

Role of class III peroxidases in stem lignification of *Zinnia elegans* Jacq.

Anastasia Tugbaeva ^{1,*}, Alexander Ermoshin ¹, Dmitry Plotnikov ¹, Hada
Wuriyanghan ² and Irina Kiseleva ¹

¹ Ural federal university, Ekaterinburg, Russia;

² Inner Mongolian University, Hohhot, China.

* Corresponding author: anastasia.tugbaeva@urfu.ru

Abstract:

Class III peroxidases (EC 1.11.1.7) have an affinity for a wide range of substrates and perform numerous functions, including the formation of lignin precursors – monolignol radicals using hydrogen peroxide. The activity and tissue localization of guaiacol (GPOX) and benzidine peroxidases (BPOX), which differ in the optimum pH (7.0 and 5.0, respectively) were determined in the first internode (relative to the hypocotyl) of *Zinnia elegans* plants of different age.

The hypocotyl length increased during 40 days from seed germination and did not change then. The Klason lignin content increased linearly up to 60 days. Histochemical analysis revealed that in juvenile plants (20 days) lignin was found mainly in protoxylem, and in adult plants (60 days) – in sclerenchyma, protoxylem and metaxylem.

Hydrogen peroxide is a marker of lignification; together with phenolic compounds, it is used by class III peroxidases to form monolignol radicals. H₂O₂ content increased in internode tissues for 40 days, and then did not change up to 60 days.

Histochemistry of enzymes revealed that BPOX was localized in endoderm, phloem, and protoxylem, while GPOX – in the metaxylem and sclerenchyma. A moderate increase of GPOX activity during internode growth was shown, and it correlated with lignin content. In contrast, BPOX activity was high at the initial growth stage, and declining to 60 days.

So the active lignification in mechanical tissue and xylem occurs during the period from 20 to 40 days. BPOX is likely involved in the processes at the early stages of growth, while GPOX is responsible for sclerenchyma and metaxylem lignification at the later stages.

Keywords: peroxidases, plant development, hydrogen peroxide, lignin, *Zinnia*

Results and Discussion

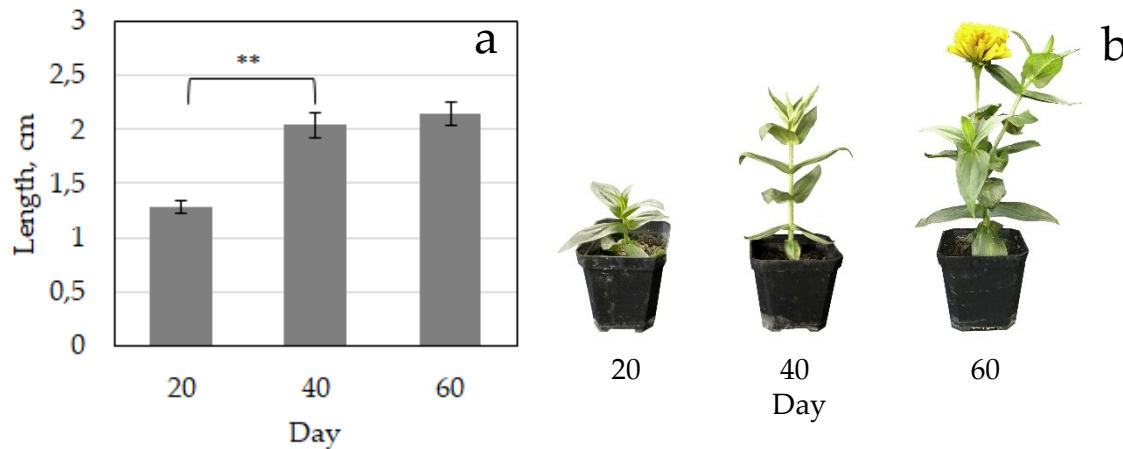


Fig. 1 *Zinnia* first internode length (a) and plants height (b) in different growth stages. The statistical significance of differences was determined by Student's t-test (**P < 0.01).

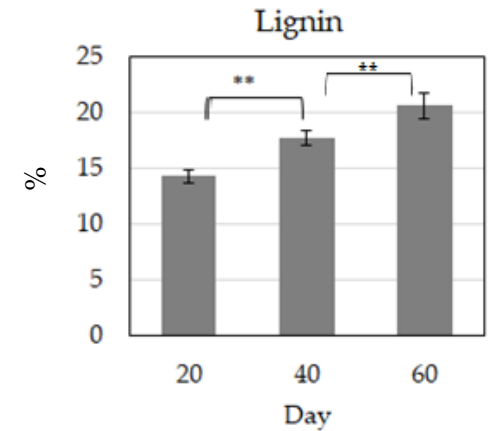


Fig. 2 The Klason lignin content in stem of *Zinnia*. The statistical significance of differences was determined by U-test (**P < 0.01).

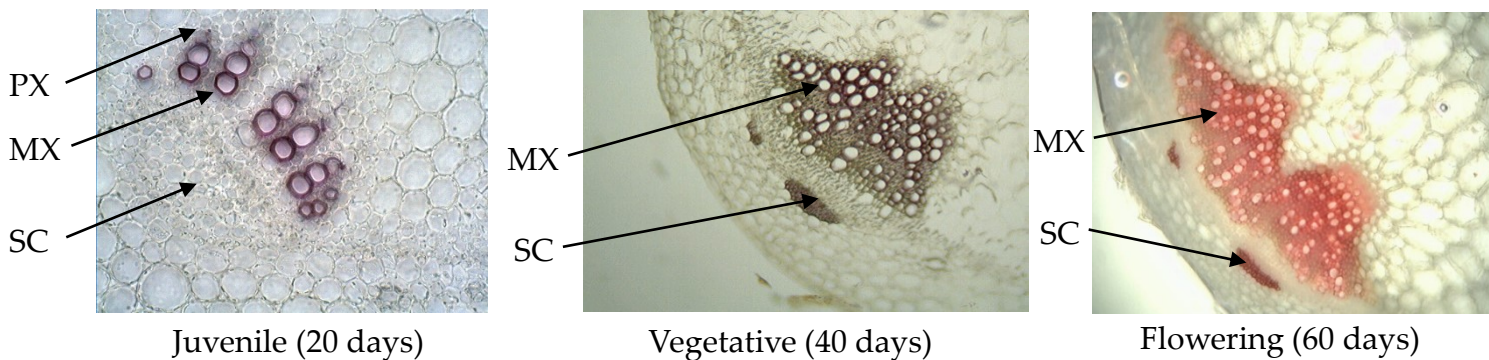


Fig. 3 Lignification of xylem and sclerenchyma in *Zinnia* plants. First internode (relative to the hypocotyl) cross section of 20, 40 and 60-days old plant were stained with phloroglucinol-HCl (protoxylem (PX), metaxylem (MX) and sclerenchyma (SC)).

Results and Discussion

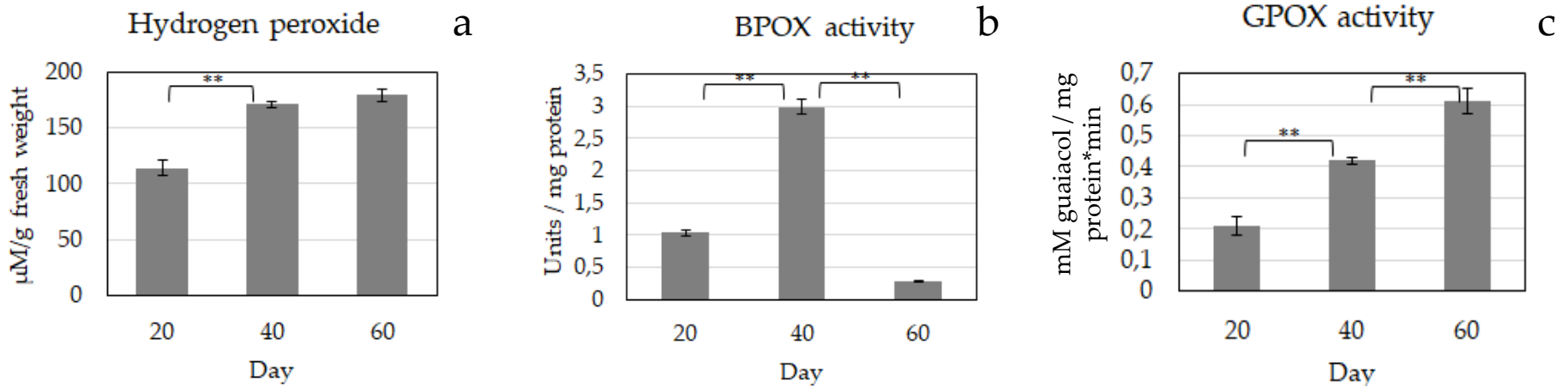


Fig. 4 H₂O₂ content (a), activities of BPOX (b) and GPOX (c) in internode tissues of *Zinnia* plants. The statistical significance of differences was determined by U-test (**P < 0.01).

According to Ros Barcelo (2004), H₂O₂ is localized mainly in non-lignified cells of stem parenchyma and protoxylem in juvenile plants, and in mechanical tissues, metaxylem, and phloem in adult.

A moderate increase of GPOX activity during internode growth was shown, and it correlated with lignin content. In contrast, BPOX activity was high at the young and vegetative growth stage, declining to flowering plants.

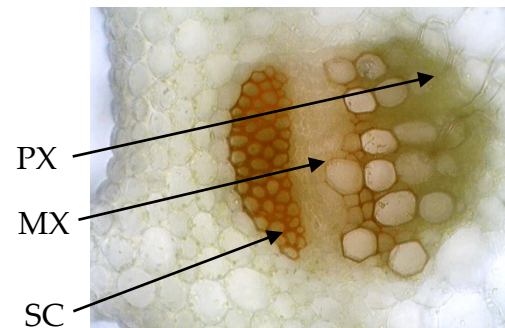
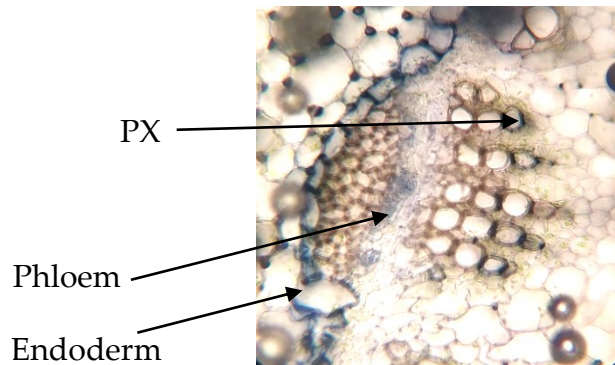


Fig. 5 Histochemical staining of BPOX (a) and GPOX (b) enzymes in *Zinnia* internode (20 day). BPOX was localized in endoderm, phloem, and protoxylem (PX), while – in the metaxylem (MX) and sclerenchyma (SC).

Conclusions

The active lignification in mechanical tissue and xylem in the first internode occurs during the period from 20 to 40 days of growth. The studied peroxidases had different tissue localisation in *Zinnia* stem. BPOX is likely involved in the processes at the early stages of growth, while GPOX is responsible for sclerenchyma and metaxylem lignification at the later stages.

Acknowledgments

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References

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