GENE NOTE

Seed legumains are expressed in stamens and vegetative legumains in seeds of *Nicotiana tabacum* L.

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Abstract

Detailed analysis of the expression pattern of seven legumain genes from *Nicotiana tabacum* L. cv. SNN revealed that it showed neither coincidences with the branches of the phylogenetic tree of legumains nor with their conventional assignment to organs. This agreed well with the fact that, so far, no functional differences could be assigned to the sequence differences reflected in the branches of the tree.

Key words: Asn-specific cysteine endopeptidases, gene expression, legumains, seeds, tobacco, vacuolar processing enzyme, vegetative organs.

Legumains are a new family of cysteine endopeptidases (C13, EC 3.4.22.34) (for a review see Münzt et al., 2002). They process polypeptide precursors by Asn-specific limited proteolysis and are involved in protein degradation. Due to their vacuolar localization and processing function, legumains have also been named vacuolar processing enzymes (VPE) (Hara-Nishimura and Maeshima, 2000).

Members of plant legumin gene families have been assigned to three different branches in a phylogenetic tree which originally were named according to differential expression patterns: E, early embryogenesis; S, seed development; and V, vegetative organs. Despite this clustering in branches no functional differences could be assigned to the underlying sequence differences (Münzt et al., 2002). Spatial and temporal expression patterns of tobacco legumains are reported here showing (i) that seed-expressed legumains are also expressed in pollen, some floral organs, seedlings, and vegetative organs, and (ii) that vegetative legumains are expressed in seeds and pollen.

Plants of *Nicotiana tabacum* L. cv. SNN were raised from seeds in pots with soil and grown in the greenhouse. Organ samples were immediately frozen in liquid nitrogen and stored at −70 °C.

Total RNA was extracted according to Chomzynsky and Sacchi (1987), except RNA extracted from seeds when the method of Becker et al. (1995) was used. Extracts were incubated with RNase-free DNase I (Roche Applied Sciences, Germany). The intactness of RNA was monitored by agarose gel electrophoresis. For semi-quantitative RT-PCR, cDNA clone-specific primer pairs were used for tobacco legumains NtPB1 (AJ238880), NtPB2 (AJ238881), NtPB3 (AJ617489), NtVPE1 (consensus primers for AB075947/075948), NtVPE2 (AB075949), and NtVPE3 (AB075950). The absence of genomic DNA was monitored by running no-RT-PCR in parallel to RT-PCR. Selectivity of the primers was confirmed by partial sequencing of DNA fragments generated by RT-PCR. Amplified fragments could also be discriminated according to ‘fragment lengths polymorphism’.

In accordance with Fischer et al. (2000) NtPB1-mRNA was detected only in 5–15 daf developing seeds whereas NtPB2 was present from 15 daf until seed maturity and in 4 dai seedlings (Fig. 1a). In addition, NtPB1 and NtPB2 genes are expressed in anthers (Fig. 1b) where NtPB2 was found at all the stages investigated, including mature pollen, but NtPB1 was found only in anthers from 2–4 cm flower buds. Mechanical dissection of anthers from 2–3 cm flower buds revealed that NtPB1-mRNA is present in maturing pollen, but not in non-pollen tissue (not shown). The presence of NtPB2 in mature pollen indicates that the signals from developing anthers are due to maturing pollen. Its presence in non-pollen anther tissue cannot be excluded. NtPB2 signals in extracts from filaments, pistils, sepals,
petals, and ovaries (Fig. 2) show that this mRNA is also present in other floral organs.

NtPB2 is a cluster S legumain. Analysis of legumain functions in *A. thaliana* and other plants (Gruis et al., 2002, 2004; Hara-Nishimura and Maeshima, 2000) had shown that cluster S legumains process seed protein precursors. Storage protein genes are expressed in pollen of various plant species (van der Geest et al., 1995; Zakharov et al., 2004). The presence of NtPB2-mRNA in tobacco pollen might indicate that NtPB2 processes storage protein precursors there as well as in seeds. Similar to δ-VPE of *A. thaliana* that does not contribute to the processing of storage globulin precursors (Gruis et al., 2002, 2004) NtPB1, which belongs to cluster E, is also thought not to be involved in this precursor processing.

All primer pairs for V-cluster legumains (NtVPE1–3) not only gave signals with extracts from vegetative organs but also from stamens (filaments, anthers, pollen), ovules, and seeds (Figs 1, 2). This agrees with the expression patterns of *A. thaliana* α- and γ-VPEs that were also found in developing seeds (Gruis et al., 2004). Conversely, weak signals of seed-specific legumains, e.g. NtPB2, could be detected in the major vegetative organs.

NtPB3 is a new tobacco legumain first detected in vegetative organs and finally cloned from 15 daf developing seeds. Its cDNA fragment encodes approximately 90% of the complete NtPB3 prepropeptide. NtPB3 like NtPB1 belongs to the E-cluster legumains. Accordingly, its expression pattern in developing seeds corresponds with that of NtPB1 except that it is also expressed in the ovules of 0 daf seeds (Fig. 1). By contrast to NtPB1, it is also expressed in vegetative organs similar to V-cluster legumains.

**Conclusions**

Similarly to storage protein genes, the tobacco legumains NtPB1 and NtPB2 are expressed not only in seeds but in pollen as well. Expression of genes encoding vegetative legumains NtVPE1–3 also happens in seeds and sexual organs of tobacco. The new legumain NtPB3 belongs to the E-cluster legumains like NtPB1, but in contrast to NtPB1 it is not only expressed during embryo- and microsporogenesis but also in vegetative organs similar to V-cluster legumains.

The classification into vegetative and seed-specific legumains according to gene expression patterns cannot be applied to *Nicotiana tabacum* legumains. This also seems to be the case with *Arabidopsis thaliana* legumains (Gruis et al., 2004; Shimada et al., 2003). The two species belong to very different taxonomic families. This raises the question as to whether, in general, the plant legumain classification according to organ specificity is still appropriate. For the present, the following nomenclature is suggested: initials of the species name followed by ‘Len’ for legumain, the letter indicating the cluster assignment (E, S or V) in the phylogenetic tree, and a number in case...
a species has more than one legumain in a cluster; for example, NtPB1, NtPB2, NtVPE3 of *Nicotiana tabacum* would become NtLenE and NtLenS and NtLenV3, according to this nomenclature. Legumains that at present cannot be assigned to one of these clusters have to be given preliminary names. Cluster names were deduced originally from the then known tissue expression patterns. The utilization of only the initial letters of these names neutralizes this original significance. The use of the general name legumains for enzymes of the asparagine-specific cysteine proteinase family C13 is recommended since these endopeptidases are not restricted to vacuoles nor are they the only processing enzymes and have only processing functions, respectively.

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**References**


