# The Role of Cytokinin in Root-Shoot Communication


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## Introduction:

The development of higher plants as supracellular organisms requires coordination between distant organs in order to adjust the developmental pattern and rate under variable environmental conditions. The plant vascular system serves as a conduit for the delivery of both nutrients and signaling molecules to the various distantly located organs. Among the known signaling molecules, are Cytokinins (CKs), a class of phytohormones known to be involved in cell division and expansion and delaying senescence (Zürcher and Müller, 2016). CKs have been regarded as root synthesized and are translocated to the shoot via the xylem. However, today we know that CKs are also synthesized in aerial parts of the plant. The first step of CK biosynthesis is controlled by Isopentenyltransferase (IPT), which is the key enzyme determining CK concentration.

Studies with CK biosynthesis mutants indicated that root derived CKs are important for normal shoot growth (Kiba et al., 2013), however, despite of intense interest in root-to-shoot signaling the complete influence of the long-distance translocated CKs on development and functioning of shoot organs such as leaf photosynthetic rate is still not clear.

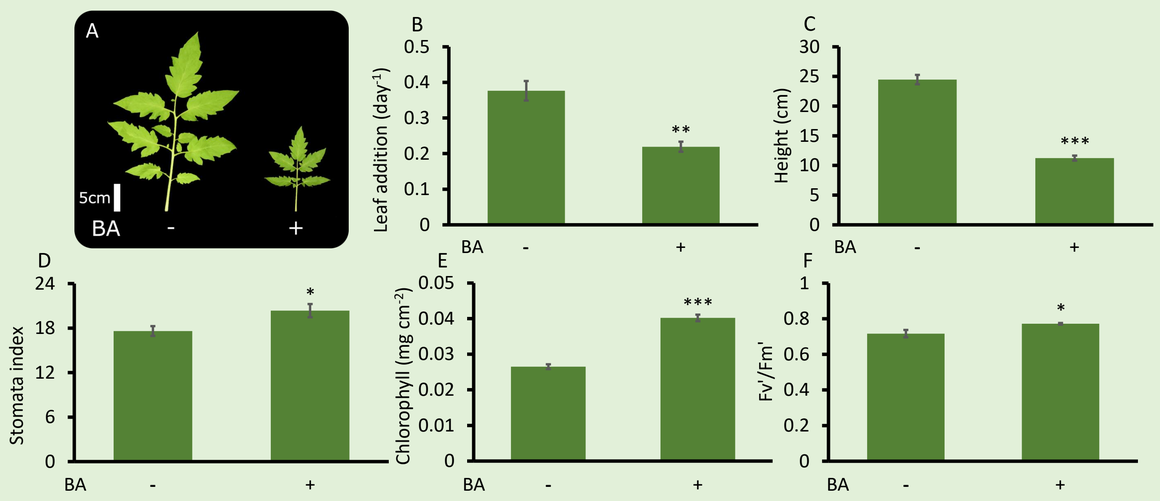


**Figure 1. Effect of BA applied to the root system on growth rate, chlorophyll concentration and photosynthetic activity of young source leaves.** Picture of young source leaf (A), leaf addition per day determined by plastochron index (B), height (C), stomata index, measured as the percentage of stomata from epidermal cells (D), chlorophyll concentration (E) and Fv'/Fm' (F), which is the measure for the efficiency of excitation capture by Photosystem II. Measurements were taken from young mature source leaves of tomato plants (var. M82) grown in a hydroponic system with and without 1μM of the synthetic cytokinin, 6-Benzyladenine (BA). Values are means ± SE (n=5-9). *P<0.05, **P<0.005, ***P<0.0001, using Student's t-test.

## research objective:

The presented research was aimed at exploring the effect of root borne CK on shoot growth, leaf development and functioning in tomato plants.

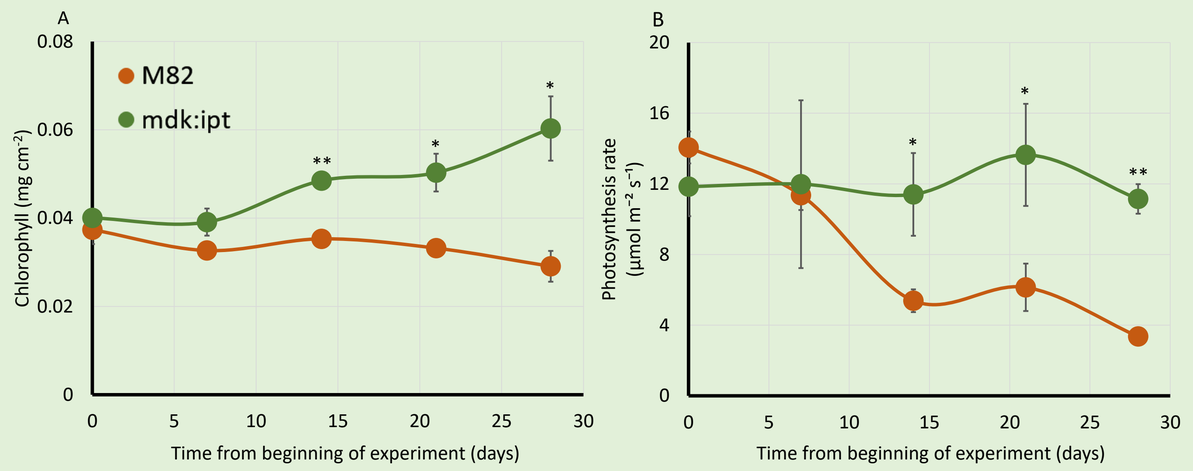


**Figure 3. Effect of root expression of *ipt* on chlorophyll concentration and photosynthesis of mature leaves.** Measurements of chlorophyll concentration (A) and photosynthesis (B), of transgenic plants expressing *ipt* under the root (*mdk*) promoter and M82 control tomato plants grown in a temperature-controlled greenhouse (25°C/18°C day/night). Values are means ± SE (n=4). *$P < 0.05$, *P<0.05, **P<0.005 using Student's t-test.

## Results:

External application of CK is a simple way to examine the influence of root-derived CK on shoot growth. To this aim, tomato plants were grown in a hydroponic system with and without a synthetic CK (6-Benzylaminopurine; BA). BA absorbed by the root caused a reduction in leaf size and shape (Fig. 1A). Growth rate of these plants was lower and they were shorter (Fig 1B,C). On the other hand, stomata index and chlorophyll content were higher (Fig 1D,E), as well as Fv'/Fm' value, which is a measure for the maximal efficiency of photosystem II (Fig 1E).

To further explore the specific influence of CK that was synthesized in the root, we over expressed the *AtIPT7* gene in transgenic tomato plants under the control of a root specific promoter (*mdk* - Lilley et al., 2011). These plants were significantly smaller and shorter than control plants (Fig 2A,C,E). It is important to note that overexpression of IPT mainly in source leaves (under the control of *FBPase* promoter) had much smaller effect on the shoot's phenotype than its overexpression in the root (Fig. 2B,D vs. A,C). Importantly, leaf chlorophyll concentration was higher in mdk:ipt plants and leaf senescence was delayed, as indicated by the constant photosynthetic rate for a period of four weeks (Fig 3A,B).

Grafting experiments confirmed that manipulation of CK biosynthesis in the roots significantly affected the development and functioning of the shoot. Again, chlorophyll concentration in leaves of control (M82) scions grafted on mdk:ipt rootstocks was higher than in homografted control plants (Fig 4A,D), and shoot growth rate was inhibited (Fig 4B,D E).

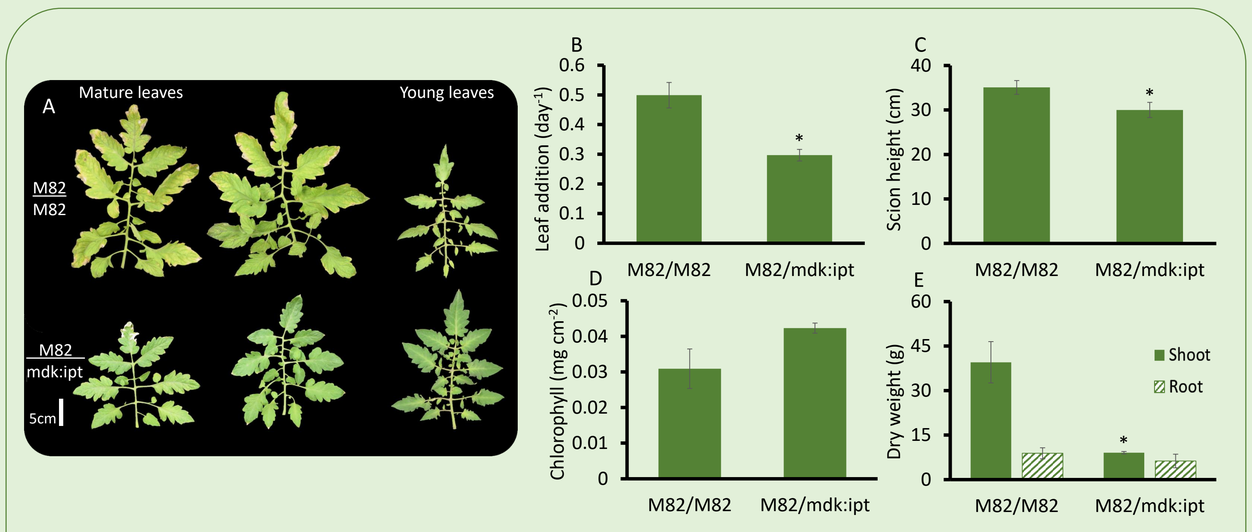


**Figure 4. Effect of rootstock expressing *ipt* on the phenotype and growth of control scions.** Picture of young and mature M82 scion leaves (A). Leaf addition per day, determined by plastochron index (B), scion height (C), chlorophyll concentration in young mature source leaves (D), shoot and root dry weight (E), of control (M82) scions grafted on transgenic plants expressing ipt under root (*mdk*) promoter or control rootstocks. Grafted plants were grown in a temperature-controlled greenhouse (25°C/18°C day/night). Values are means ± SE (n=3-6). *$P < 0.05$, using Student's t-test.

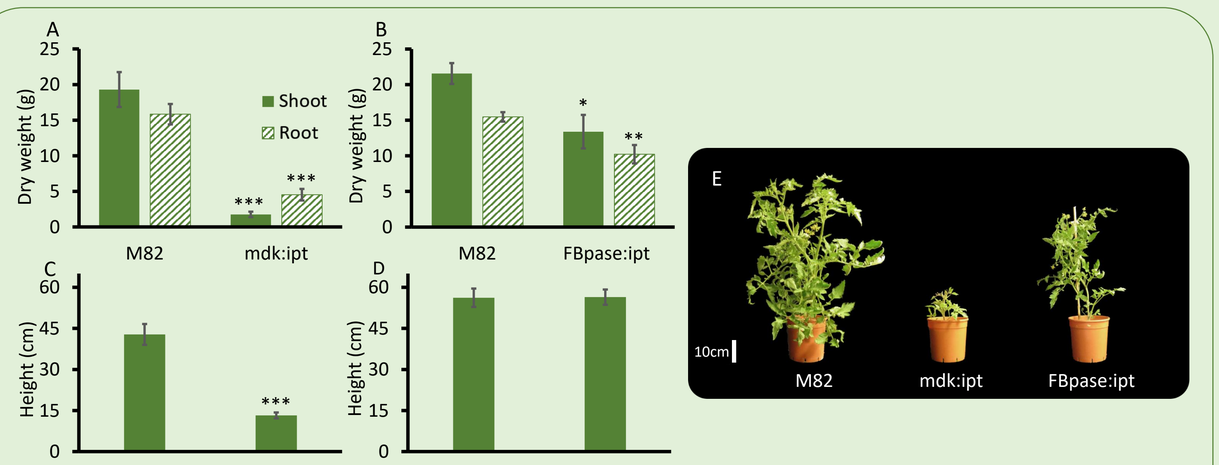


**Figure 2. Effect of root or source leaf expression of *ipt* on plant growth.**
Shoot and root dry weight (A,B) and height (C,D) of transgenic tomato plants expressing *ipt* under the root (*mdk*) promoter (A,C) or source leaf (*FBpase*) promoter (B,D) as compared with M82 control plants. Picture of 49 day-old control (M82), mdk:ipt and FBpase:ipt transgenic plants (E), grown in a temperature-controlled greenhouse (25°C/18°C day/night). Values are means ± SE (n=5-8). *P<0.05, **P<0.005, ***P<0.0001, using Student's t-test.

## Conclusions:

Root specific expression of *ipt* and grafting experiments established that root-derived cytokinin is a key player in modulating shoot growth and leaf functioning. Trans-zeatin (tZ) type molecules are the major xylem translocated CK (Kiba et al., 2013). Our current research is aimed at manipulating the biosynthesis of tZ in the roots. An increase in translocation of tZ to the developing shoot may inhibit leaf senescence and increase photosynthetic activity.

Understanding of the endogenous mechanisms stimulating CK translocation to the shoot may enable the development of biotechnology strategies to increase productivity and crop yield.

## References:


**Kiba T, Takei K, Kojima M, Sakakibara H** (2013) Side-chain modification of cytokinins controls shoot growth in Arabidopsis. Dev Cell **27**: 452–461

**Lilley CJ, Wang D, Atkinson HJ, Urwin PE** (2011) Effective delivery of a nematode-repellent peptide using a root-cap-specific promoter. Plant Biotechnol J **9**: 151–161

**Zürcher E, Müller B** (2016) Cytokinin synthesis, signaling, and function-advances and new insights. Int Rev Cell Mol Biol **324**: 1–38