Translocation and accumulation of nicotine via distinct spatio-temporal regulation of nicotine transporters in *Nicotiana tabacum*

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**Keywords:** alkaloid, MATE, nicotine, tobacco, translocation, transporter

**Abbreviations:** MATE, multidrug and toxic compound extrusion; MeJA, methyl jasmonate.

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In plants, secondary metabolites play important roles in adaptation to the environment. Nicotine, a pyridine alkaloid in *Nicotiana tabacum*, functions as a chemical barrier against herbivores. Nicotine produced in the root undergoes long-distance transport and accumulates mainly in the leaves. Since production of such defensive compounds is costly, plants must regulate the allocation of the products to their tissues; however, the molecular mechanism of nicotine translocation remains unclear. Our recent studies identified a novel multidrug and toxic compound extrusion (MATE)-type nicotine transporter, JAT2 (jasmonate-inducible alkaloid transporter 2). This transporter is specifically expressed in leaves, localizes to the tonoplast, and transports nicotine as its substrate. The specific induction of JAT2 expression in leaves by methyl jasmonate (MeJA) treatment suggests that this transporter plays an important role in nicotine distribution to leaves, especially under herbivore attack, by transporting nicotine into the vacuole. Considering JAT2, together with the previously identified MATE transporters JAT1, MATE1, and MATE2, and the PUP (purine permease) transporter NUP1 (nicotine uptake permease1), we show a model of nicotine translocation and accumulation via distinct spatio-temporal regulation of nicotine transporter expression. Furthermore, we discuss the possible role of nicotine transporters in determining outcrossing rates and seed production.

Plants produce a vast number of secondary metabolites with specific activities (such as the anti-bacterial and anti-herbivore activities of some alkaloids) that likely play important roles in plant adaptation to environmental stresses. Because the production of secondary metabolites as a chemical defense is costly to plants, the biosynthesis of these metabolites is systematically controlled and some are specifically induced by environmental changes such as herbivore attack. Earlier studies reported that the allocation of chemical defense compounds within a plant is correlated with the value of tissues; that is, tissues of high value receive more secondary metabolites to improve their defenses. Several transport systems and multiple transporters likely function in the distribution of these metabolites from the organ of biosynthesis to other organs for accumulation, as reported for long-distance transport of sucrose and auxin. Several transporters responsible for the transport of a single secondary metabolite have been identified; however, how spatial and temporal regulation of expression of such transporters regulate inter-organ movement of a secondary metabolite remains unclear. Metabolite transport requires a plasma membrane-localized efflux or influx transporter in the biosynthetic tissue, and both plasma and vacuolar membrane-localized transporters in the tissue that accumulates the metabolite. Several findings about nicotine translocation, including our recent identification of a leaf-specific nicotine transporter, provided insights into this mechanism.

*Nicotiana tabacum* plants produce the pyridine alkaloid nicotine only in root tissues; nicotine translocates to the aerial parts of the plant via the xylem. Due to the strong toxicity of nicotine to the
nervous system of insects, nicotine accumulation in *Nicotiana* species functions as a defense mechanism. Since production of nicotine has high metabolic costs, its production remains at basal levels under normal conditions, but increases to higher levels in plants attacked by insects or herbivores. This induction occurs through methyl jasmonate (MeJA) signaling. Upon up-regulation of nicotine production, nicotine content in the xylem fluid increases, and ultimately nicotine concentration in leaves also increases. Therefore, nicotine transport from source tissues to sink organs is an excellent model system to analyze the organization of multiple nicotine transporters.

Our recent study identified a novel nicotine transporter, JAT2 (jasmonate-inducible alkaloid transporter) (Fig. 1). This transporter belongs to the multidrug and toxic compound extrusion (MATE) transporter family, which has diverse physiological functions, including in transport of divergent secondary metabolites, translocation of iron, and plant hormone signaling. JAT2 is specifically expressed in leaves, localizes to the tonoplast and shows nicotine transport activity. These characteristics suggest that JAT2 acts in nicotine sequestration in the leaf vacuole (Fig. 1). In addition, previous work has identified and characterized 3 other MATE transporters, MATE1, MATE2, and JAT1, and one PUP (purine permease) transporter, NUP1 (nicotine uptake permease) (Fig. 1). MATE1 and MATE2 show high sequence similarity, are specifically expressed in roots, and function in vacuolar accumulation of nicotine in roots, where nicotine biosynthesis takes place. NUP1 is abundantly expressed in root tips, where nicotine biosynthesis is highly active. This plasma membrane-localized transporter takes up nicotine into the cells from the apoplast. NUP1 increases nicotine production in roots by positively regulating the expression of ERF189, a transcription factor controlling nicotine biosynthesis. JAT1 is expressed in roots, stems and leaves, localizes to the tonoplast in the leaves, and shows nicotine/proton antiport activity. JAT1 may function in vacuolar nicotine accumulation, at least in green leaves.

All MATE transporters are rapidly (within 2 h) induced by MeJA treatment, enabling the efficient transport of increased amounts of nicotine. An early study reported the selective distribution of nicotine to younger leaves, which are tissues of high fitness value, at the elongation stage. Enhanced expression of JAT2 in both younger and older leaves suggests the involvement of this transporter in distribution of regulated amounts of nicotine to leaves (Fig. 1). Surprisingly, JAT1 protein seems to localize to the plasma membrane or endosomes in the root tissues. JAT1 might function in nicotine efflux from cells and loading into the xylem in roots, although this will require further investigation. Understanding the mechanism of nicotine efflux at the plasma membrane of root cells and the mechanism of nicotine import at the plasma membrane of leaf cells will clarify
the mechanism of nicotine translocation throughout the plant.

At the flowering stage, nicotine is also allocated to reproductive tissues. In flowers, benzyl acetone attracts pollinators and nicotine repels pollinators, thus affecting rates of outcrossing and seed production. MATE1 and MATE2 transcripts are present at very low levels in flowers. JAT1 shows high expression in flowers during flower development, and is expressed in petals, pistils, and stamens of fully open flowers at stage 12 (Fig. 2). In contrast, JAT2 is faintly expressed in the petal, and its protein is slightly smaller than that found in leaves. These expression patterns suggest that tobacco nicotine transporters might affect reproductive success by transporting nicotine in flowers.

These findings provide, to our knowledge, the first example that several transporters play important roles in the translocation and accumulation of a single secondary metabolite via distinct spatial and temporal regulation of their expression patterns, and contribute to our understanding of plant mechanisms of adaptation to the environment through multiple transporters.

Research on transport of secondary metabolites has emerged as a hot topic of late, and several transporters have been identified. These transporters may have applications in engineering of metabolite transport for production of valuable compounds. Further identification and characterization of transporters of secondary metabolites would be useful for the molecular breeding of transgenic plants with high contents of bioactive metabolites in specific organs.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Figure 2. Expression of Nt-JAT1 and Nt-JAT2 proteins during tobacco flower development. Flowers were sampled at stage 1 (bud), stage 7 (corolla tube bulge at tip of calx), and stage 12 (fully open flower); stages defined previously. Senesced flowers were defined as stage 13. Petals, stamens, and pistils were sampled from flowers of stage 12. Membrane proteins were extracted from each part and subjected to SDS-PAGE and immunoblot analyses using anti-Nt-JAT1 and Nt-JAT2 antibodies.
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