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SHORT COMMUNICATION



# Comparative effects of L-DOPA and velvet bean seed extract on soybean lignification

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**ABSTRACT**

Velvet bean (*Mucuna pruriens*) is an efficient cover forage that controls weeds, pathogens and nematodes, and the non-protein amino acid L-3,4-dihydroxyphenylalanine (L-DOPA) is its main allelochemical. The effects of 3 g $L^{-1}$ of an aqueous extract of velvet bean seeds, along with 0.5 mM L-DOPA for comparison, were evaluated in roots, stems and leaves of soybean (*Glycine max*). The activities of phenylalanine ammonia lyase (PAL) and cinnamyl alcohol dehydrogenase (CAD) were determined, along with the lignin content and its monomeric composition. The results revealed similar effects caused by L-DOPA and the aqueous extract. Both treatments reduced PAL and CAD activities, lignin, and lignin monomer contents in roots; PAL and CAD activities in stems, and CAD activity in leaves. These findings provide further evidence that the effects of velvet bean cover forage on root lignification were due to the L-DOPA, its major allelochemical.

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L-3,4-dihydroxyphenylalanine; *Glycine max*; lignin; *Mucuna pruriens*; non-protein amino acid

## Introduction

One of the most dynamic and flexible processes related to plant growth is cell wall lignification, which comprises the anchorage of lignin in the secondary wall of specialized cells such as xylem vessels, tracheids, and fibers. Lignin is a heterogeneous polymer generated by combinatorial radical coupling of *p*-hydroxycinnamyl alcohols, also termed monolignols.[1] Lignin gives rigidity to cell walls, working as an efficient and resistant glue, keeping different cells together, making the cell wall hydrophobic, and protecting the plant against abiotic and biotic stresses.[2]

Like secondary compounds such as hydroxycinnamic acids and esters, lignans, tannins, coumarins and flavonoids, lignin is an end-product of the phenylpropanoid pathway. Briefly, the first reaction of this metabolic route is the non-oxidative deamination of L-phenylalanine by phenylalanine ammonia-lyase (PAL) yielding *t*-cinnamate; a rate-limiting step. After sequential enzymatic reactions of hydroxylation, methoxylation, esterification and reduction, hydroxycinnamates (*p*-coumarate, caffeate, ferulate and sinapate) are converted in their respective aldehydes. These are reduced to monolignols (*p*-coumaryl, coniferyl and sinapyl alcohols) by the action of cinnamyl alcohol dehydrogenase (CAD). Finally, oxidative polymerization of these three monolignols by the action of peroxidase/laccase generates the *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units of lignin.[1,3] Increased lignin content in plants has been considered to be a defense against stresses. In fact, there is evidence that many allelochemicals cause impaired plant growth, associated with changes in the enzyme activities of the phenylpropanoid pathway and premature cell wall lignification.[4-10] As a consequence, stress-induced lignification can limit cell expansion, the capacity for nutrient uptake and the ability to sustain plant growth.

Velvet bean (*Mucuna pruriens* L. DC. var. *utilis*) is an efficient cover forage used in intercropping with *Zea mays*, *Sorghum bicolor*, and *Pennisetum glaucum*, that controls weeds, pathogens, and nematodes.[11] It produces many secondary compounds, the most important being the non-protein amino acid L-3,4-dihydroxyphenylalanine (L-DOPA). L-DOPA constitutes about 1% of the fresh leaf weight and 6–9% of the dry seed weight of velvet bean, and it has attributes typical of an allelochemical. In fact, after its release from the roots of velvet bean,[12] L-DOPA can accumulate in high concentrations (100–450 kg $ha^{-1}$) in the soil and, thus, influence the growth of neighboring plants (allelopathy). Many plant species are sensitive to L-DOPA, its effect being to reduce seed germination and root growth.[13-19]

Analyzing a DNA microarray of *Arabidopsis thaliana*, Golisz et al.[20] reported that L-DOPA affects gene expression related to several biological processes, including lignification. In short-term (24 h) experiments, we previously observed that L-DOPA reduced soybean (*Glycine max*) root growth, associated with lignification and increased activities of PAL and peroxidase, two key enzymes of the phenylpropanoid pathway.[21] Because the effects of L-DOPA were tested only in roots, our aim in this study was to compare the effects of L-DOPA and a velvet bean seed extract on leaves, stems and roots of soybean, in longer-term (22 d) experiments. For this purpose, we determined the PAL and CAD activities and the contents of lignin and its main monomers, *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S).

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## Results and discussion

The HPLC quantification procedure indicated that the 3 g $L^{-1}$ aqueous extracts of velvet bean seeds contain about 0.5 mM of L-DOPA, in agreement with the results reported by Marchiosi et al.[22]

In soybean roots, both L-DOPA and the aqueous extract reduced the PAL and CAD activities (Fig. 1A, B) and lignin contents (Fig. 2). With L-DOPA treatment, PAL and CAD activities and lignin content were decreased by 54.6%, 52.5% and 43.1%, respectively, in comparison to the control. The aqueous extract of velvet bean seeds reduced these same parameters by 79.7%, 75.3% and 54.3%, respectively. In stems, compared with the control, L-DOPA reduced the PAL and CAD activities by 61.1% and 54.1%, respectively, while aqueous extract of velvet bean extract reduced their activities by 76.5% and 44.4%, respectively. Stem lignins were not affected by L-DOPA or the aqueous extract. In leaves, L-DOPA and the aqueous extract of velvet bean only reduced the CAD activities, by 63.4% and 46.9%, respectively, compared with the control.

Lignification is a primordial process related to the plant growth. It occurs during secondary plant cell wall development and the lignin deposition hardens the stem walls. This process provides structural support, enabling increased plant growth.[23] Lignin biosynthesis involves the polymerization of monolignols that are primarily derived from the phenylpropanoid pathway.[1,24] Thus, the findings that L-DOPA and the aqueous velvet bean extract reduced the PAL and CAD activities (Fig. 1A, B) and lignin contents (Fig. 2) are relevant. While PAL is the first rate-limiting enzyme of the phenylpropanoid pathway, CAD is the last and is considered a marker for lignification.[1]

Following the treatment of soybean roots with L-DOPA and the aqueous velvet bean extract, the H monomer contents decreased by 56.4% and 23.9%, respectively, compared with the control (Fig. 3A). The G monomer contents decreased by 27.5% and 57.1%, respectively, while the S monomer contents decreased by 42.0% and 71.3%, respectively. Based on these data, the lignin content (the sum of H+G+S) decreased by 22.4% (L-DOPA) and 53.3% (aqueous extract of velvet bean) compared with the control. Neither L-DOPA nor the aqueous velvet bean affected the lignin contents in stems (Fig. 3B) or leaves, except for a 23.3% reduction in the G monomer content (Fig. 3C).

The more evident effects noted herein were in roots, *i.e.*, decreased activities of PAL and CAD were associated with a reduction in lignin contents (Figs. 1A, B & 2). These results were in agreement with those for lignin monomer composition

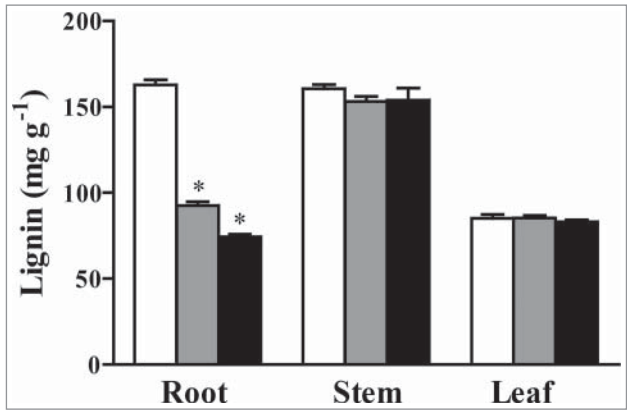


**Figure 2.** Effects of L-DOPA and aqueous velvet bean extract on lignin contents in roots, stems and leaves of soybean after 22 d. *Means ($N = 3$ to 5 ± standard error of the mean) significantly ($P \leq 0.05$) smaller than the control experiment (Dunnett's test).

since H, G, S and H+G+S contents also decreased under the action of both L-DOPA and the aqueous extract (Fig. 3A). In stems, the activities of PAL and CAD decreased, but without any impact on lignin (Fig. 2) or lignin monomer contents (Fig. 3B). In leaves, the CAD activity decreased, but lignin (Fig. 2) and lignin monomer (Fig. 3B) contents were unchanged by treatment with L-DOPA or the aqueous extract, except for an unexplained effect on the G monomer (Fig. 3C). In general, the findings support the role of these enzymes on the metabolic flux towards lignin synthesis.[23,25,26] Several lines of evidence support this enzymatic role; for example, decreased PAL and CAD activities lead to reduced lignin content in *Populus* × *euuamericana*,[27] *Sorghum bicolor*,[28] soybean,[29] *Arabidopsis*[30,31] and *Brachypodium*.[32]

Contrary to our previous report,[21] the results herein indicate that L-DOPA decreased the PAL activity associated with reduced lignin and monomer content in soybean roots. These results are not necessarily contradictory based on the following aspects. First, the earlier study[21] only considered a short time period (24 h), while the current research is a longer-term (22 d) study. Second, herein the nutrient solutions containing L-DOPA or aqueous velvet bean extract were renewed every other day from 10th to 22nd day of cultivation. Thus, a prominent uptake of L-DOPA by the roots can affect the PAL activity (Fig. 1A) and lignification (Figs. 2 & 3A). Finally, it is possibly that effects of L-DOPA on lignin production and related enzymes are different during differentiation processes of roots, stems and leaves. However, this interesting approach requires a further detailed investigation.

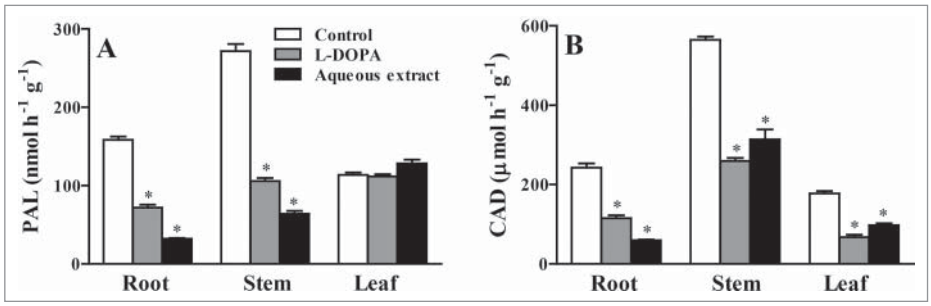


**Figure 1.** Effects of L-DOPA and aqueous velvet bean extract on PAL (A) and CAD (B) activities in roots, stems and leaves of soybean after 22 d. *Means ($N = 3$ to 5 ± standard error of the mean) significantly ($P \leq 0.05$) smaller than the control experiment (Dunnett's test).

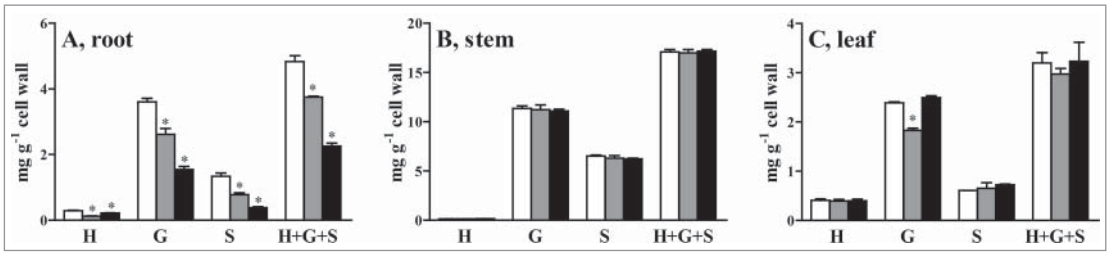


**Figure 3.** Effects of L-DOPA and aqueous velvet bean extract on lignin monomer composition in roots (A), stems (B) and leaves (C) of soybean after 22 d. *p*-hydroxyphenyl (H); guaiacyl (G) and syringyl (S) monomers. *Means ($N = 3$ to 5 $\pm$ standard error of the mean) significantly ($P \leq 0.05$) smaller than the control experiment (Dunnett's test).

Another data showed here are related to the reduced activity of CAD, especially in stems and leaves (Fig. 1B) without alter lignin (Fig. 2) and its monomeric composition (Fig. 3B, C), when compared to the corresponding controls. It is not surprisingly, because plants are able to circumvent an eventual block in CAD activity by shipping the substrates cinnamaldehydes to the cell wall for polymerization.[1] At least for stems and leaves, this is an indicative that the impact of CAD on lignin production may not be critical.[6] As an evidence, the cross-coupling of hydroxycinnamyl aldehydes into lignin can compensate the reduced availability of monolignols in CAD-deficient plants.[33,34]

In summary, the results obtained in the current research revealed a similarity between the effects of treatment with 0.5 mM of L-DOPA or 3 g $L^{-1}$ aqueous velvet bean extract for the parameters evaluated (Figs. 1, 2, & 3). Taken together, the current investigation provides further evidence that: 1) in longer-term experiments, the L-DOPA allelochemical and aqueous velvet bean extract affect PAL and CAD activities and lignin contents in roots more than in stems and leaves of soybean; 2) the observed effects of the aqueous extract can be explained by the presence of L-DOPA, the major allelochemical of velvet bean.

## Materials and methods

### Plant material and general procedures

Soybean (*Glycine max* L. Merr. cv. BRS-232) seeds were surface-sterilized with 2% sodium hypochlorite, rinsed extensively with deionized water and dark-germinated at 25°C on two sheets of moistened filter paper. Three-day-old seedlings of uniform size were supported by an adjustable Styrofoam plate and the roots were immersed in 350 ml of a 1/6-strength nutrient solution (pH 6.0) in an 8 × 15-cm acrylic container.[35] The container was kept in a growth room (at 25°C, with a light/dark photoperiod of 14/10 h and a photon flux density of 400 $\mu$mol $m^{-2}$ $s^{-1}$). On the fourth day of cultivation, the solution was replaced by a 1/3-strength nutrient solution (pH 6.0), and on the eighth day by a half-strength solution (pH 6.0). On the 10th day of cultivation, the solution was replaced by half-strength nutrient solution (pH 6.0), or half-strength nutrient solution containing 0.5 mM L-DOPA or 3 g $L^{-1}$ of aqueous extract of velvet bean seeds. These nutrient solutions were renewed every other day, and the experiment was concluded after 22 d of cultivation. The L-DOPA concentration was selected based on a survey of the literature,[13,15,19,21,36,37] and the concentration of L-DOPA in the aqueous extract was determined by high performance liquid chromatography (HPLC), as described below. L-DOPA was purchased from Sigma-Aldrich (St. Louis, MO, USA), and all other reagents used were of the purest grade available.

### Extraction and quantification of L-DOPA from velvet bean seeds

Aqueous extracts of velvet bean seeds were obtained as described by St-Laurent et al.[38] Briefly, 0.105 g of seeds was homogenized in 10 ml of deionized water in each of ten glass tubes. Extractions were performed by immersing the tubes in a sonication bath for 5 min, followed by centrifugation of the homogenates at 2,200 × g for 2 min. The supernatants were bulked and made up to a volume of 350 ml with deionized water resulting in a 3 g $L^{-1}$ aqueous extract.

For L-DOPA quantification, samples of the aqueous extracts were filtered through a 0.45 $\mu$m disposable syringe filter and analyzed (20 $\mu$l) in a high-performance liquid chromatography system (LC-20 Prominence HPLC system, Shimadzu®, Japan) equipped with an LC-20AT quaternary gradient pump, a SIL-20A autosampler and an SPD-M20A photo diode array detector. A reversed-phase Shimpack® CLC-ODS column (150 × 4.6 mm, 5 $\mu$m), protected with an equivalent pre-column (10 × 4.6 mm) was used at 30°C. The mobile phase consisted of a mixture of 0.1% phosphoric acid, 2% methanol and 97.9% water, with a flow rate of 0.5 ml $min^{-1}$ for an isocratic run of 30 min. Quantification of L-DOPA was performed at 280 nm using the corresponding standard.

### Enzymatic assays

After the cultivation period with the aqueous extract of velvet bean or L-DOPA, fresh roots, stems and leaves of the soybean seedlings were detached and the enzymes extracted. Phenylalanine ammonia-lyase (PAL) was extracted as described by Ferrarese et al..[39] Tissues (1.0 g) were ground at 4°C in 0.1 M sodium borate buffer (pH 8.8), homogenates were centrifuged (2,200 × g, 15 min), and the supernatant was used as the enzyme preparation. The reaction mixture (100 $\mu$moles sodium borate buffer, pH 8.7, and a suitable amount of enzyme extract in a final volume of 1.55 ml) was pre-incubated at 40°C for 5 min before the PAL activity assay. L-Phenylalanine (15 $\mu$moles) was added to start the reaction, which was stopped after one hour of incubation by the addition of 50 $\mu$l of 5 M HCl. Samples were filtered through a 0.45-$\mu$m disposable syringe filter (Hamilton® Co., Nevada, USA) and 20 $\mu$l aliquots

were analyzed in a high-performance liquid chromatography (HPLC) system (Shimadzu® 10AVP, Tokyo, Japan) equipped with an LC-10AD pump, a Rheodyne® injector, an SPD-10A UV detector, a CBM-101 Communications Bus Module, and a Class-CR10 workstation system. A reversed-phase Shimpack® CLC-ODS column (150 × 4.6 mm, 5 $\mu$m) protected with an equivalent pre-column (10 × 4.6 mm) was used at 30°C. The mobile phase was 70% methanol (v/v), with a flow rate of 0.8 ml $min^{-1}$ for an isocratic run of 10 min. UV detection was carried out at 275 nm. Data collection and integration were performed with Class-CR10 software (Shimadzu®, Tokyo, Japan). *t*-Cinnamate, the product of PAL, was identified by comparing its retention time with a standard compound. Parallel controls without L-phenylalanine or with *t*-cinnamate (added as an internal standard in the reaction mixture) were performed as described elsewhere.[39] PAL activity was expressed as $\mu$mol *t*-cinnamate $min^{-1}$ $g^{-1}$ of fresh weight.

Cinnamyl alcohol dehydrogenase (CAD) was extracted from fresh roots, stems or leaves (2 g) with 3 ml of an extraction medium containing 40 mM of $\beta$-mercaptoethanol and 100 mM potassium phosphate buffer (pH 7.3). The homogenate was centrifuged at 2,200 × g for 15 min, and the supernatant was used as enzyme preparation.[40] CAD was assayed chromatographically by the reduction of sinapaldehyde to sinapyl alcohol. The assay was carried out at 30°C in 1 ml of a reaction mixture containing 200 $\mu$l of crude enzyme preparation (≤0.35 mg of protein), 104 nmol NADPH, and 150 nmol Tris–HCl buffer (pH 8.0). At the start, 50 nmol of sinapaldehyde was added, and the reaction was stopped after 3 min of incubation by adding 50 $\mu$L of 5 M HCl. Parallel controls with sinapaldehyde added into the reaction mixture (without NADPH) were also prepared. All samples were filtered through a 0.45–$\mu$m disposable syringe filter (Hamilton® Co., NV, USA) and analyzed (20 $\mu$l) in a Shimadzu® Liquid Chromatograph (Tokyo Japan), as described above. The mobile phase 4% was methanol/acetic acid in water (20:80, v/v), with a flow rate of 1.2 ml $min^{-1}$ for an isocratic run of 20 min. The absorbance was measured at 345 nm. Sinapyl alcohol was identified by comparing its retention time with standard values. CAD activity was expressed as $\mu$mol sinapaldehyde $min^{-1}$ $g^{-1}$ of fresh weight.

### *Quantification of lignin and monomer composition*

After the cultivation period, dry roots (0.3 g) were homogenized in a mortar and pestle in 50 mM potassium phosphate buffer (7 ml, pH 7.0) and transferred into a centrifuge tube.[41] The homogenate was obtained and defined as a protein-free cell wall fraction. Lignin was assayed by the acetyl bromide method.[42] Samples (20 mg) of the protein-free cell wall fraction were placed into a screw-cap centrifuge tube containing 0.5 ml of 25% acetyl bromide (v/v in glacial acetic acid) and incubated at 70°C for 30 min. After complete digestion, the samples were quickly cooled on ice, mixed with 0.9 ml 2 M NaOH, 0.1 ml 7.5 M hydroxylamine-HCl and 2 ml glacial acetic acid. After centrifugation (1,000 × g, 5 min), the absorbance of the supernatant was measured at 280 nm and the lignin content was expressed as mg $g^{-1}$ cell wall.

The oxidation of alkaline nitrobenzene was used to determine the lignin monomer composition.[7] The protein-free cell wall fraction (50 mg) obtained above was sealed in a Pyrex® ampule containing 1 ml of 2 M NaOH and 1 ml of nitrobenzene, prior to heating at 170°C for 90 min with occasional shaking during the reaction. The sample was then cooled at room temperature, washed twice with chloroform, acidified to pH 2.0 with 2 M HCl, and extracted twice with chloroform. The organic extracts were combined, dried and resuspended in 1 ml of a mixture of methanol and 4% acetic acid in water (20:80, v/v). All of the samples were filtered through a 0.45-$\mu$m disposable syringe filter (Hamilton® Co., Nevada, USA), and 20 $\mu$l aliquots were analyzed in a HPLC system (Shimadzu® 10AVP, Tokyo, Japan), as described earlier. A reversed-phase Shimpack® CLC-ODS column (150 × 4.6 mm, 5 $\mu$m), protected with an equivalent pre-column (10 × 4.6 mm) was used at 30°C. The mobile phase consisted of a mixture of methanol and 4% acetic acid in water (20:80, v/v), with a flow rate of 1.2 ml $min^{-1}$ for an isocratic run of 20 min. Quantification of the monomer aldehyde products (*p*-hydroxybenzaldehyde, vanillin and syringaldehyde) released by nitrobenzene oxidation was performed at 290 nm using the corresponding standards. The results were expressed as mg monomer $g^{-1}$ cell wall.

### *Statistical analysis*

The experimental design was completely randomized, and each plot was represented by one acrylic container containing one seedling. The data were expressed as the means of three or six independent experiments ± the standard error of the mean. One-way analysis of variance (ANOVA) was performed to test the significance of the observed differences using the *GraphPad Prism*® package (Version 5.01, GraphPad Software Inc., USA). The differences between the parameters were evaluated using Dunnett's test, and *P* values ≤ 0.05 were considered as statistically significant.

## Abbreviations

| | |
|---|---|
| CAD | cinnamyl alcohol dehydrogenase |
| G | guaiacyl |
| H | *p*-hydroxyphenyl |
| L-DOPA | L-3,4-dihydroxyphenylalanine |
| PAL | phenylalanine ammonia-lyase |
| S | syringyl |

## Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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## References

1. Boerjan W, Ralph J, Baucher M. Lignin biosynthesis. Ann Rev Plant Biol. 2003;54:519–46. doi: 10.1146/annurev.arplant.54.031902.134938.
2. Calvo-Flores FG, Dobado JA, Isac-García J, et al. Lignin and lignans as renewable raw materials. Chemistry, technology and applications. Chichester: John Wiley & Sons (UK); 2015.

3. Mottiar Y, Vanholme R, Boerjan W, Ralph J, Mansfield SD. Designer lignins: harnessing the plasticity of lignification. Curr Opin Biotechnol. 2016;37:190–200. doi: 10.1016/j.copbio.2015.10.009.
4. Politycka B. Ethylene-dependent activity of phenylalanine ammonia-lyase and lignin formation in cucumber roots exposed to phenolic allelochemicals. Acta Soc Bot Pol. 1999;68:123–7.
5. dos Santos WD, Ferrarese MLL, Finger A, Teixeira AC, Ferrarese-Filho O. Lignification and related enzymes in soybean root growth-inhibition by ferulic acid. J Chem Ecol. 2004;30:1199–208. doi: 10.1023/B:JOEC.0000030272.83794.f0.
6. dos Santos WD, Ferrarese MLL, Nakamura CV, Mourão KS, Mangolin CA, Ferrarese-Filho O. Soybean (*Glycine max*) root lignification induced by ferulic acid. The possible mode of action. J Chem Ecol. 2008;34:1230–41. doi:10.1007/s10886-008-9522-3.
7. Zanardo DIL, Lima RB, Ferrarese MLL, et al. Soybean root growth inhibition induced by *p*-coumaric acid. Environ Exp Bot. 2009;66:25–30. doi:10.1016/j.envexpbot.2008.12.014.
8. Bubna GA, Lima RB, Zanardo DYL, et al. Exogenous caffeic acid inhibits the growth and enhances the lignification of the roots of soybean (*Glycine max*). J Plant Physiol. 2011;168:1627–33. doi: 10.1016/j.jplph.2011.03.005.
9. Lima RB, Salvador VH, dos Santos WD, Bubna GA, Finger-Teixeira A, Soares AR, Marchiosi R, Ferrarese Mde L, Ferrarese-Filho O. Enhanced lignin monomer production caused by cinnamic acid and its hydroxylated derivatives inhibits soybean root growth. PLoS One. 2013;8:e80542. doi: 10.1371/journal.pone.0080542.
10. Ferro AP, Marchiosi R, Siqueira-Soares RC, et al. Effects of cinnamic and ferulic acids on growth and lignification of maize roots. J Allel Inter. 2015;1:29–38.
11. Pugalenthi M, Vadivel V, Siddhuraju P. Alternative food/feed perspectives of an underutilized legume *Mucuna pruriens* var. utilis – a review. Plant Foods Hum Nutr. 2005;60:201–18. doi: 10.1007/s11130-005-8620-4.
12. Furubayashi A, Hiradate S, Fujii Y. Role of catechol structure on the adsorption and transformation reactions of L-DOPA in soils. J Chem Ecol. 2007;33:239–50. doi: 10.1007/s10886-006-9218-5.
13. Fujii Y, Shibuya T, Yasuda T. L-3,4-Dihydroxyphenylalanine as an allelochemical candidate from *Mucuna pruriens* (L.) DC. var. *utilis*. Agric Biol Chem. 1991;55:617–8. doi: 10.1271/bbb1961.55.617.
14. Nakajima N, Hiradate S, Fujii Y. Characteristics of growth inhibitory effect of L-3, 4-dihydroxyphenylalanine (L-DOPA) on cucumber seedling. J Weed Sci Technol. 1999;44:132–8.
15. Hachinohe M, Sunohara Y, Matsumoto H. Absorption, translocation and metabolism of L-DOPA in barnyardgrass and lettuce: their involvement in species-selective phytotoxic action. Plant Growth Reg. 2004;43:237–43. doi: 10.1023/B:GROW.0000045996.72922.1b.
16. Nishihara E, Parvez MM, Araya H, et al. Germination growth response of different plant species to the allelochemical L-3,4-dihydroxyphenylalanine (L-DOPA). Plant Growth Reg. 2004;42:181–9. doi: 10.1023/B:GROW.0000017483.76365.27.
17. Nishihara E, Parvez MM, Araya H, et al. L-3-(3,4-dihydroxyphenyl) alanine (L-DOPA), an allelochemical exuded from velvetbean (*Mucuna pruriens*) roots. Plant Growth Reg. 2005;45:113–20. doi: 10.1007/s10725-005-0610-x.
18. Topal S, Kocaçaliskan I. Allelopathic effects of DOPA against four weed species. DPU Fen Bil Enst. 2006;11:27–32.
19. Soares AR, Marchiosi R, Siqueira-Soares RC, et al. The role of L-DOPA in plants. Plant Signal Behav. 2014;9:e28275. doi: 10.4161/psb.28275.
20. Golisz A, Sugano M, Hiradate S, Fujii Y. Microarray analysis of Arabidopsis plants in response to allelochemical L-DOPA. Planta. 2011;233:231–40. doi: 10.1007/s00425-010-1294-7.
21. Soares AR, Ferrarese MLL, Siqueira RC, et al. L-DOPA increases lignification associated with *Glycine max* root growth-inhibition. J Chem Ecol. 2007;33:265–75. doi: 10.1007/s10886-006-9227-4.
22. Marchiosi R, Bido GS, Bohm PAF, et al. Photosynthetic response of soybean to L-DOPA and aqueous extracts of velvet bean. Plant Growth Reg. 2016;79:1–12. doi: 10.1007/s10725-016-0154-2.
23. Barros J, Serk H, Granlund I, Pesquet E. The cell biology of lignification in higher plants. Ann Bot. 2015;115:1053–74. doi: 10.1093/aob/mcv046.
24. Bonawitz ND, Chapple C. The genetics of lignin biosynthesis: Connecting genotype to phenotype. Ann Rev Gen. 2010;44:337–63. doi: 10.1146/annurev-genet-102209-163508.
25. Bell AA. Biochemical mechanisms of disease resistance. Ann Rev Plant Physiol. 1981;32:21–81.
26. Baucher M, Chabbert B, Pilate C, et al. Red xylem and higher lignin extractability by down-regulating a cinnamyl alcohol dehydrogenase in poplar. Plant Physiol. 1996;112:1479–90.
27. Grand C, Sarni F, Boudet AM. Inhibition of cinnamyl alcohol-dehydrogenase activity and lignin synthesis in poplar *(Populus* X *euuamericana* Dode) tissues by two organic compounds. Planta. 1985;163:232–7.
28. Pillonel C, Mulder MM, Boon JJ, Forster B, Binder A. Involvement of cinnamyl-alcohol dehydrogenase in the control of lignin formation in *Sorghum bicolor* L. Moench. Planta. 1991;185:538–44. doi: 10.1007/BF00202964.
29. Andrade AB, Ferrarese MLL, Teixeira AF, et al. Mimosine-inhibited soybean (*Glycine max*) root growth, lignification and related enzymes. Allelopathy J. 2008;21:145–54.
30. Huang J, Gu M, Lai Z, Fan B, Shi K, Zhou YH, Yu JQ, Chen Z. Functional analysis of the *Arabidopsis PAL* gene family in plant growth, development, and response to environmental stress. Plant Physiol. 2010;153:1526–38. doi: 10.1104/pp.110.157370.
31. Zhang X, Liu C-J. Multifaceted regulations of gateway enzyme phenylalanine ammonia-lyase in the biosynthesis of phenylpropanoids. Mol Plant. 2015;8:17–27. doi: 10.1093/mp/ssu134.
32. Cass CL, Peraldi A, Dowd PF, Mottiar Y, Santoro N, Karlen SD, Bukhman YV, Foster CE, Thrower N, Bruno LC. Effects of phenylalanine ammonia lyase (PAL) knockdown on cell wall composition, biomass digestibility, and biotic and abiotic stress responses in *Brachypodium*. J Exp Bot. 2015;66:4317–35. doi: 10.1093/jxb/erv269.
33. Kim H, Ralph J, Yahiaoui N, Pean M, Boudet AM. Cross-coupling of hydroxycinnamyl aldehydes into lignins. Org Lett. 2000;2:2197–200. doi 10.1021/ol005906o.
34. Li L, Cheng XF, Leshkevich J, Umezawa T, Harding SA, Chiang VL. The last step of syringyl monolignols biosynthesis in angiosperms is regulated by a novel gene encoding sinapyl alcohol dehydrogenase. Plant Cell. 2001;13:1567–85. doi 10.1105/TPC.010111.
35. Dong J, Wu F, Zhang G. Influence of cadmium on antioxidant capacity and four microelement concentrations in tomato seedlings (*Lycopersicon esculentum*). Chemosphere. 2006;64:1659–66. doi: 10.1016/j.chemosphere.2006.01.030.
36. Soares AR, Ferrarese MLL, Siqueira-Soares RC, et al. The allelochemical L-DOPA increases melanin production and reduces reactive oxygen species in soybean roots. J Chem Ecol. 2011;37:891–8. doi: 10.1007/s10886-011-9988-2.
37. Soares AR, Siqueira-Soares RC, Salvador VH, et al. The effects of L-DOPA on root growth, lignification and enzyme activity in soybean seedlings. Acta Physiol Plant. 2012;34:1811–7. doi: 10.1007/s11738-012-0979-x.
38. St-Laurent L, Livesey J, Arnason JT, et al. Variation in L-dopa concentration in accessions of *Mucuna pruriens* (L.) DC. and in *Mucuna brachycarpa* Rech. In: Flores MB, Eilittä M, Myhrman R, Carew LB, Carsky RJ, editors. Proceedings of the International Workshop "Food and Feed from *Mucuna*: Current Uses and the Qay Forward". Tegucigalpa (Honduras): CIDICCO; 2002. p. 352–74.
39. Ferrarese MLL, Rodrigues JD, Ferrarese-Filho. Phenylalanine ammonia lyase activity in soybean roots extract measured by reverse-phase high performance liquid chromatography. Plant Biol. 2000;2:152–3. doi: 10.1055/s-2000-9162.
40. dos Santos WD, Ferrarese MLL, Ferrarese-Filho O. High performance liquid chromatography method for the determination of cinnamyl alcohol dehydrogenase in soybean roots. Plant Physiol Biochem. 2006;44:511–5. doi: 10.1016/j.plaphy.2006.08.004.
41. Ferrarese MLL, Zottis A, Ferrarese-Filho O. Protein-free lignin quantification in soybean (*Glycine max*) roots. Biologia. 2002;57:541–3.
42. Moreira-Vilar FC, Siqueira-Soares RC, Finger-Teixeira A, et al. The acetyl bromide method is faster, simpler and presents best recovery of lignin in different herbaceous tissues than Klason and thioglycolic acid methods. PloS One. 2014;9:e110000. doi: 10.1371/journal.pone.0110000.