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Interactions among tobacco sieve element occlusion (SEO) proteins

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Angiosperms transport their photoassimilates through sieve tubes, which comprise longitudinally-connected sieve elements. In dicots and also some monocots, the sieve elements contain parietal structural proteins known as phloem proteins or P-proteins. Following injury, P-proteins disperse and accumulate as viscous plugs at the sieve plates to prevent the loss of valuable transport sugars. Tobacco (*Nicotiana tabacum*) P-proteins are multimeric complexes comprising subunits encoded by members of the *SEO* (*sieve element occlusion*) gene family. The existence of multiple subunits suggests that P-protein assembly involves interactions between SEO proteins, but this process is largely uncharacterized and it is unclear whether the different subunits perform unique roles or are redundant. We therefore extended our analysis of the tobacco P-proteins NtSEO1 and NtSEO2 to investigate potential interactions between them, and found that both proteins can form homomeric and heteromeric complexes *in planta*.

Keywords: sieve element occlusion, P-protein, forisome, phloem, wound response, interaction, BiFC

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SEO Genes Encode Structural Phloem Proteins in Angiosperms

SEO genes were initially described to encode forisomes, the specialized P-proteins found only in leguminous plants, which are capable of reversible conformational changes.[1-3] The discovery of *SEO* genes in other plants lacking forisomes led to speculation that they might encode the common P-proteins found in all dicots.[4-6] We therefore analyzed two tobacco SEO proteins (NtSEO1 and NtSEO2) to determine whether they were P-protein components.[7] We found that the *NtSEO1* and *NtSEO2* promoters restricted reporter gene expression to immature sieve elements, matching the expression profiles determined for these and other *SEO* genes.[3,4,8,9] P-protein subunits first become visible in immature sieve elements and accumulate as large bodies before forming a parietal layer in fully-differentiated sieve elements.[10] When expressed as hrGFP fusion proteins in tobacco phloem, both NtSEO1 and NtSEO2 assembled into native P-protein structures revealed as fluorescent protein bodies in immature sieve elements or as parietal agglomerates or large fluorescent plugs at the sieve plates of mature phloem. The heterologous expression of NtSEO1 or NtSEO2 in a non-phloem background (*Nicotiana benthamiana* epidermal cells) resulted in the formation of large complexes composed of longitudinally arranged fibrillar subunits, clearly resembling native P-protein bodies. Finally, tobacco RNAi plants showing a significant knockdown of both *NtSEO1* and *NtSEO2* were lacking the typical P-protein structures in sieve elements, finally confirming the identity between SEO proteins and P-proteins in tobacco.[7]

The generation of *NtSEO*-knockdown plants lacking P-protein structures also allowed us to test whether P-protein plugs formed at sieve plates as a consequence of wounding can prevent the loss of photosynthate as anticipated. We therefore performed exudation experiments and compared the loss of photoassimilates from NtSEO-RNAi and wild-type tobacco plants. Plants depleted for P-proteins were shown to lose nine times more sucrose

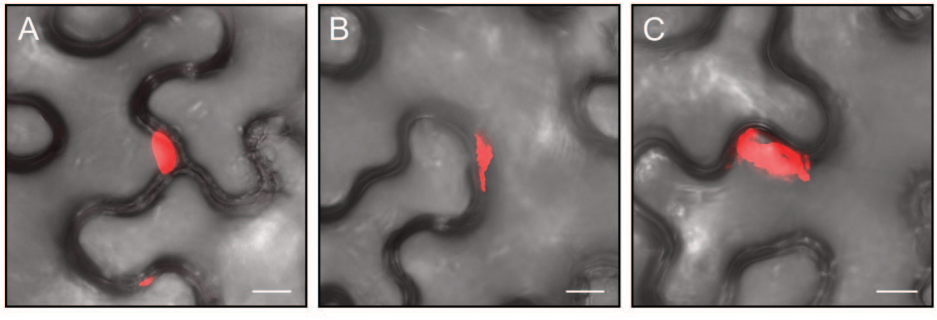

Figure 1. NtSEO-derived interaction complexes in epidermal cells of agroinfiltrated *N. benthamiana* leaves. BiFC analysis confirmed homomeric interactions for both NtSEO1 (A, the combination NmRFP:NtSEO1 + NtSEO1:CmRFP is shown as an example) and NtSEO2 (B, the combination NmRFP:NtSEO2 + CmRFP:NtSEO2 is shown as an example) as well as heteromeric interactions between NtSEO1 and NtSEO2 (C, the combination NtSEO1:NmRFP + CmRFP:NtSEO2 is shown as an example). The images are overlays of mRFP fluorescence and the corresponding transmitted light pictures. Scale bars: 10 μm.

than corresponding wild-type plants upon injury, confirming the ability of P-proteins to seal the phloem, although additional functions are certainly conceivable.[7]

NtSEO1 and NtSEO2 Form Heteromeric Complexes

Many angiosperm genomes contain multiple *SEO* genes,[4,5] raising the question of potential functional redundancy of SEO proteins within one species. This was recently addressed in a study dealing with the *Arabidopsis thaliana SEO* genes *At3g01670* and *At3g01680*.[11] We recommend that the original names (*AtSEOR1* and *AtSEOR2*, for *s*ieve *e*lement *o*cclusion *r*elated) are replaced with *AtSEOa* (*At3g01670*) and *AtSEOb* (*At3g01680*) as we proposed earlier,[4] because their direct role in sieve tube sealing is now acknowledged.[7] The analysis of T-DNA insertion mutants and corresponding complementation lines strongly indicated that interactions between AtSEOa and AtSEOb are necessary to form phloem filaments as no P-protein structures could be observed when either AtSEOa or AtSEOb was knocked out. However, yeast two-hybrid experiments demonstrated strong homomeric but no heteromeric interactions between AtSEOa and AtSEOb, contradicting the in vivo observations.[11] To address this inconsistency, we analyzed interactions between NtSEO1 and NtSEO2 *in planta* using bimolecular fluorescence complementation (BiFC).[12,13] Our previous agroinfiltration experiments addressing the assembly of NtSEO complexes demonstrated the suitability of the *N. benthamiana* background for such experiments.[7] To reduce any potential impact of the reporter fragment position, we fused split variants of the monomeric red fluorescent protein mRFP1-Q66T[14] to each end of NtSEO1 and NtSEO2 in separate constructs, using GATEWAY-compatible pBatTL vectors (resulting in pBatTL-*NmRFP*:*NtSEO1/2*, pBatTL-*CmRFP*:*NtSEO1/2*, pBatTL-*NtSEO1/2*:*NmRFP*, pBatTL-*NtSEO1/2*:*CmRFP*). Agroinfiltration experiments were performed using the 16 possible homomeric and heteromeric combinations, and infiltrated leaf discs were analyzed 4 d post-infiltration by confocal laser scanning microscopy as described before.[7] In agreement with our previous work, both NtSEO1 and NtSEO2 formed homomeric complexes in epidermal cells (**Fig. 1A, B**) but we also observed large heteromeric complexes containing both proteins (**Fig. 1C**). Indeed, NtSEO interactions were observed in all the combinations we tested.

In summary, NtSEO proteins are highly interactive, forming both homomeric and (more notably) heteromeric complexes in *N. benthamiana* leaf epidermal cells. However, the formation of heteromers could not be demonstrated for AtSEO proteins.[11] A potential explanation for the successful demonstration of SEO heteromer formation in this study might be the plant background. To examine whether this is the case or if the differing observations are species-specific, BiFC experiments should be performed with AtSEOa and AtSEOb (preferably in an Arabidopsis background).[15] The mode of interaction is an interesting aspect regarding the further characterization of SEO proteins as this would provide further insight into the functional mechanisms of P-proteins and forisomes.

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