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BRIEF COMMUNICATION

Catechin promotes growth of *Arabidopsis thaliana* with concomitant changes in vascular system, photosynthesis and hormone content

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Abstract

Catechin is associated with several functions in animal and plant systems, with little information available regarding its role in plant growth. Low concentrations of catechin (50 and 100 μM) were found to enhance length of primary and lateral roots, number of lateral roots, fresh and dry masses of shoots and roots, leaf area, water potential of leaf and root tissues, the number of vascular bundles in the inflorescence, and leaf thickness in *Arabidopsis thaliana* ecotype Col-0. A significant increase in net photosynthetic rate, stomatal conductance and concentration of indole-3-acetic acid was also observed in catechin treated plants.

Additional key words: indole-3-acetic acid, net photosynthetic rate, primary and lateral roots, stomatal conductance, xylogenesis.

Catechin, found in many fruits, vegetables and beverages such as tea (Scalbert and Williamson 2000), and callus/suspension culture cells of *Polygonum hydropiper* (Ono *et al*. 1998), is water soluble polyhydroxylated flavonoid compound, synthesized through phenylpropanoid and flavonoid pathways in plants (Rani *et al*. 2009). Higher catechin concentration (100 μg cm^{-3}) is reported to inhibit root growth of *Arabidopsis thaliana*, whereas lower concentration (25 μg cm^{-3}) doubled the plant biomass as compared to the control within 2 weeks of the treatment (Prithiviraj *et al*. 2007). There are reports that flavonoids enhanced plant growth, *e.g.*, callus growth in *Prunus avium* (Feucht and Nachit 1977) and growth of flower pedicels in beech tree (Feucht *et al*. 1997), possibly through redistribution of indole-3-acetic acid (IAA) and indole-3-butyric acid (Rusak *et al*. 2010).

To further study the mode of action of catechin on growth and development of *Arabidopsis thaliana*, ecotype Columbia-0 (Col-0) was grown aseptically in agar-gelled medium (0.7 %; m/v) essentially as described by Signora *et al*. (2001). Catechin (50 and 100 μM) was filter sterilized for inclusion into the medium.

On day 7 of germination, seedlings of uniform size were transferred to fresh plates containing media without or with catechin and placed in culture room at 16-h photoperiod (temperature of 25 ± 1 ºC, irradiance of 60 - 70 μmol m^{-2} s^{-1}). Samples were harvested weekly between 09:00 and 11:00 till day 28, dipped immediately in liquid nitrogen and stored at -80 °C for further studies. Morphological parameters such as fresh and dry masss of roots and shoots, length of primary and lateral roots, lateral root number and leaf area were regularly determined. Samples were oven dried at 70 °C for 96 h to record dry mass. Leaf area was calculated by plotting on a graph paper. For histological studies, tissues were fixed in formaldehyde + acetic acid + 50 % ethanol (1:1:18) and dehydrated in *t*-butylalcohol series. Sections (12 μm thick) were stained with safranin-fast green, and the slides were mounted in *DPX* (80 g distrene + 5 cm^3 dibutylphthalate + 35 cm^3 xylene; Ruzin 1999). The photographs were taken

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Abbreviations: g_s - stomatal conductance; IAA - indole-3-acetic acid; P_N - net photosynthetic rate; Ψ_w - water potential.

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under a light microscope (*Nikon*, Tokyo, Japan) using a digital camera (*Nikon DXM 1200*).

Water potential (Ψ_w) was measured using a dew point microvoltmeter *HR 33T* (*Wescor*, Logan, USA) as described by Kumar *et al.* (2006). IAA was extracted in 70 % (v/v) methanol and measured using a high pressure liquid chromatography system (on a *RP-C18* column) against the standard IAA dissolved in methanol (Kelen *et al.* 2004). Total catechins were measured using diazotized sulfanilamide based procedure as described by Singh *et al.* (1999).

Net photosynthetic rate (P_N) and stomatal conductance (g_s) were measured by an infra-red gas analyser (*LI-6400*, *Li-Cor*, Lincoln, NE, USA) as described by Kumar *et al.* (2006) at irradiance of 500 μmol m^{-2} s^{-1}, leaf temperature of 25 °C, and CO_2 concentration of 400 ± 5 μmol(CO_2) mol^{-1}. In this experiment, *Arabidopsis* plants were grown in *HIKO* trays, since plants grown in Petri plates were too small for such measurements. One month old plants were treated with different concentrations of catechin (50, 100, 200, 500 and 1000 µM) using a stock solution prepared in 70 % ethanol and diluted to required concentration with water.

Data was analyzed by factorial analysis using *SPSS* statistical package (*SPSS*, Chicago, USA) and general linear model procedures. Significant results were compared using Gabriel *post hoc* comparison. Differences between means were tested against critical difference at $P < 0.05$.

Plants grown in Petri plates showed that lower concentration (50, 100 µM) enhanced, whereas higher concentrations (200 and 500 µM) severely retarded the growth with no lateral roots emergence. At 1000 µM of catechin, seeds did not germinate. Since higher concentrations (>100 µM) were toxic, further experiments were conducted with 50 and 100 µM of catechin.

Catechin (50 and 100 µM) treated seedlings showed a significant ($P < 0.05$) increase in the content of catechin in root and leaf tissue upto day 21 of the treatment (data not shown). On day 28, catechin content in leaf and root tissues declined but was still significantly higher as compared to the control (Table 1). Catechin treated seedlings showed a significant increase in different morphological, physiological and biochemical parameters as compared the untreated control, except for fresh and dry masses of root (Table 1). IAA content in catechin treated seedlings showed a significant increase upto day 21 of the treatment followed by a decrease on day 28 (Table 1).

For plants grown in *HIKO* trays, higher catechin concentrations (200, 500 and 1000 µM) did not produce any adverse effect on plant growth, possibly due to its dilution/absorption/adsorption by the soil, and hence gas exchange data was collected for plants grown at the above concentrations of catechin treatment. P_N and g_s showed significant increase in response to 50, 100, 200, 500 and 1000 µM catechin as compared to the control at day 5 of the treatment (Table 2).

Leaf thickness across the vascular bundles was higher by 11.6 and 16.3 % at 50 and 100 µM catechin, respectively on 14th day of the treatment, which did not change appreciably on day 21. The number of cell layers, however, remained unchanged at all stages of observation. The number of vascular bundles in inflorescences

Table 1. Effect of 50 and 100 µM catechin on different parameters in *Arabidopsis thaliana* on day 28 of the treatment (35-d-old plants). Values are the means ± SE; number of replicates is shown in the parenthesis. Values marked with different letters show significant difference at $P < 0.05$ when analyzed over a period of 28 d with data collected at weekly intervals as compared to the respective control data. A significant increase in IAA content was recorded at day 21 of the treatment. These values were 309.7 ± 6.0, 363.7 ± 7.6 and 415.0 ± 8.8 ng g^{-1}(f.m.) for leaf tissue at 0, 50 and 100 µM catechin, respectively.

Parameters	Control	50 µM	100 µM
Primary root length [mm] (27)	80.6 ± 7.0c	89.2 ± 8.2b	92.4 ± 6.0a
Lateral root length [mm] (25)	15.4 ± 4.4b	22.6 ± 2.9a	24.0 ± 2.3a
Lateral roots number (22)	8.6 ± 0.9b	9.3 ± 1.3a	9.6 ± 1.5a
Leaf area [mm^2] (3)	7.7 ± 0.4b	9.2 ± 0.5b	9.2 ± 0.6a
Seedling fresh mass [mg] (6)	42.0 ± 4.4b	62.0 ± 3.6a	56.0 ± 4.7a
Shoot fresh mass [mg] (6)	30.2 ± 4.5b	49.6 ± 3.2a	43.8 ± 5.6a
Root fresh mass [mg] (6)	11.8 ± 0.9a	12.4 ± 1.1a	12.2 ± 1.1a
Seedling dry mass [mg] (6)	5.5 ± 0.7b	6.2 ± 0.4a	6.2 ± 0.9a
Shoot dry mass [mg] (6)	3.9 ± 0.5b	4.9 ± 0.6a	4.8 ± 0.6a
Root dry mass [mg] (6)	1.1 ± 0.09a	1.2 ± 0.09a	1.2 ± 0.2a
Leaf water potential [-MPa) (3)	1.36 ± 0.40c	0.92 ± 0.3b	0.61 ± 0.1a
Root water potential [-MPa) (3)	1.08 ± 0.48b	0.66 ± 0.16a	0.66 ± 0.16a
Leaf catechin content [ng g^{-1}(f.m.)] (3)	413.2 ± 18.0c	503.3 ± 13.2b	547.0 ± 13.8a
Root catechin content [ng g^{-1}(f.m.)] (3)	328.0 ± 17.6c	370.0 ± 14.4b	405.0 ± 15.0a
Leaf IAA content [ng g^{-1}(f.m.)] (3)	336.7 ± 9.0c	262.7 ± 10.0b	169.3 ± 8.0a

Table 2. Net Photosynthetic rate and stomatal conductance of *Arabidopsis thaliana* leaves in response to 5-d catechin treatment. Means ± SE, $n = 15$. Different letters in the column showed significant difference at $P < 0.05$. Numbers in parentheses are % increase over the control value.

Catechin [μM]	P_N [μmol(CO_2) m^{-2} s^{-1}]	g_s [mmol(H_2O) m^{-2} s^{-1}]
0	6.75 ± 0.92a	0.32 ± 0.05a
50	7.65 ± 1.23b (13.3)	0.39 ± 0.08b (21.9)
100	7.87 ± 0.78bc (16.6)	0.38 ± 0.04b (18.8)
200	8.36 ± 0.97cd (23.9)	0.41 ± 0.07bc (29.0)
500	8.50 ± 0.85de (25.9)	0.41 ± 0.09bc (29.0)
1000	9.03 ± 1.24e (33.8)	0.43 ± 0.09bc (34.4)

of catechin treated tissue was 7, as compared to 4 in the control. Also, there was formation of interfascicular fibre that made a continuous ring of vascular bundles.

Results in the present study showed that catechin led to significant increase in various growth parameters, such as length of primary and lateral roots, number of lateral roots, shoot fresh and dry masses, leaf area, and Ψ_w of leaf and root tissues. Growth involves an increase in cell volume (dV/dt) and an extension of the cell wall (Cleland 1986). Lockhart (1965) showed that growth rate is the product of wall extensibility (m) and effective pressure potential (Ψp - Y), where Ψp is the pressure potential and Y is the wall yield threshold and can be characterized by the expression: $dV/dt = m(\Psi p - Y)$. At higher pressure potential and lower yield threshold cell expansion is larger than at lower pressure potential and higher yield threshold (Cleland 1986). The latter is determined by cell wall properties, particularly the extent of lignification (Dencheva and Kilsurska 1982). Increase in pressure potential causes a certain amount of elastic stretching of the cell wall that leads to increase in cell volume. As the volume increases, there is a dilution and consequent rise in the osmotic potential of the cell sap. This increases the Ψ_w and leads to cell expansion and hence growth. In our experiment, catechin mediated increase in Ψ_w was evident. This could lead to overall cell expansion and hence the observed increase in various growth parameters. Anatomical studies supported this view since cell enlargement was observed in response to catechin treatment, while the cell number remained unaffected.

Catechin mediated increase in the length of primary and lateral roots, number of lateral roots, and leaf area suggested catechin to mimic or modulate the auxin content. IAA is an abundant natural auxin required for many important plant functions, including lateral root initiation, vascular tissue differentiation (Buer and Muday 2004), and secondary xylem formation (Sachs 2000). It has been reported that auxin can induce the formation of new vascular strands from parenchymatic cells (Sachs 1981). Our results showed that catechin treated tissue had increased content of IAA, thus raising the possibility that catechin might operate through IAA in modulating growth and development in *Arabidopsis*. Formation of continuous ring of secondary xylem (xylogenesis) in response to catechin treatment was supportive of the above stated observation, since IAA is known to induce xylogenesis (Wilson and Wilson 1991). Increased xylogenesis would support increased nutrient uptake (Dabydeen and Charran 1990), possibly due to increased length of primary root, length of lateral root and number of laterals *i.e.*, overall increase in root area (Table 1). Increase in IAA content in catechin treated tissue could be brought about due to catechin mediated IAA stabilization, possibly by reducing IAA oxidation (Feucht *et al.* 1997). Possibility also exists that catechin influenced the distribution of IAA to mediate such effects; for example flavonoids differentially affected distribution of IAA and indole-3-butyric acid in roots and leaves and modulated the root growth and gravitropism in *Arabidopsis* (Rusak *et al.* 2010).

Catechin also affected gas exchange parameters in *Arabidopsis*, showing significant increases in both P_N and g_S. The role of g_S in affecting P_N is well established (Mott *et al.* 1982). A significant increase in g_S would facilitate greater inflow of CO_2 thus raising the probability of enhanced P_N. It is likely that catechin induced enhanced content of IAA has a role in enhancing P_N. IAA mediated enhancement in P_N has been reported earlier in *Phaseolus vulgaris, Fragaria chiloensis, Betula vulgaris, Helianthus annuus, Pelargonium hortorum* and *Polystichum acrostichoides* (Bidwell and Turner 1966).

To conclude, our results showed that catechin modulated growth of *Arabidopsis* through changes in root and shoot morphology, gas exchange characteristics and IAA content.

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