Bridging the Gap:

Berberine Bridge Enzyme-like Proteins in *Arabidopsis thaliana*

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BACKGROUND

Berberine bridge enzyme-like proteins (BBE-like) are flavin-dependent oxidoreductases that play a role in plant defense. In *Arabidopsis thaliana*, BBE-like enzymes were found to oxidize cell wall-derived molecules, such as oligogalacturonides and cellodextrins[1]. However, the biological function of most of the *At*BBE-like enzymes remains enigmatic[2].

We found members of *At*BBE-like subgroup 6 to oxidize canonical monolignols to their corresponding aldehydes *in vitro*[3] and therefore propose an involvement in the regulation of the extracellular phenolics pool.

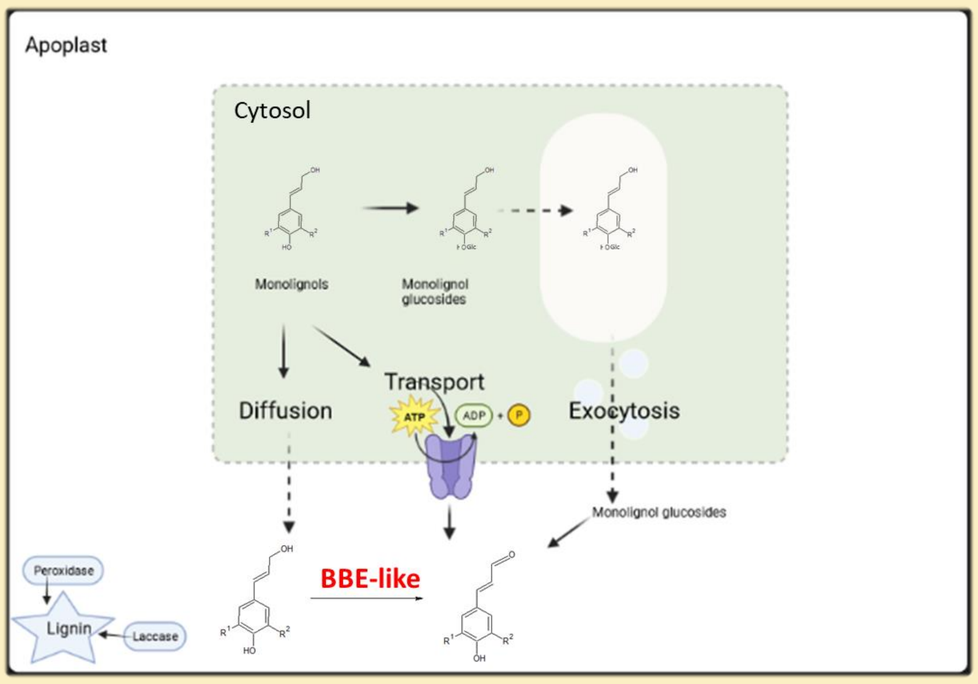

Fig. 1: Schematic overview of the monolignol pathway. Monolignols are synthesized in the cytosol and transported to the apoplast via diffusion or transporters. Monolignol glucosides are stored in the vacuole and transported to the apoplast by exocytosis. Extracellular monolignol alcohols are converted into radicals by peroxidases and laccases and subsequently incorporated into the lignin polymer. In order to maintain an extracellular monolignol alcohol- aldehyde homeostasis, BBE-like enzymes may play a key role. Created with BioRender.

RESULTS

Transcriptional GUS reporter lines show expression in seedling roots for all genes from subgroup 6.

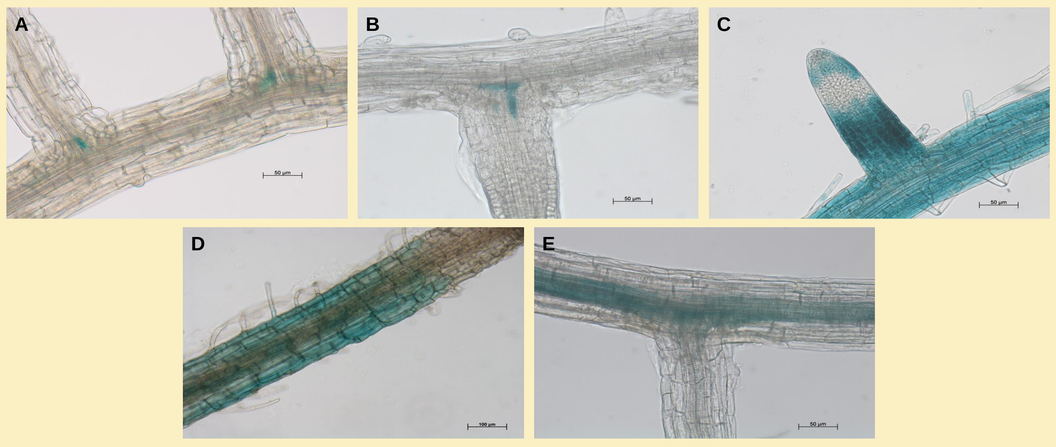

Fig. 2: GUS staining in seedling roots. A: *pAtBBE-like13:GUS*; B: *pAtBBE-like15:GUS*; C: *pAtBBE-like24:GUS*; D: *pAtBBE-like25:GUS*; E: *pAtBBE-like26:GUS*. A, B, C, E: Bar = 50 µm; D: Bar = 100 µm.

Loss-of-function of *AtBBE-like13, 15, 24* and *25* as well as the double mutants *Atbbe13,15* and *Atbbe24,25* do not exhibit obvious phenotypical changes.

RESULTS

Expression of the GUS reporters are visible in additional plant organs.

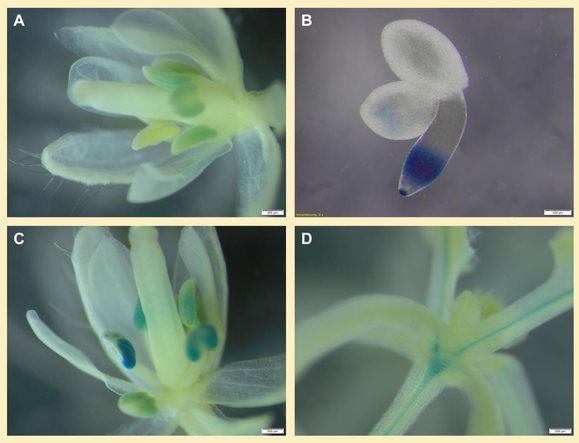

Fig. 3: GUS staining of *At*BBE-like promoter-GUS reporter lines. *A: pAtBBE-like15:GUS*; Flower with pollen staining in anthers. B: *pAtBBE-like24:GUS*; embryo with stained radicle and root tip. C: *pAtBBE-like26:GUS*; Flower with stained anthers. D: *pAtBBE-like26:GUS*; Stained conductive tissue. A, C, D: Bar = 200 µm; B: Bar = 100 µm

***At*BBE-like13, 15 and 24 are predominantly localized in the apoplast.**

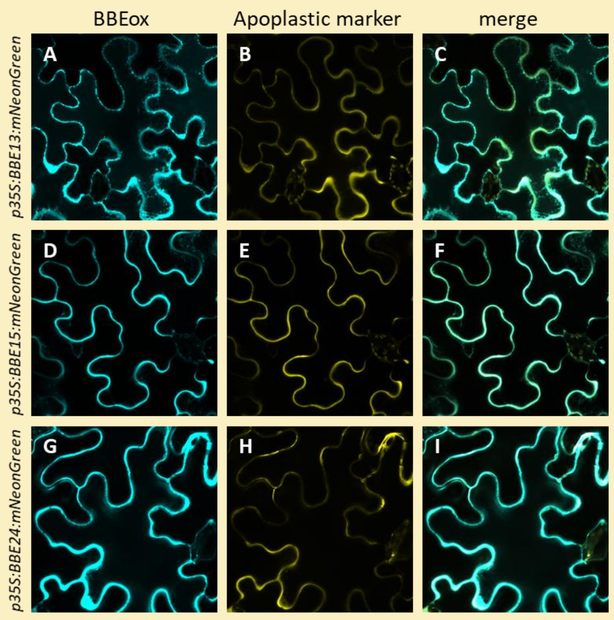

Fig. 4: Apoplastic localization of transiently expressed *At*BBE-like proteins in *Nicotiana benthamina* leaves. A-C: *At*BBE-like13; D-F: *At*BBE-like15, G-I: *At*BBE-like24. A, D, G: *p35S:AtBBE-like:mNeonGreen* fluorescent overexpressing lines (BBEox). B, E, H: apoplast localized signal peptide fused to mCherry. C, F, I: merge.

SUMMARY AND OUTLOOK

- Expression patterns of the GUS reporter are very distinct for the individual *AtBBE*-like genes from subgroup 6.
- Transcriptional reporter lines show expression in pollen (*pAtBBE-like15:GUS*), embryos (*pAtBBE-like24:GUS*) anthers and conductive tissue (*pAtBBE-like26:GUS*).
- No altered phenotype was observed in loss-of-function lines.
- A quintuple loss-of-function of all genes from subgroup 6 will be generated
- *At*BBE-like13, 15 and 24 are predominately apoplastic proteins.
- Metabolomic profiling of *Atbbe-like13, 15* and *Atbbe-like13,15* did not show changes in phenolics content.
- Translational reporter lines will be generated for *AtBBE-like25* and *26*

[1] Scortica et al. (2022) Molecular plant- microbe interactions MPMI 35 (10)

[2] Eggers et al. (2021) Phytochemistry 189. 112822

[2] Daniel et al. (2015) The Journal of Biological Chemistry 290: 18770–1878

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