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Research article

Localization of tobacco germin-like protein 1 in leaf intercellular space

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ABSTRACT

To characterize leaf cell wall proteins relating the architectural changes of leaves, we analyzed *Nicotiana tabacum* leaf cell wall proteins that were extracted by the treatment with lithium chloride. Some of these proteins showed amino acid sequence homology to some germin-like proteins (GLP). Based of those sequences, we isolated the cDNA encoding the GLPs (NtGLP1-1, NtGLP2-1). Phylogenetic analysis including *de novo* assembled tobacco GLPs using EST clones, revealed that tobacco GLPs belong to at least 5 different subgroups of GLP and both NtGLP1 and NtGLP2 belong to GLP subfamily 3. We showed that the NtGLP1 actually localizes to cell wall and revealed that it predominantly localized at specific sites on the leaf cell wall where intercellular attachment was just bifurcated. Expression of the NtGLP1 mRNA was mainly detected in leaves especially at elongating stage. NtGLP1 is possibly relevant to development of leaf intercellular space.

1. Introduction

During development of leaves especially at the sink–source transition, several changes occur on cell shape and cell wall attachment. One of the most significant architectural changes in leaves is the increase in thickness from base to the tip with large increase in the volume of intercellular spaces and decrease in the intercellular attachment (Roberts et al., 2001). Changes in plasmodesmatal densities and architecture are also related to sink–source transition and to decrease in the intercellular attachment (Roberts et al., 2001). Though, there is little knowledge about proteins relating to these architectural changes.

In our previous study (Kishi-Kaboshi et al., 2005), we developed a method to differentially extract tobacco mosaic virus movement protein (MP), which specifically localized to branched plasmodesmata. MP tightly binds to plasmodesmata, and could be extracted after the treatment with 8 M lithium chloride. We named this MP extractable treatment and its fraction as lithium-free (LF) treatment and LF fraction, respectively. The protein profiles of LF fraction changes along leaf developmental phases. It is assumed that these proteins possibly related to cell wall changes during leaf development. Therefore, we examined the composition of protein in LF fraction and found three putative germin-like proteins (GLPs) in this report.

GLPs are plant specific members of the cupin superfamily (Khuri et al., 2001). Recently, one tobacco GLP have been reported to localize to plasmodesmata (Ham et al., 2012), but no other tobacco GLP is analyzed in respect to their localization. To characterize the property of GLPs found in LF fraction, we phylogenetically analyzed the ESTs of tobacco GLPs, raised specific antibody and analyzed its localization in detail.

2. Materials & methods

2.1. Plant materials

Nicotiana tabacum cv. Xanthi plants were maintained in a greenhouse at 22 °C with a 17 h photoperiod. Harvested from 50 to 55-day old plants were leaves shorter than 1.5 cm in length (1st leaves), 2.5–4.0 cm (2nd leaves), 5.0–9.0 cm (3rd leaves), 10–14 cm (4th leaves), 12–16 cm (5th leaves) and slightly yellow, drooping leaves (old leaves, 10–15 cm), stems between 4th and 6th leaves, and roots before the following analysis. For whole leaf analysis, second and 3rd leaves were used as “young leaf” and 5th leaf was used as “mature leaf”.

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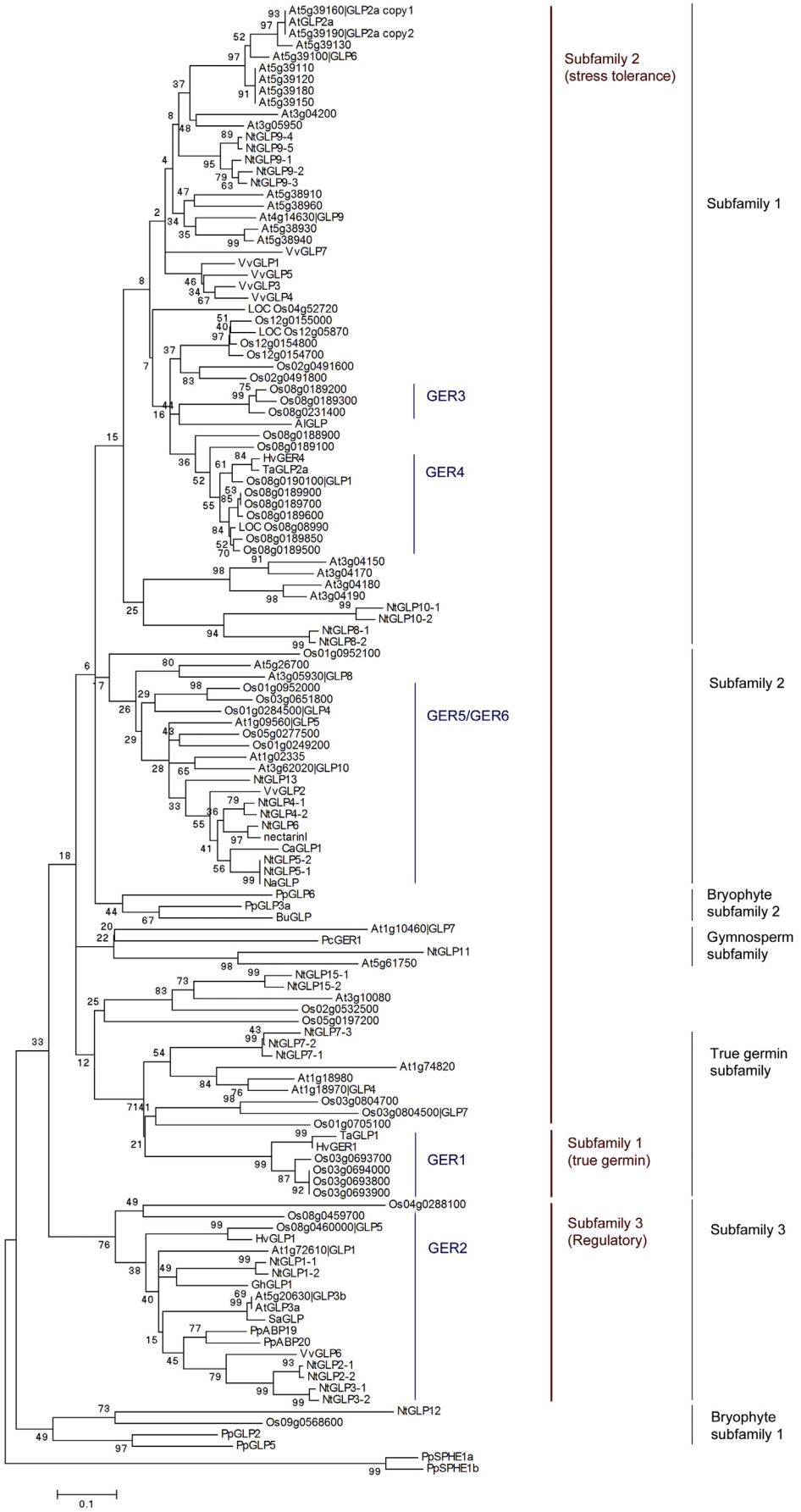
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At5g39150
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NtGLP9-3
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At3g62020|GLP10
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VvGLP2
NtGLP4-1
NtGLP4-2
NtGLP6
nectarinI
CaGLP1
NtGLP5-2
NtGLP5-1
NaGLP
GER5/GER6
PpGLP6
PpGLP3a
BuGLP
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PcGER1
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Subfamily 1
Subfamily 2
Bryophyte
subfamily 2
Gymnosperm
subfamily
True germin
subfamily
Subfamily 1
(true germin)
Subfamily 3
(Regulatory)
Subfamily 3
Bryophyte
subfamily 1