 10 % of the total protein content in potato tubers cv. Kuras (1). In the tuber, lox is located in the vacuole even though it contains no recognizable transport signal. Therefore, it is a mystery how lox without any transport signals gets into the vacuole. The mechanism of import was attempted solved by investigating different lox fragments. The 3D structure of a soybean lox, GmLox1, (2) was examined in order to design six constructs containing a lox fragment. A positive construct with GFP was also designed. Besides the gene encoding sequences, all the constructs contained a hexapeptide coding sequence (CCPPGCC), which gives a green fluorescence together with the arsen dye, Fluorescein Arsenical Hairpin (FlAsH).

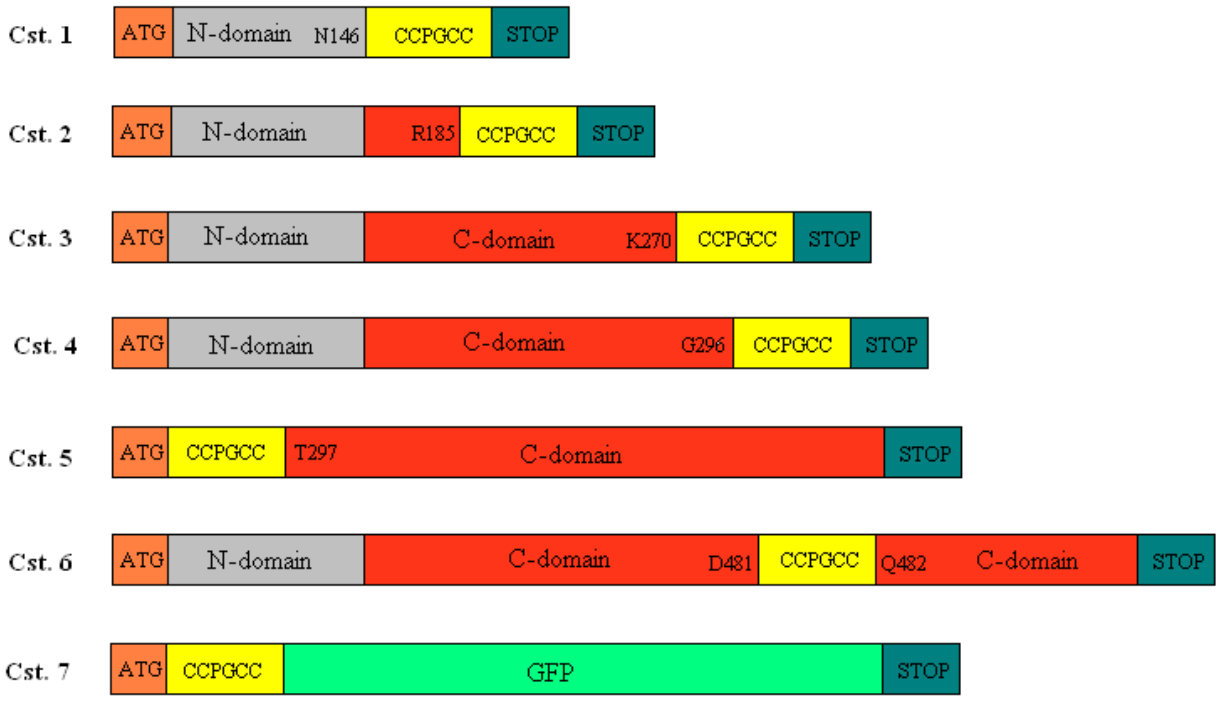

FIGURE 1: The content of the seven constructs designed for plant expression. Construct 1-4 (Cst. 1-4) contain different structural domains of the N-terminal part of potato lox. Cst. 5 contains a part of the C-terminal, while Cst. 6 contains the entire lox sequence. Cst. 7 is made as a positive control for transient expression and contains a GFP molecule.

pBluescript SK plasmids containing a Kuras potato lox (StLox) insert (071H07) were purified from TOP10 *E. coli* cells and the lox insert was sequenced. The seven constructs were engineered by carrying out different PCR experiments. The PCR constructs were then cloned into the plant expression vector pCAMBIA230035Su using the method Uracil Specific Excision Reagent (USER) cloning. All the seven constructs were successfully cloned. Unfortunately a deletion was discovered in the sequenced 071H07 potato lox insert, affecting four of the seven constructs. Only the three unaffected constructs, construct 1, 5 and 7 (GFP), were utilized to transform *Agrobacterium tumefaciens*, which was used for transient expression. The transient transformation, called agro infiltration, was executed in leaves from *Nicotiana benthamiana*. RT-PCR was performed on purified mRNA from infiltrated leaves and showed that all the constructs were transcribed, indicating a successful transformation. The protein expression of all the constructs was investigated with SDS-PAGE, fluorescence microscopy (FM) and confocal laser scanning microscopy (CLSM). All three experiments showed a high protein expression of the positive GFP controls, but a low or no protein expression of construct 1 and 5. More investigations are needed to conduct a conclusion about the mechanism of lox import to the potato vacuole.

RT-PCR and GFP detection

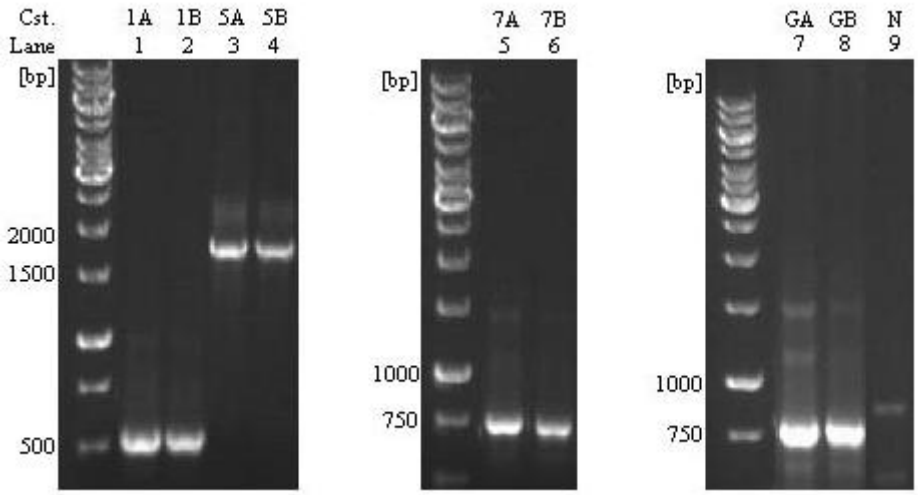

FIGURE 2: Agarose gelelectrophoresis of RT-PCR products conducted with construct specific primers and mRNA from infiltrated tobacco leaves as template.

The PCR reaction was carried out with 30 cycles. mRNA from two different leaves (A end B) infiltrated with each of the 4 constructs was investigated. In lane 1-2 RT-PCR products from tobacco leaves infiltrated with Cst. 1 are shown to contain bands at 500 bp (theoretical value 529 bp). Lane 3-4 contain RT-PCR products of Cst. 5 with bands near 1700 bp (1744 bp) and Lane 5-6 with RT-PCR products from leaves infiltrated with Cst. 7 contain bands at 750 bp (744 bp). RT-PCR products of tobacco infiltrated with Cst. G, GFP molecule, also contain bands around 750 bp (744 bp). All the RT-PCR products show a band with a size near the expected fragment size. A negative control (N)

consisting of a non-infiltrated leaf is shown in lane 9. The gels all contain a O'GeneRuler™ 1 kb DNA ladder.

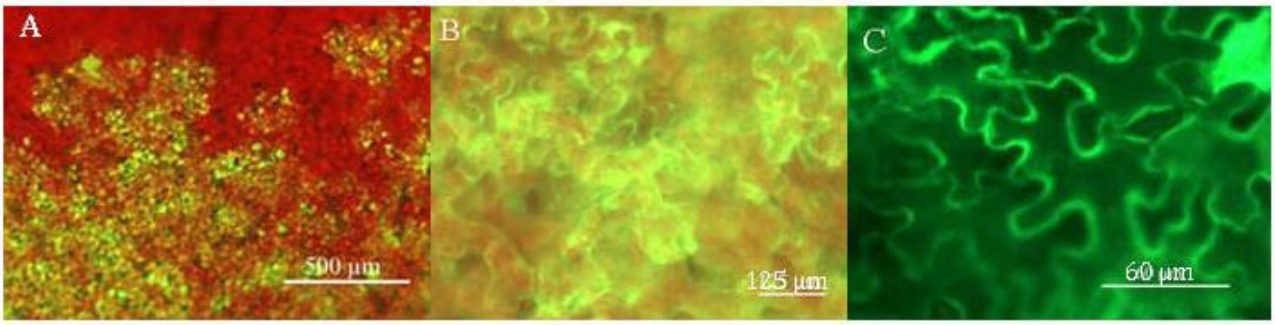

FIGURE 3: Investigation of GFP expression in leaves infiltrated with GFP.

The fluorescence of GFP and autofluorescence of the leaf samples were detected with fluorescence microscopy. A) A spot on a leaf infiltrated with GFP 4 days post infiltration (dpi). The GFP expression is seen as green fluorescence, while the red fluorescence is autofluorescence from chlorophyll. B) A higher magnification than A) but still with a GFP expression from 4 dpi. The GFP is seen in the cells and also near the cell wall. C) GFP expression from 8 dpi. Puzzle-shaped cells with green GFP signal near the cell wall.

FlAsH staining

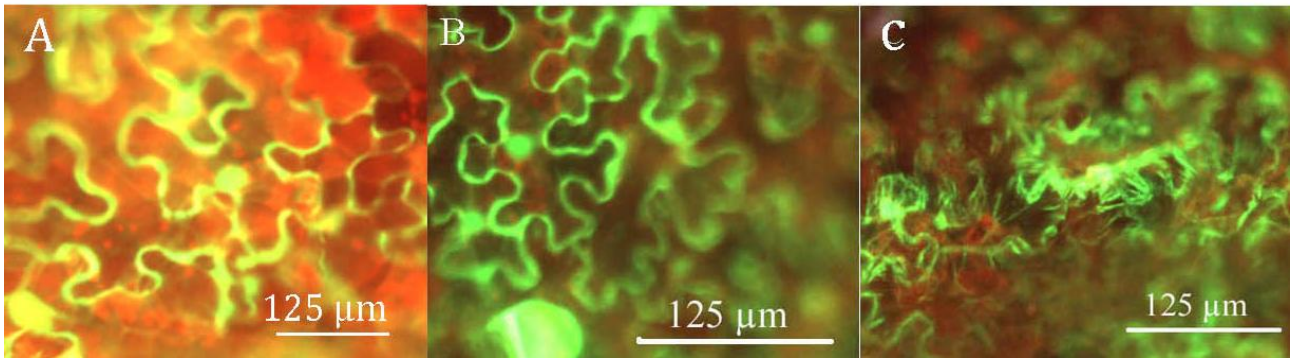

FIGURE 4: Fluorescence microscopy detection of GFP, FlAsH and red autofluorescence from leaves infiltrated with Cst. 7.

The leaves were investigated 4 dpi and bars indicate the magnitude of the pictures. A) An unstained leaf piece infiltrated with Cst. 7 shows puzzle-shaped cells with green fluorescence from GFP in Cst. 7 at the cytoplasm near the cell wall. B) and C) are from the same leaf piece stained with FlAsH dye. B) Cst. 7 is found at the cytoplasm of the puzzle shaped plant cells, which is similar to the fluorescence pattern in A). C) Has been exposed to UV a bit longer than B) and contains crystallised green spots.

Expression of constructs

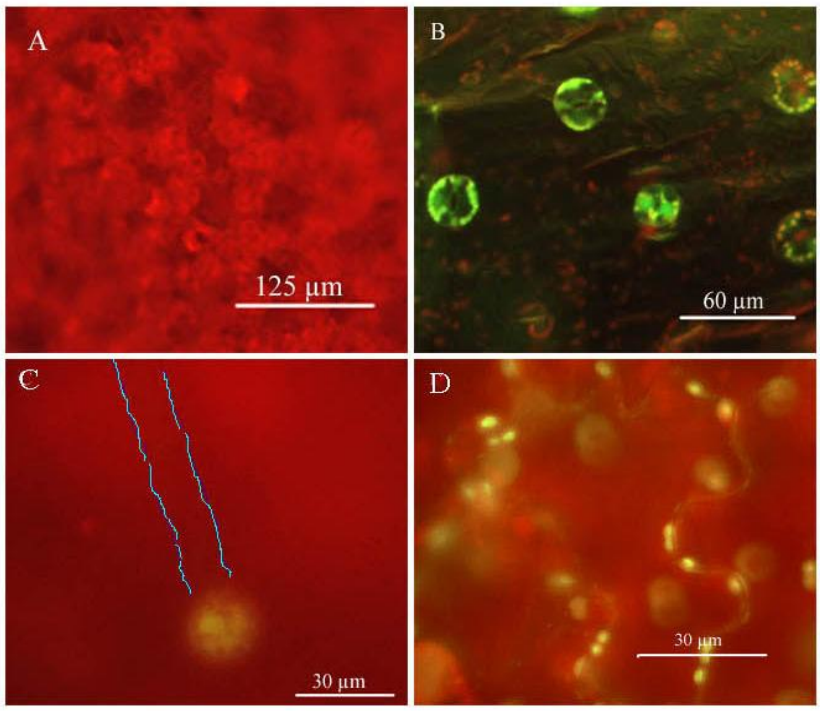

FIGURE 5: Fluorescence microscopy images of non-infiltrated leaves (negative control) stained with FlAsH.

A) A magnification were only red auto-fluorescence from chlorophylls is seen. B) Weak green signals detected in stomata cells found in the epidermis layer. C) Green fluorescence is seen in the gland-tip of finger hairs surrounded by red auto-fluorescence. The finger hair is indicated with blue. D) Small and lager green spots are seen in the epidermis layer.

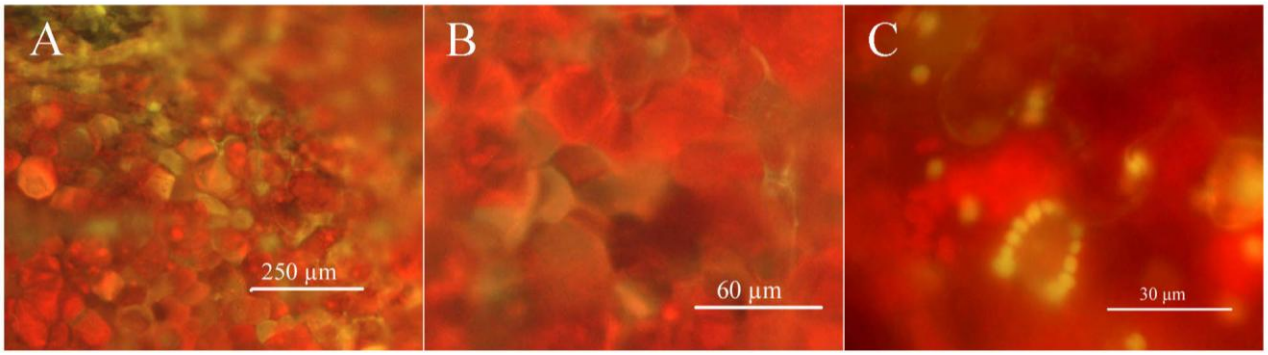

FIGURE 6: Fluorescence investigations of FlAsH stained leaf samples infiltrated with Cst. 1.

A) Green spots from bound FlAsH are shown inside the different cells, but also reflection from cells beneath the first cell layer is shown. B) A higher magnification which also shows the same green spots

as detected in A). C) Weak green spots surrounded by red auto-fluorescence found in the epidermis layer of Cst. 1 infiltrated leaf samples.

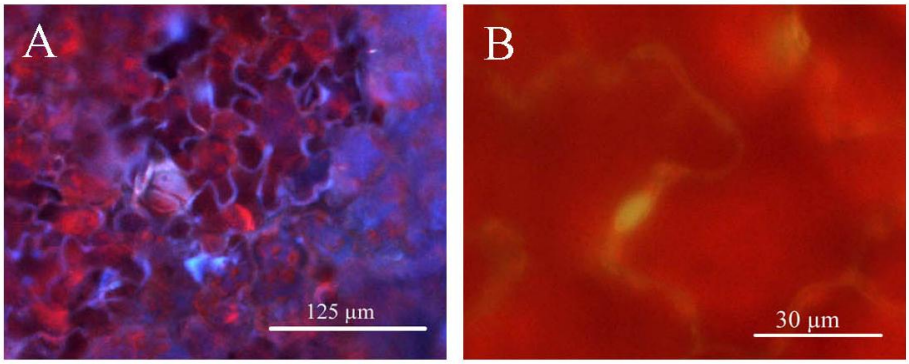

FIGURE 7: FlAsH stained leave samples 5 dpi with Cst. 5 investigated with FM.

A) Blue fluorescent signals from bound FlAsH are discovered in the cytoplasm of puzzle shaped cells. The image was taken with another filter set up (ex. 340-380 nm and em. >425 nm). B) Green spots are detected in the epidermis layer of the leaf sample.

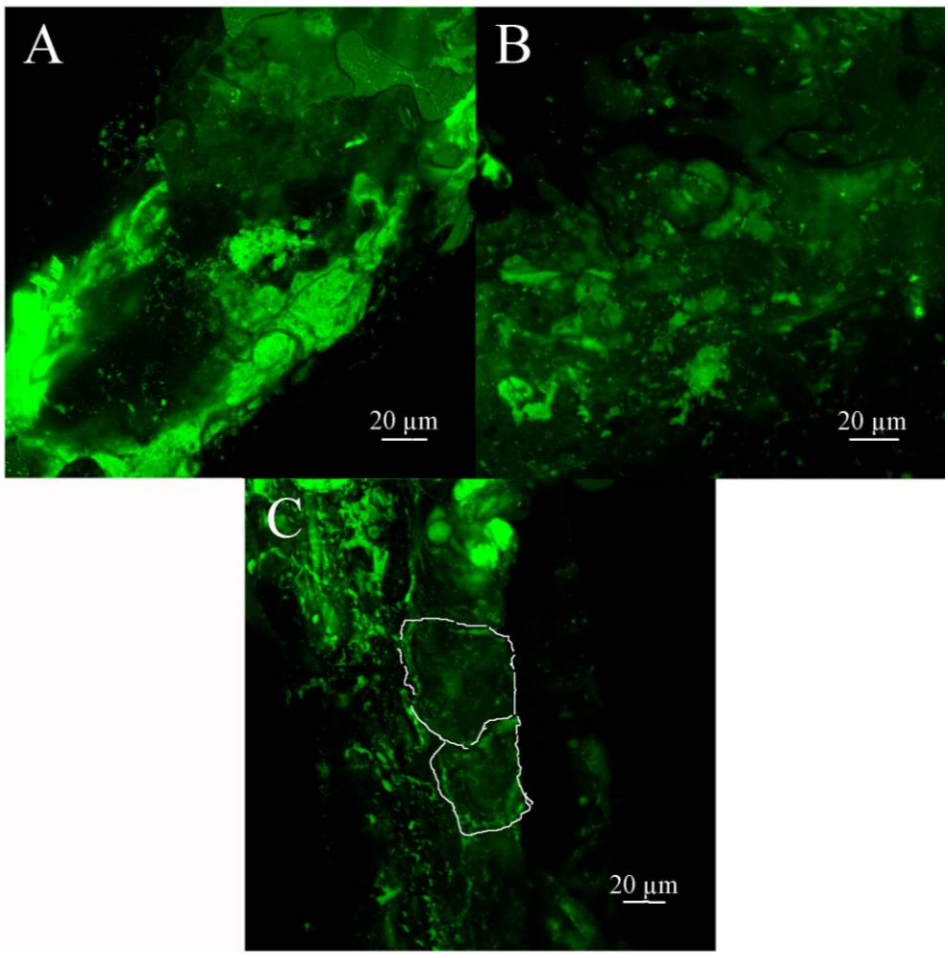

FIGURE 8: FlAsH stain leaf samples with no infiltration (negative), infiltrated with Cst. 1 and infiltrated with Cst. 5 investigated with CLSM.

All three CLSM images of the leaf samples were taken 5 dpi. A) A negative control containing both green spots and green fluorescence background signals. B) Epidermis layer of leaf sample infiltrated with Cst. 1 shows green spots and a weak green background signal from bound FlAsH. C) Cst. 5 infiltrated leaf sample shows also green spots and a weak green background signal.

References

1. Jørgensen, M., Bauw, G., and Welinder, K. G. (2006) *J Agric Food Chem* **54**, 9389-9397
2. Minor W., Steczko J., Stec B., *et al.* (1996) *Biochemistry* **35**, 10687-10701