Supplementary Data:

The Intra-Cellular Localization of the Vanillin Biosynthetic Machinery in Pods of *Vanilla planifolia*

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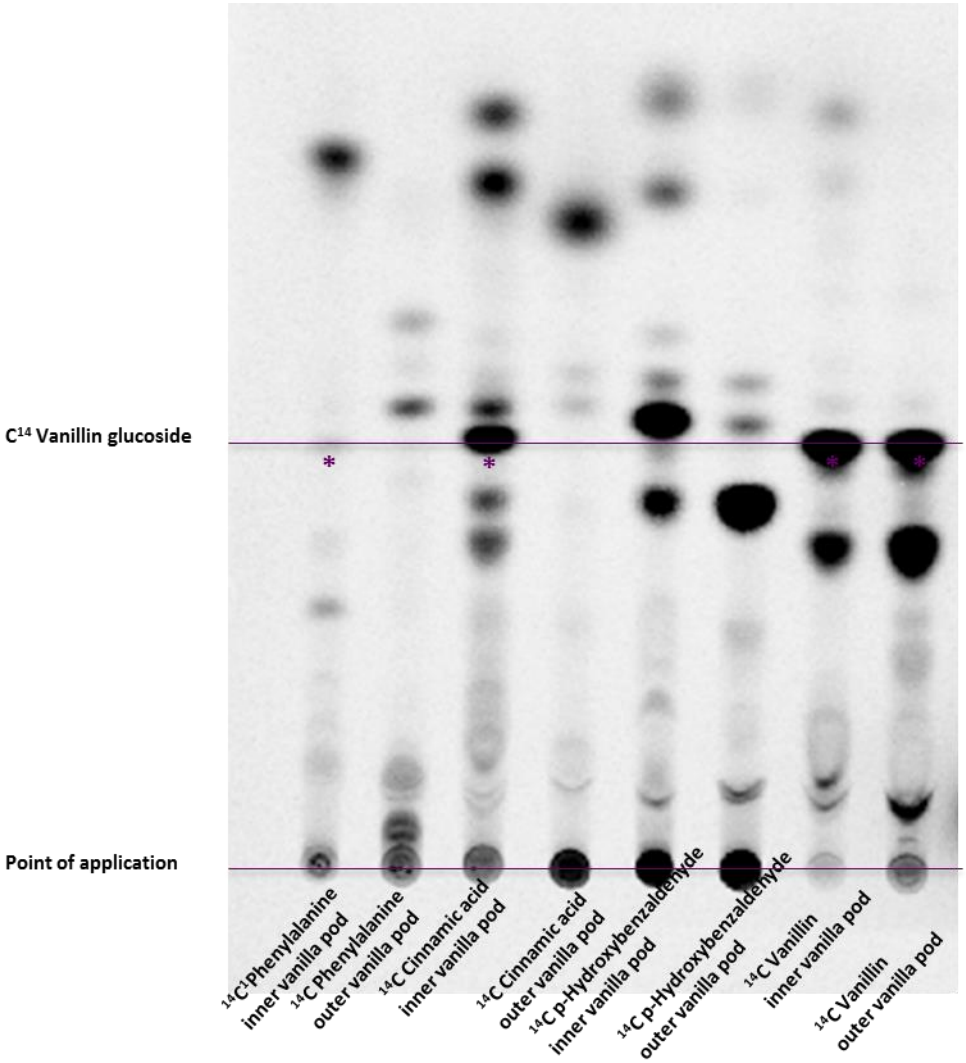
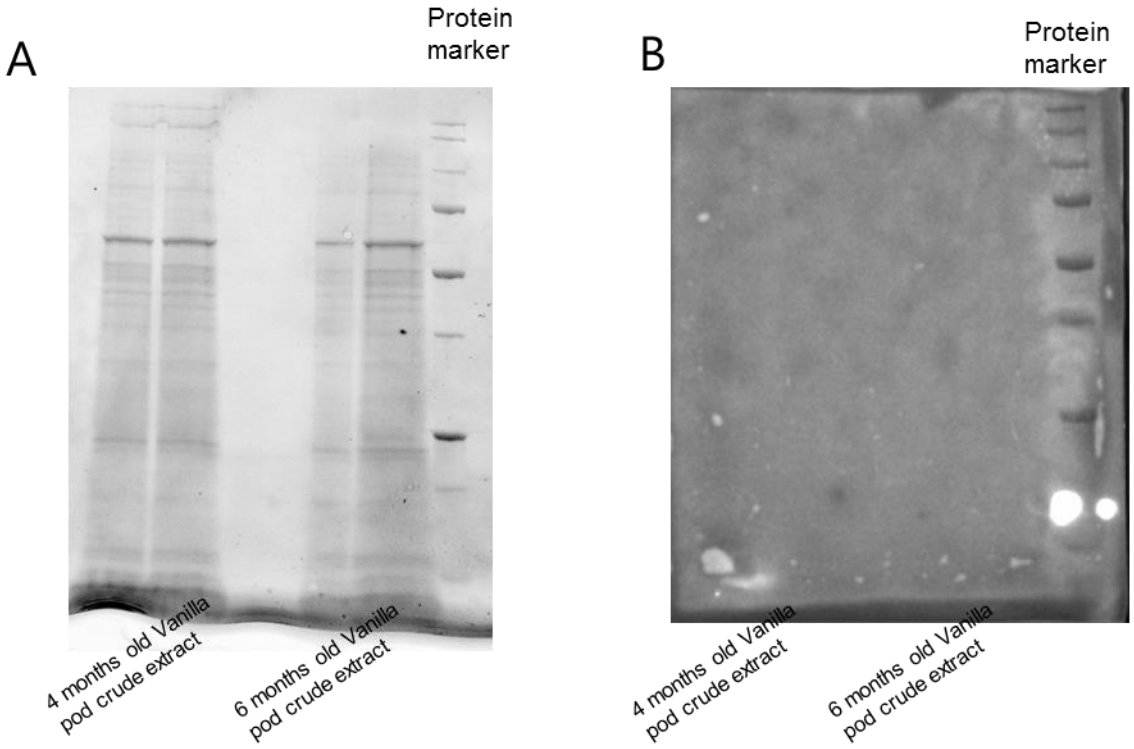
Supplementary Fig. S1: Vanillin biosynthetic activity is detected in the inner part of the pod during pod development from three- to nine-months. The vanilla pods used in this experiment were seven-months-old.



Radiolabelled putative vanillin precursors were administered to tissue slices of a vanilla pod. Formation of radiolabelled [^{14}C]-vanillin glucoside was observed following administration of [^{14}C]-phenylalanine and [^{14}C]-cinnamic acid to the inner part of tissue slices of a fresh seven-months-old vanilla pod as monitored by TLC analysis. In the inner as well as outer part of the pods, [^{14}C]-vanillin and [^{14}C]-p-hydroxybenzaldehyde were converted to [^{14}C]-vanillin glucoside and [^{14}C]-p-hydroxybenzaldehyde glucoside, respectively. (Vanillin glucoside marked with *)

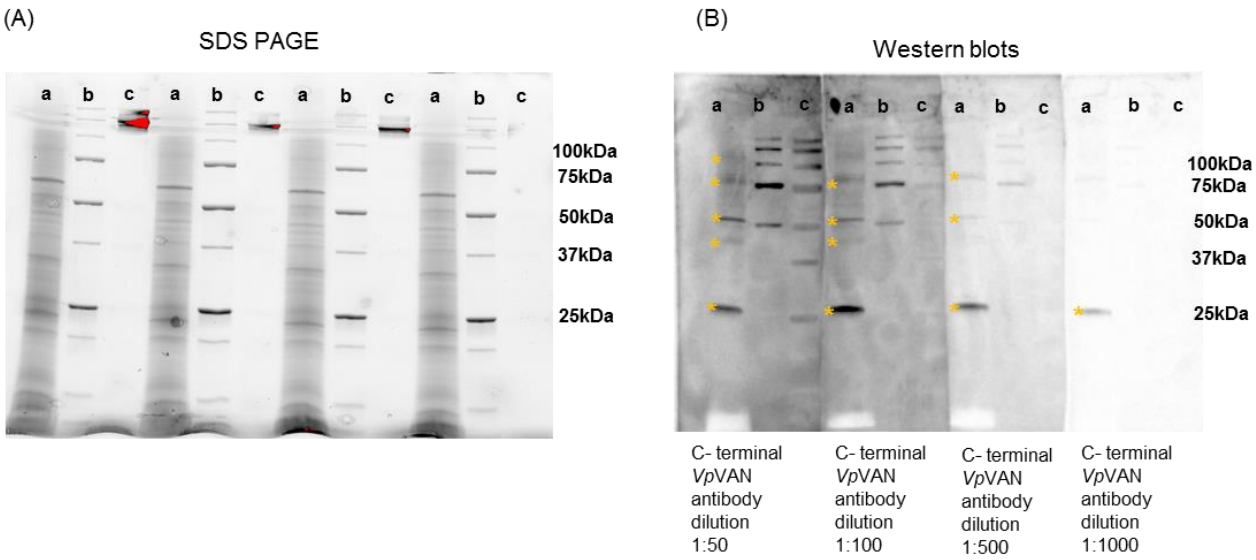
Supplementary Fig. S2: Pre-immune serum from the rabbit used to produce the *Vp*VAN antibody did not show unspecific binding to proteins present in a crude protein extract from *V. planifolia*.



Panel A: SDS-PAGE gel (TGX Stain-Free™ Precast Gels (BioRad, US) visualized using ChemiDoc MP Imaging System (Bio-Rad, US) and Panel B: Western blot

Crude proteins were extracted from four- and six-months-old *V. planifolia* pods and separated by SDS-PAGE (Panel A). Western blot of the same protein samples probed with the pre-immune serum from the rabbit used to prepare the antibody against *Vp*VAN (Panel B). About 10µg protein were applied to each lane. Pre-immune serum was used in 1:500 dilutions as requested by the producer (Agrisera). Anti-rabbit HRP secondary antibody was used in 1:5,000 dilutions. No background reactions were observed using the pre-immune serum.

Supplementary Fig. S3: Mature *Vp*VAN oligomers detected in the crude *V. planifolia* extracts as demonstrated by Western blot analysis



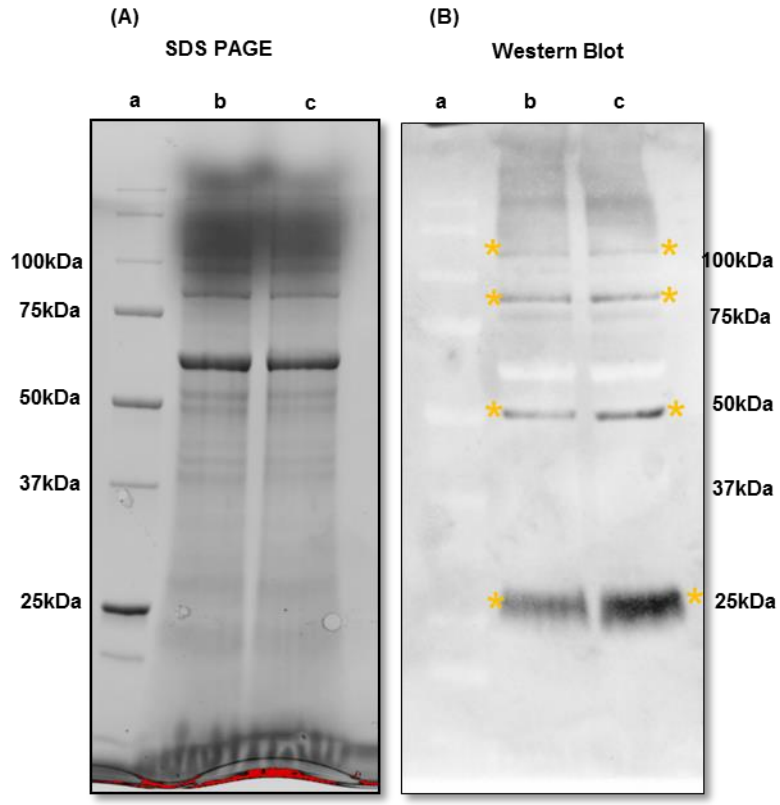
The proteins in the crude protein extract from a seven-months-old *V. planifolia* pod were separated by SDS-PAGE (Panel A) and then probed with the C- terminal specific *Vp*VAN antibody in different concentrations (1:50, 1:100, 1:500, 1:1,000) (Panel B). Anti-rabbit HRP secondary antibody was used in 1:5,000 dilutions in all samples. *Vp*VAN C-terminal specific antibody reactive bands are marked with *.

Lane (a) Crude protein extract from a seven-months-old vanilla pod from *V. planifolia*

Lane (b) Pre-stained protein ladder Bio-Rad

Lane (c) Un-stained protein ladder Bio-Rad

Supplementary Fig. S4: The ratio between monomeric and putative oligomeric forms of *Vp*VAN as analysed by treatments with reductant and oxidant as monitored by SDS-PAGE followed by Western blot analysis.



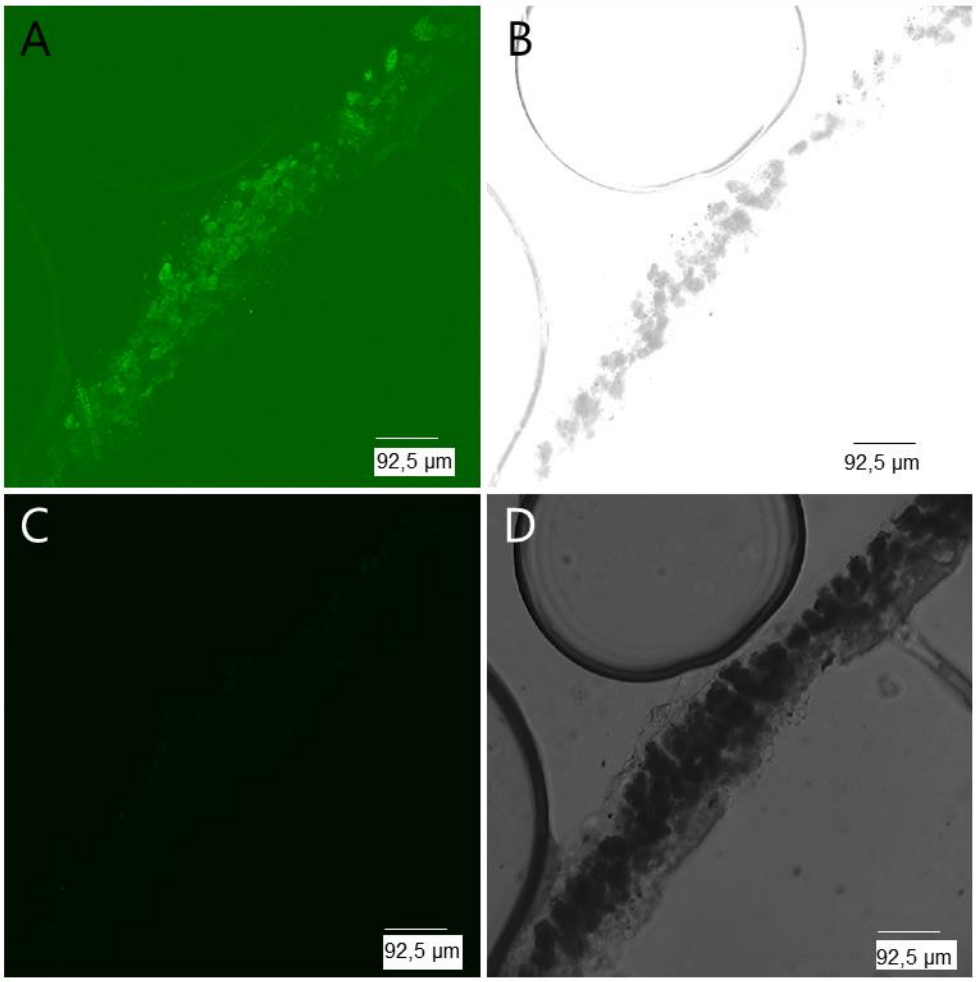
The proteins in a crude protein extract from eight-months-old *V. planifolia* pods were separated by SDS PAGE (Panel A) and analyzed by Western blotting (Panel B). Protein bands recognized by the *Vp*VAN antibody are marked with *.

Lane a: Pre-stained protein ladder Bio-Rad

Lane b: Treatment with 5mM TCEP for 15 min

Lane c: Treatment with 5mM TCEP for 15 min followed by 10mM potasium fericyanide for 30 min

Supplementary Fig. S5: The series of images collected for immunohistochemical localization of *Vp*VAN in pod discs by confocal microscopy. The instrument gain was set so that no background fluorescence was apparent in the untreated control sample.

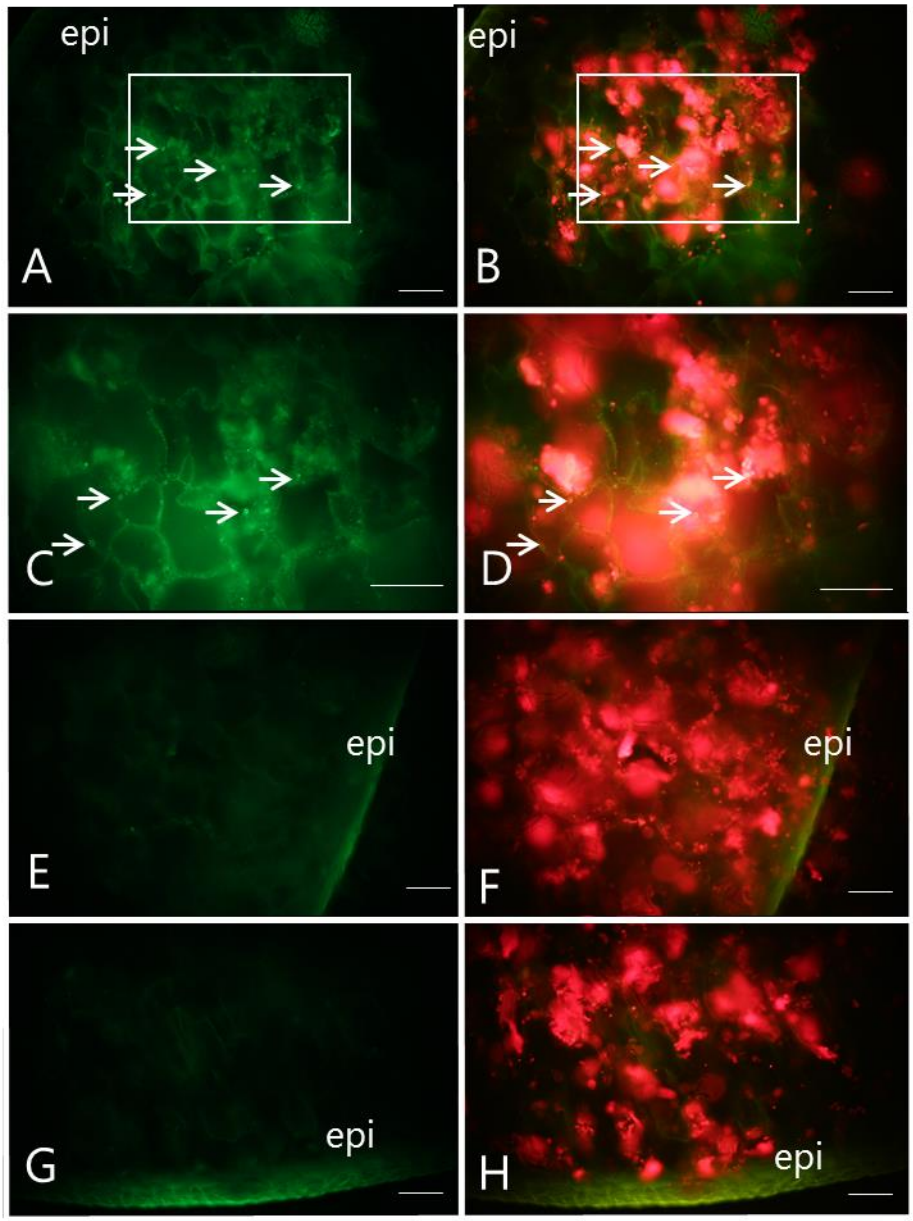


Panel A: Untreated *V. planifolia* pod discs before the gain was adjusted;

Panel B: Same *V. planifolia* pod discs after gain set.

Panel C and D: Corresponding translucent pictures for panels A and B, respectively.

Supplementary Fig. S6: Immunohistochemical analysis using a C-terminal specific antibody targeting the immature and mature form of *Vp*VAN in cross sections of a six-months-old *V. planifolia* pod monitored by light microscopy. Controls were conducted using pre-immune serum obtained from the rabbits used to produce the antibodies to the C-terminal sequence.

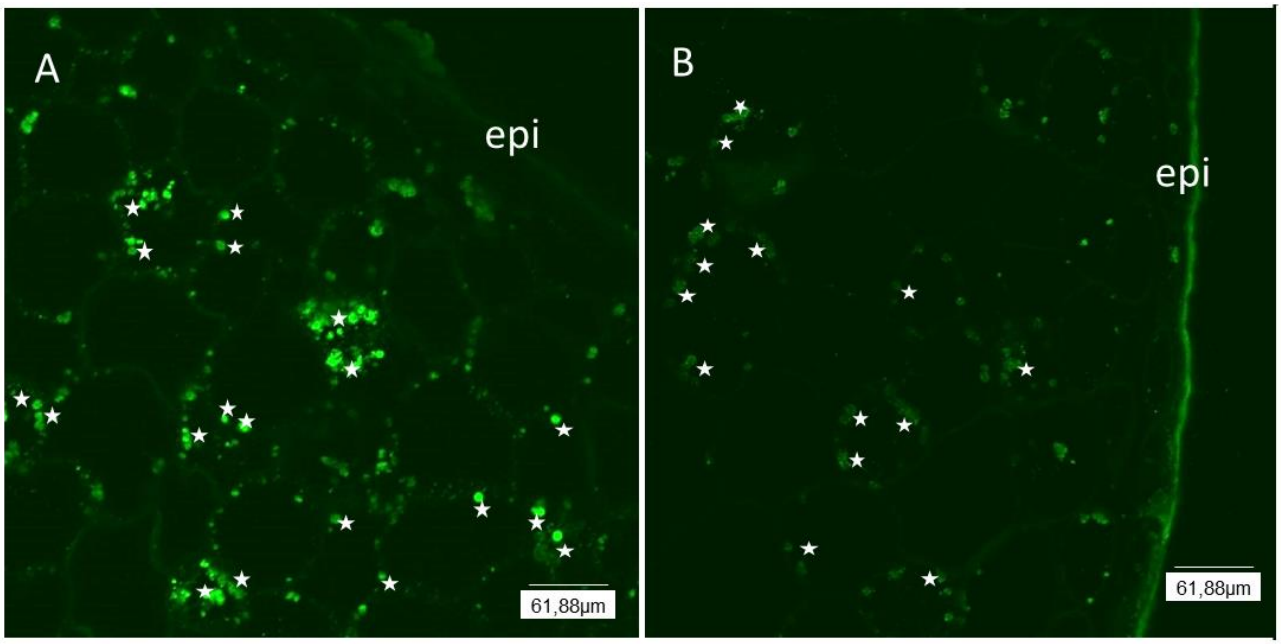


Panel A: Immunohistochemical localization of *Vp*VAN in cross section of a *V. planifolia* pod as monitored by fluorescein isothiocyanate **(**FITC) fluorescense; Panel C: close-up of the immunohistochemical localization of *Vp*VAN; Panel E: Control experiment using pre-immune serum; Panel G: Background reaction, autofluorescence and unspecific binding of the secondary antibody. Panels B, D, F and H represent the same *V. planifolia* section as used in panels A, C, E and G, respectively, but using a filter setting enabling simultaneous detection of FITC fluorescence as well as

chlorophyll autofluorescence. For all panels, the instrument gain was set to obtain no background reaction with an untreated control sample (see Suppl. Figure 5)**.**

Scale bars correspond to 100 µm. Abbreviations: epi: epicarp

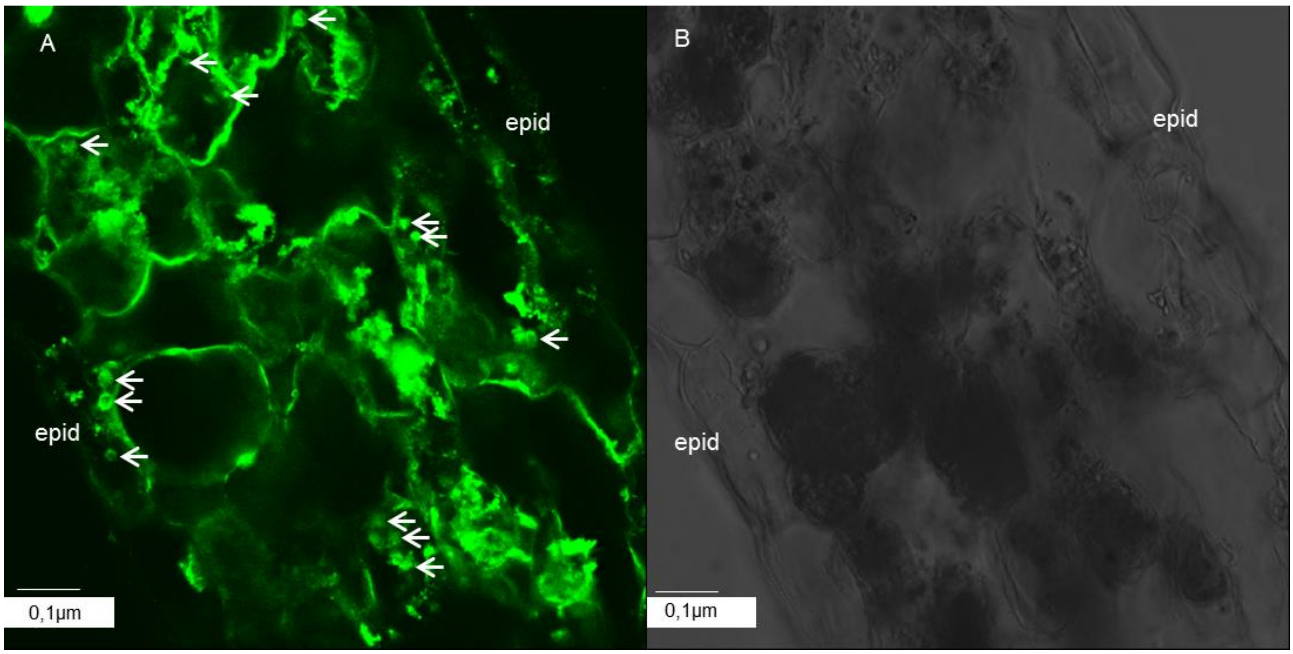
Supplementary Fig. S7: A comparison of immunohistochemical analysis using a C-terminal specific antibody targeting the immature and mature form of *Vp*VAN in cross sections of a five-months-old and a seven-months-old *V. planifolia* pods, respectively.



Panel A: Immunohistochemical analysis using a C-terminal specific antibody targeting the immature and mature form of *Vp*VAN in cross sections of a five-months-old *V. planifolia* monitored by light microscopy. Selected chloroplasts are indicated by white stars.

Panel B: Immunohistochemical analysis using a C-terminal specific antibody targeting the immature and mature form of *Vp*VAN in cross sections of a seven-months-old *V. planifolia* monitored by light microscopy. Selected chloroplasts are indicated by white stars. Abbreviations: epi: epicarp

Supplementary Fig. S8: Immunohistochemical localization of *Vp*VAN in a cross section of a tobacco leaf transiently expressing the gene encoding *Vp*VAN monitored by confocal microscopy.

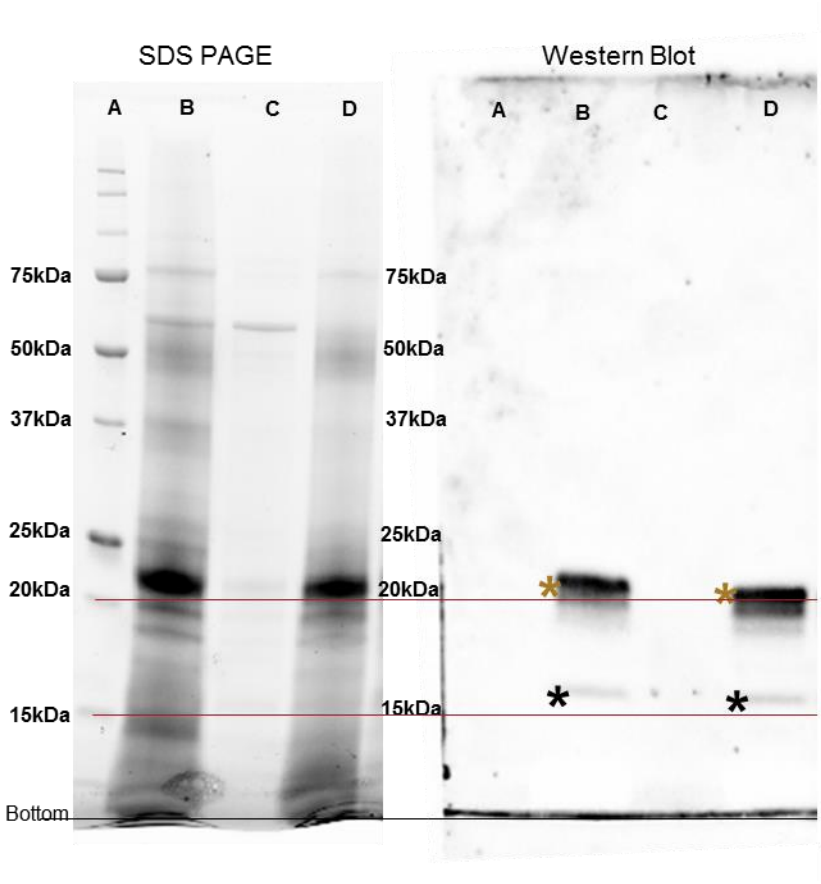


Panel A: Immunohistochemical localization of *Vp*VAN to the chloroplasts of a tobacco leaf in which the gene encoding *Vp*VAN was transiently expressed and with the antibody reaction being visualized by FITC staining and monitored by confocal microscopy.

Panels B: Same tissue section observed with translucent light.

When probed with the antibody targeting the C-terminal sequence of *Vp*VAN, the signal is primarily located in the chloroplasts (indicated by arrows) in the *Vp*VAN transiently expressed tobacco. Plant cell walls are clearly observed by their strong background fluorescence, which was also observed in control experiments in the absence of the antibody targeting the C-terminal sequence of *Vp*VAN. Abbreviations: epid: epidermis

Supplementary Fig. S9: The intact chloroplasts isolated from eight-months-old *V. planifolia* pods were analysed by SDS PAGE followed by Western blotting



The proteins in a crude protein extract of intact chloroplasts isolated from eight-months-old *V. planifolia* pods were separated by SDS-PAGE using stained free SDS gel (BioRad, US). Western blot analysis was carried out using an antibody for PSI-D, dilution 1: 10,000 (the D subunit of photosystem I). Expected mass was 18 kD (Haldrup et al., 2003). PSI-D specific binding is marked with * and unspecific binding of the antibody is marked with *.

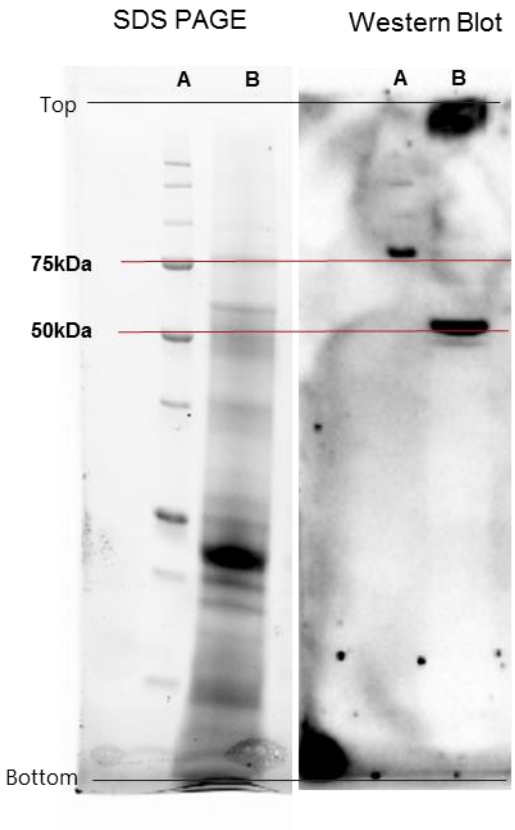
Lane A: Pre-stained protein ladder Bio-Rad

Lane B: Western blot analysis of *V. planifolia* intact chloroplast proteins

Lane C: Western blot analysis of stroma fraction of *V. planifolia* intact chloroplast proteins

Lane D: Western blot analysis of thylakoid fraction of *V. planifolia* intact chloroplast proteins

Supplementary Fig. S10: Homodimer for mature *Vp*VAN was detected in the intact chloroplasts isolated from eight-months-old *V. planifolia* pods



The proteins in a crude protein extract of intact chloroplasts isolated from eight-months-old *V. planifolia* pods were separated by SDS-PAGE using stained free SDS gel (BioRad, US) followed by Western blotting using *Vp*VAN C-terminal specific antibody 1: 300.

Lane A: Pre-stained protein ladder Bio-Rad

Lane B: Crude proteins from intact chloroplasts isolated from eight-months-old *V. planifolia* pods

Reference:

HALDRUP, A., LUNDE, C. & SCHELLER, H. V. 2003. Arabidopsis thaliana plants lacking the PSI-D subunit of photosystem I suffer severe photoinhibition, have unstable photosystem I complexes, and altered redox homeostasis in the chloroplast stroma. *J Biol Chem.* 278**,** 33276-83.