

Thigmomorphogenesis in *Solanum lycopersicum*

Morphological and biochemical responses in stem after mechanical stimulation

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The activation of the phenylpropanoid pathway in plants by environmental stimuli is one of the most universal biochemical stress responses known. In tomato plant, rubbing applied to a young internode inhibit elongation of the rubbed internode and his neighboring one. These morphological changes were correlated with an increase in lignification enzyme activities, phenylalanine ammonia-lyase (PAL), cinnamyl alcohol dehydrogenase (CAD) and peroxidases (POD), 24 hours after rubbing of the forth internode. Furthermore, a decrease in indole-3-acetic acid (IAA) content was detected in the rubbed internode and the upper one. Taken together, our results suggest that decrease in rubbed internode length is a consequence of IAA oxidation, increases in enzyme activities (PAL, CAD and POD), and cell wall rigidification associated with induction of lignification process.

In their environment, plants are constantly submitted to several stimuli such as wind, rain and wounding. The growth response of plants to such stimuli was termed thigmomorphogenesis and was observed in a wide range of plants.¹⁻³ The most common thigmomorphogenetic response is a retardation of tissue elongation accompanied by an increase in thickness.⁴ The plant response to mechanical perturbation is mainly restricted to the young developing internode, since no influence can be detected when the internode has reached its final length.^{5,6} These plant growth modifications, which characterize

thigmomorphogenesis, are related to biochemical events associated with lignification process⁷ and ethylene production.^{8,9}

In tomato plant the length of internodes 4 (N4) and 5 (N5) was measured 14 days after rubbing of the fourth internode. Results reported in **Figure 1** show that rubbing led to a significant reduction of elongation of the stressed internode (N4) (decrease of N4 length from 4.3 cm in the control plant to 2.9 in the rubbed one). This effect was not limited to the rubbed area but affected also the elongation of the neighboring internodes (N5) that were shorter in rubbed plants than in control ones.

Results reported in **Figure 2** show an increase in PAL activity in both internodes N4 and N5, 24 hours after mechanical stress application as compared with corresponding controls. CAD activity was also investigated in N4 and N5, 24 h after rubbing of the fourth internode. Results presented in **Figure 3** show that mechanical stress application induces a strong increase of CAD activity in the rubbed internode N4 (5.3 nkatal μg^{-1} protein) with an approximately two-fold increase when compared to control tomato internodes (2.3 nkatal μg^{-1} protein). Further, CAD activity in N5 was also increased in the rubbed internode (5.538 nkatal μg^{-1} protein) as compared with the control one (3.256 nkatal μg^{-1} protein).

Syringaldazine (S-POD) and gallicol (G-POD) peroxidase activities were measured in tomato N4 and N5. Results reported in **Figure 4** show an increase in soluble peroxidase activity with both

Key words: Mechanical stimulation, PAL, CAD, POD, IAA

Abbreviations: PAL, phenylalanine ammonia-lyase; CAD, cinnamyl alcohol dehydrogenase; POD, peroxidases; IAA, auxine

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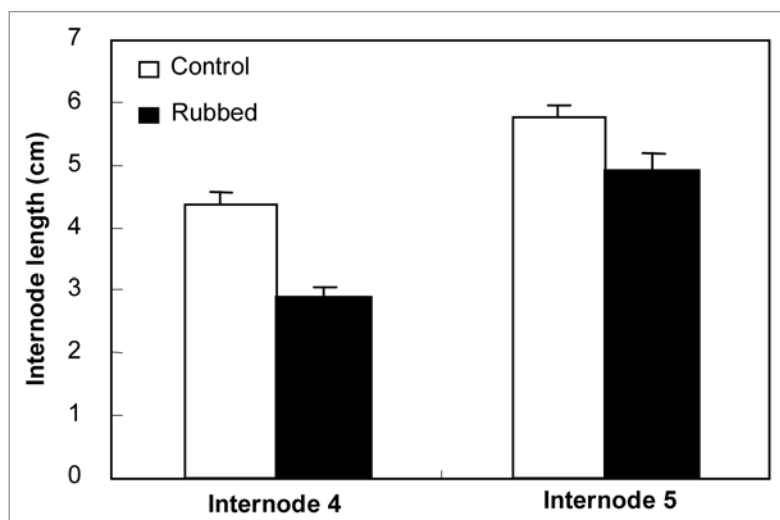


Figure 1. Internode lengths of control and rubbed plants measured 14 day after mechanical stress applied to the fourth internode. Standard errors are indicated by vertical bars.

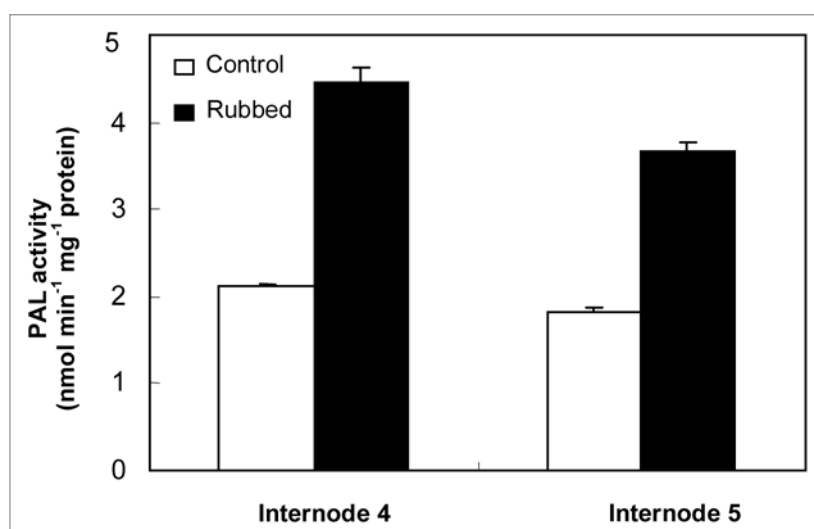


Figure 2. PAL activity of internode 4, and 5 in control and rubbed plants 24 h after rubbing of the fourth internode. Standard errors are indicated by vertical bars.

substrates in the rubbed internode N4 as compared with control plant. Enhancement in peroxidase activities in N4 was more pronounced with gallicol (80.7 U) as an electron donor than syringaldazine (33.8 U). Similar results were observed in internode 5 as compared with control one (Fig. 4).

IAA was quantified in control and rubbed plant internodes 24 h after rubbing of the fourth internode. Results reported in figure 5 show that in control sample and as expected, the content of IAA was found to be higher in the younger internode (N5) as

compared to the older one (N4). Rubbing led to a significant decrease in IAA levels in N4 (5.06 nmol g⁻¹ MF⁻¹) as compared with corresponding controls (7.27 nmol g⁻¹ MF⁻¹). Similar results were observed in internode 5, where IAA content was reduced from 16.52 nmol g⁻¹ MF⁻¹ in control internode to 12.35 nmol g⁻¹ MF⁻¹ in the rubbed internode (Fig. 5).

The results reported here establish an evident correlation between growth limitation of the rubbed internode and their degree of lignification, the increase in lignification enzymes activities and

auxin degradation after mechanical stress application.

Auxin seems to be involved in thigmomorphogenesis.¹⁰ It was proposed that MIS (Mechanically-induced stress) has opposite effects on auxin levels in the two species studied to date, *Phaseolus vulgaris*¹⁰ and *Bryonia dioica*.^{11,12} Auxin level as measured by bioassay, increased in *Phaseolus vulgaris* following rubbing of the stem.¹⁰ It was proposed that a build up of auxin may result from the reduced polar transport of IAA at the rubbed internode, causing a build up of IAA in the stem tissue. Exogenous IAA did not reverse the MIS inhibition of growth in *Phaseolus vulgaris* and high levels of IAA retarded growth in non-stressed plants.¹⁰ Thus, retardation of extension growth in *Phaseolus vulgaris* may have been caused by high levels of endogenous auxin and the increase in stem diameter by increased ethylene production.⁴ However, ethylene increases radial growth only if auxin is present.¹³

Boyer¹¹ reported a decrease in auxin-like activity in *Bryonia dioica* following MIS and this was confirmed in the same species by Hofinger et al.¹² who reported a decrease in IAA using gas chromatography-mass spectrometry. Auxin catabolism was accompanied with changes in both soluble and ionically bound cell wall basic peroxidases¹⁴ and the appearance of an additional peroxidase. This can suggest that in *Bryonia*, auxin catabolism is hastened by mechanical stimulated peroxidase. In addition, Boyer et al.¹⁵ reported that lithium pre-treatment prevents both thigmomorphogenesis and appearance of specific cathodic isoperoxidase in *Bryonia* plants subjected to MIS. This is give further credence to the possibility that the peroxidase-auxin system is involved in *Bryonia* thigmomorphogenesis. In addition, ethylene increases peroxidase activity which reduces the auxin content in the tissue to a level low enough not to support normal growth. We have evidence that decrease of auxin level contribute to mechanism leading to tomato internode inhibition subjected to mechanical stress.

Growth inhibition has been suggested to be the result of tissues lignification.⁶ As the initial enzyme in the monolignol biosynthesis pathway, PAL has a direct influence on lignin accumulation.¹⁶

The characteristics of lignin differ among cell wall tissues and plant organs.¹⁷ It comprises polyphenolic polymers derived from the oxidative polymerization of different monolignols, including p-coumaryl, coniferyl and sinapyl alcohols via a side pathway of phenylalanine metabolism leading to lignin synthesis.¹⁸ The increase in lignin content in the rubbed tomato internode could be a response mechanism to mechanical damage caused by rubbing.³ It is known that plants create a natural barrier that includes lignin and suberin synthesis, components directly linked to support systems.^{19,20}

The increase in lignin content of rubbed tomato internode³ is paralleled by a rise in CAD activity and whilst such direct proportionality between CAD activity and lignin accumulation does not always agree with the results in the literature, it clearly is responding in ways similar to those of the other enzymes in the pathway.²¹

Mechanical stress-induced membrane depolarization would generate different species of free radicals and peroxides, which in turn initiate lipid peroxidation.²² The degradation of cell membranes is suggested to bring about rapid changes in ionic flux, especially release of K⁺ which would result in an enhanced endogenous Ca/K ratio and in leakage of solutes, among them electron donors such as ascorbic acid and phenolic substances. The increased intracellular relative calcium level activated secretion of basic peroxidases²³ into the free space where, in association with the electron donors and may be with the circulating IAA, they eliminate the peroxides, and facilitated binding of basic peroxidases to membrane structures allowing a role as 1-aminocyclopropane-1-carboxylic acid (ACC)-oxidases. The resulting IAA and ACC oxidase-mediated changes in ethylene production²⁴ would further induce (this time through the protein synthesis machinery) an increase in activity of phenylalanine ammonia-lyase and peroxidases. The resulting lignification and cell wall rigidification determines the growth response of tomato internode to the mechanical stress.

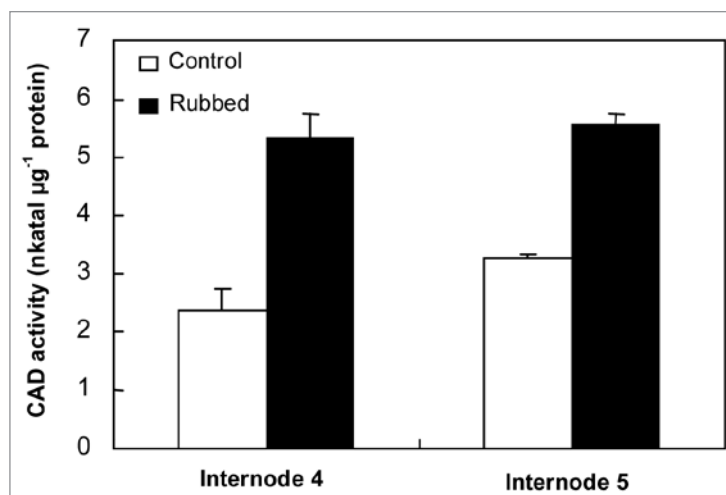


Figure 3. CAD activity of internode 4, and 5 in control and rubbed plants 24 h after rubbing of the fourth internode. Standard errors are indicated by vertical bars.

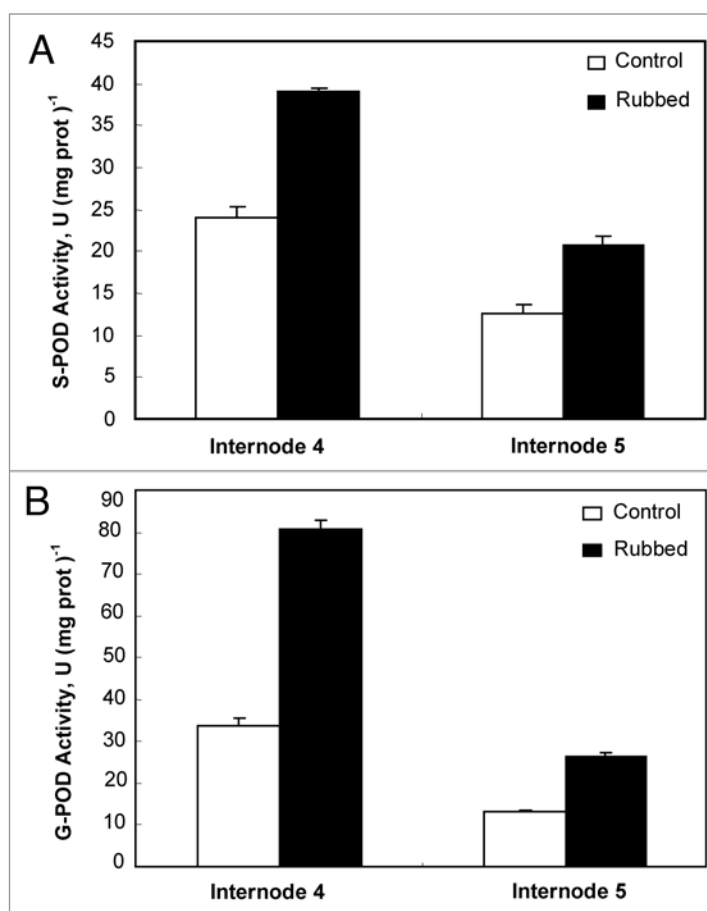


Figure 4. (A) Syringaldazine-POD (Syr-POD) activity of internode 4 and 5 in control and rubbed plants 24 h after rubbing of the fourth internode. Standard errors are indicated by vertical bars. (B) Gaiacol-POD (G-POD) activity of internode 4 and 5 in control and rubbed plants 24 h after rubbing of the fourth internode. Standard errors are indicated by vertical bars.

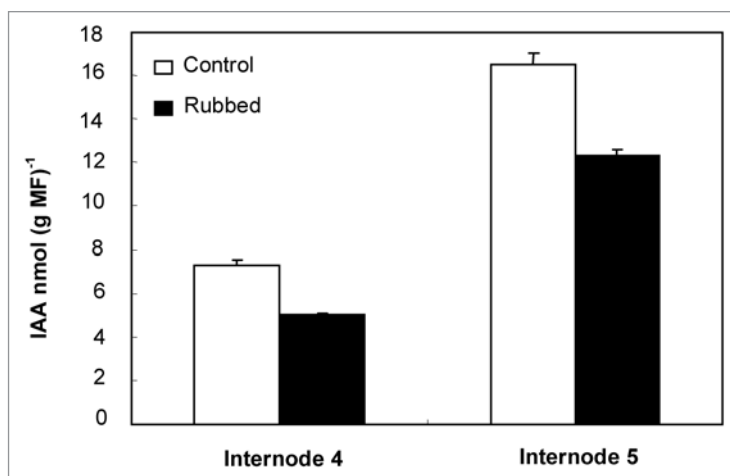


Figure 5. IAA Level of internode 4 and 5 in control and rubbed plants 24 h after rubbing of the fourth internode. Standard errors are indicated by vertical bars.

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