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Article Addendum

Multiple Signals Regulate Nicotine Synthesis in Tobacco Plant

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Addendum to:

Nicotine Synthesis in* Nicotiana tabacum L. *Induced by Mechanical Wounding is Regulated by Auxin

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ABSTRACT

After wounding of tobacco plants, roots synthesize a large amount of nicotine to be transported to the shoot. Jasmonic acid (JA) acts as a long-distance signal between the wounding stimulus and response in tobacco plants. In addition, another phloem-mobile signal (auxin) plays a role in the transmission of the message triggered by wounding. Auxin can serve as a negative signal to regulate nicotine synthesis in roots of tobacco plants, even when plants are not wounded. Furthermore, removing the shoot apex after girdling the stem base still stimulates nicotine synthesis in roots. Since girdling prevented the involvement of signals transported in the phloem, this wound likely induced a response of nicotine synthesis in roots regulated by a signal transported via an alternative pathway. The results suggest that there are multiple signals in tobacco plant to regulate nicotine synthesis, depending on the treatment.

Unlike animals, which can escape or move to a more suitable place, or food and water, when under attack by other animals or from environmental stresses, higher plants cannot change site after germination, because their roots are anchored in the soil, and hence plants have to adjust to biotic or abiotic stress factor they are exposed to. Indeed, plants do not simply endure these stresses, but acclimate with different strategies. For example, plants produce a suite of secondary metabolites, some of which functioning as defenses against herbivores by reducing their performance, survival, or reproduction.[1] The increase in nicotine synthesis in tobacco roots upon wounding of the shoot is one of the typical responses of plants to avoid further herbivore attack.[2] For transduction of the stimulus and response in different organs of a plant, long-distance signals are necessary. Higher plants use different signals that are transported over a larger distance, including electric, hydraulic and chemical signals.[3-8] Jasmonic acid (JA), an endogenous plant signaling molecule is produced in wounded tissue, and acts as a long-distance signal regulating nicotine synthesis in roots of tobacco. Mechanical wounding of tobacco leaves induces a 10-fold increase in JA concentration in damaged leaves within 90 min, and systemically in the roots (3.5-fold) 180 min after wounding; nicotine concentrations in leaves reaches its peak 5 days after wounding.[9-13]

We recently demonstrated that besides JA, a second long-distance signal, i.e., auxin, plays a role in tobacco plants to transmit the wounding signal and to regulate nicotine synthesis. We found that the nicotine production in tobacco plant is not correlated with the degree of wounding, but highly dependent on auxin status in the plant, since removal of the apical meristems (either only the shoot apex or also the lateral buds), and thus a source of auxin, had a much stronger effect on nicotine synthesis in the root than either a much more severe wounding of the young leaves or excision of the mature leaves.[14] Auxin can serve as a plant-specific 'neurotransmitter'[15] and inhibits a number of wound-induced responses.[16–19] Our results suggest that there are at least two kinds of signal molecules, i.e., JA and auxin, involved in long-distance signaling in tobacco plants to transduce the stimulus in the shoot and enhance the nicotine synthesis in the roots. Similarly, in tomato the severity of the wounding is a major determinant of the type of wound signaling. The wounding response following mechanical wounding is the systemic synthesis of defense proteins, known as proteinase inhibitors (PIs). A chemical elicitor of PI synthesis transported in the phloem is induced by minor wounding; while severe wounding produces outcomes that are consistent with the distribution of elicitors of PI synthesis and of electrical activity by hydraulic dispersal in the xylem.[15]

Further study revealed that application of *N*-1-naphthylphthalamic acid (NPA), an auxin-transport inhibitor, around the stem directly under the shoot apex of intact plants caused an increase in nicotine concentration in the whole plant, although the treatment

Table 1 **IAA and JA concentration in stem tissue 90 min after removing the shoot apex or 210 min after application of NPA**

	Treatments		
	Control	Removing apex	NPA
IAA concentration (ng g^{-1} FW)	82 ± 19	59 ± 13	37 ± 12
JA concentration (ng g^{-1} FW)	5.6 ± 0.5	133.2 ± 25.2	3.7 ± 0.4

Samples of different treatments were taken at the same position, either below the cut-surface after excision of the shoot apex, or below the position of NPA application. GC-MS (Trace 2000-Voyager, Finnigan, Thermo-Quest, USA) was employed to analyze IAA and JA concentration. Means ± SE, n = 3.

did not involve any wounding of the plant.[14] In comparison with control plant, the NPA treatment caused a marked decrease in IAA concentration, but did not affect the JA concentration in the stem tissue below the position of NPA application (Table 1), implying that JA was not involved in the regulation of nicotine synthesis caused by NPA application. These results support the contention that auxin serves as a negative signal to regulate nicotine synthesis in roots of tobacco plants. Moreover, removing the shoot apex after girdling with a 1 cm-long device at the stem base still stimulated nicotine synthesis in roots of tobacco (C. Li, W. Teng, unpublished results). Since the girdling excluded involvement of a phloem-mobile signal, this wound-induced response of nicotine synthesis in roots may have been regulated by electric or hydraulic signals transported via the xylem, as in tomato after severe wounds.[15] Together with the effect of JA and auxin in the regulation of nicotine synthesis mentioned above, the results suggest that there is more than one signal type in tobacco to regulate the same process, i.e., nicotine synthesis, depending on the treatments. The interactions between these signal types remain to be illustrated.

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