LIGNIN AND POLYPHENOLS FROM VEGETAL WASTES AS KEY MODULATORS OF METABOLIC PATHWAYS DURING PLANT DEVELOPMENT

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The development of plants is highly influenced by exogenous substances, which can act as biostimulators or inhibitors of the growth process. Among them, a very large group of aromatic compounds comprising lignins and polyphenols seem to have phytohormonal properties. Based on current knowledge, our objective was to investigate the action of flax lignin and polyphenols extracted from spruce bark upon the development of *Phaseolus vulgaris* L. plantules, along with their influence upon some enzymatic systems involved in glucidic metabolism and cellular respiration. Our findings show that the tested substances possess phytohormonal activity, translated into higher biomass accumulation by 20-40% compared with the reference and an increased germination capacity on the average by 10-20%. Moreover, when the concentration of the biologically active compound ranged between 0.05 and 0.1 g/L, the increase in the germination capacity, along with biomass accumulation, was related to increased activities of amylase, ascorbatoxidase and polyphenol oxidase, on the average by 15-25% comparative with the reference, with a decreased activity of catalase. The obtained results clearly demonstrate that polyphenols act in a dose dependent manner upon biological media by stimulation of anabolic pathways, which is translated into biosynthesis of proteins and distinguished by biomass accumulation.

Keywords: flax lignin, polyphenols, spruce bark, amylase, catalase, ascorbatoxidase, polyphenol oxidase

INTRODUCTION

Modern methods used in science and agricultural techniques recognize the importance of acceleration or temporary inhibition of plant growth processes, development and metabolism with the aid of physical and chemical factors. Among these factors, substances that act as plant biostimulators have gained, along the time, a very large applicability in plant cultivation.[1,2]

Research undertaken in recent years, with the objective of discovering new green biostimulators compatible with the environment, has revealed that some natural products separated from phytomass could interfere with plant growth processes.[3-6] The most abundant and ubiquitously distributed extractives in the plant kingdom are polyphenols and lignins, which are essential for plant growth. They have been proved to have a large range of biological properties, such as antimicrobial and antioxidant capacities.[7-14] Being involved in the plant life cycle and regulating the transport of auxins, these compounds have similar hormonal properties to those of cytokinines.[15]

Moreover, one of the most important features of lignins and polyphenols is that they are non-toxic, biodegradable and are present in almost all plants. The quantity of green biomass globally generated every year (only from agriculture) has been estimated to be about 140 billion tones, from which almost 60% is lost or wasted.[16] From the viewpoint of biorefining or complex processing of biomass, the recovery of such chemicals from vegetable wastes is of great importance both for sustainable development and for economic reasons.

An important source of polyphenols originates in the pulp and paper industry in the form of spruce bark, which represents more than 12% of the processed mass of wood. Another source of potential plant bioregulators is represented by flax lignin, which results as a by-product from the

textile industry during high-quality fiber refining.[17,18]

The intrinsic abilities of such substances to act as stimulators or inhibitors of metabolic processes in plants are often correlated with the concentration of these compounds in the culture media. Thus, the presence of these biological active compounds in low concentrations may stimulate plant development in the vegetative stage or, on the other hand, a higher concentration may cause inhibition phenomena. The dose dependent response is something usual for almost all substances that interfere with a living system.

Unfortunately, little is known about how these substances interfere with metabolic processes during plant development once they are introduced in the culture media. Plant growth is closely related with the biochemical reactions that occur in all their morphological segments. Thus, it becomes of interest not only to study the influence of polyphenols and lignins as bioregulators, but also to find out if they act upon some enzymatic systems involved in glucidic metabolism and cell respiration, and if so, what are the mechanisms of their action.

The aim of this paper is to investigate the influence of flax lignin and spruce bark polyphenols upon germination and biomass accumulation of *Phaseolus vulgaris* L. beans, along with their biochemical interference with some key enzymes involved in glucidic metabolism and cellular respiration. Amylase was selected because this enzyme hydrolyzes starch to sugars, which are necessary for bean embryo development; catalase, ascorbatoxidaze and polyphenol oxidase as indicators of intracellular redox potential and cell respiration. At the same time, lignin and polyphenols were chemically modified by nitration to improve their biological activities.

EXPERIMENTAL

Plant material

Spruce bark (*Picea abies* L.) was obtained from "Somes S.A.", Dej, Romania. Flax lignin was kindly offered by "Granit S.A." Switzerland and beans (*Phaseolus vulgaris* L., variety Ami, 4 years old) were received from the Research and Production Station, Podu-Iloaiei, Romania.

Spruce bark polyphenols

Spruce bark was ground and extracted with diethyl ether to remove greasy substances, such as volatile oils, glycerides, resins, waxes and superior acids or alcohols. Then, the plant material was extracted with 1% NaOH (v/w 10) at room temperature for 3 hours and filtered. Hemicelluloses were precipitated from the filtrate by addition of acetone, followed by centrifugation. Acetone was removed from the supernatant by distillation. The alkaline solution was passed through a column packed with a cationic resin (Vionit S32) to remove sodium cations and the extract was dried under vacuum at 40 °C. The obtained solid material had a dark brown color; yield – 17.51%.

Characterization of spruce bark extract

Total phenolic content was determined by the Folin Ciocâlteu method[19] and expressed in gallic acid equivalents (47.3% referring to dry matter).

Total content of flavonoids was determined by the aluminium chloride method[20,21] based on the formation of a complex of flavonoids with aluminium, which has a maximum absorption at 510 nm. The amount of flavonoids was expressed in quercetin and was found to be 39.36%.

Nitration of lignin and polyphenols

An amount of 1 g of material (lignin or polyphenols) and 20 mL sulfonitric mixture (HNO_3:H_2SO_4 = 1:4, v/v) were kept at 50 °C for 120 min. Then, the reaction mass was poured on a water and ice mixture when the nitro-derivative precipitated. The product was filtered on a Buchner funnel, rinsed with water and air dried. The yield was of 40.1% for spruce bark polyphenols and of 81.7% for flax lignin.

Germination tests

Shortly, for each concentration of the tested biologically active compounds (0.05, 0.1, 0.25 and 0.5 g/L), 10 Petri dishes were used with 10 beans/dish. Each dish received 10 mL of solution, corresponding to the established concentration, and 10 mL of distilled water for reference. Petri dishes were incubated at 25 °C in a thermostat and germination was monitored for 10 days. After that, the number of germinated beans/dish was counted and the green biomass for each dish was weighed and recorded.

Enzymes assay

The activities of amylase, catalase, ascorbatoxidase and polyphenol oxidase were determined by current protocols used in enzymology, and well described by "Worthington Enzyme Manual".[22] In brief, the weighed plant material was crushed in a mortar with a specific buffer solution, filtered off of debris and the filtrate was adjusted to a specific volume. Then, spectrophotometric measurements were performed and enzyme activity was expressed in specific units for each kind of enzyme, in accordance with International Union of Biochemistry.

RESULTS AND DISCUSSION

This experiment was focused on establishing the correlation between any modification of the

activity of some enzymatic systems, under the action of biologically active compounds separated from vegetal wastes, and the response of germinative material to this action.

Figure 1 presents the influence of lignin and polyphenols, along with their corresponding nitro-derivatives, upon the germination capacity of *Phaseolus vulgaris* L. beans.

As can be seen, a low concentration of flax lignin in the culture medium has no effect, in spite of the fact that nitration improves somehow the germination capacity. On the other hand, polyphenols and especially nitrated polyphenols clearly improve the germination process by 10-20%, in comparison with the reference, the optimal dose being between 0.05-0.1 g/L.

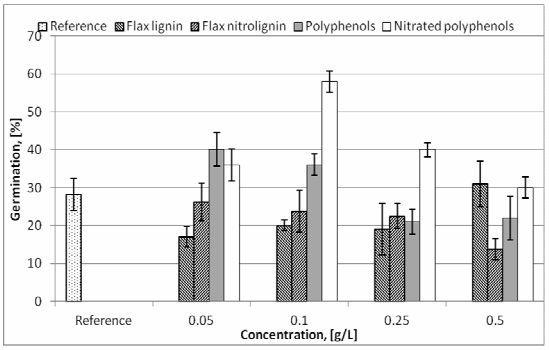

Figure 1: Influence of bioactive compound addition on the germination capacity of *Phaseolus vulgaris* L. beans

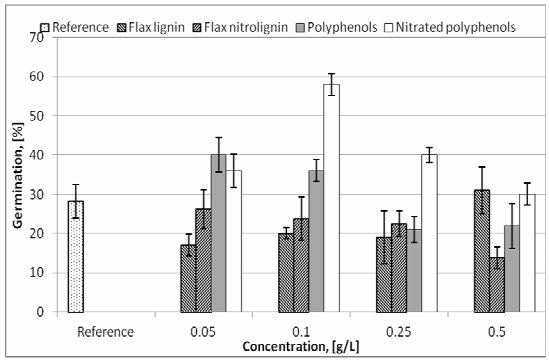

Figure 2: Quantity of biomass as a result of bioactive compound addition in culture media

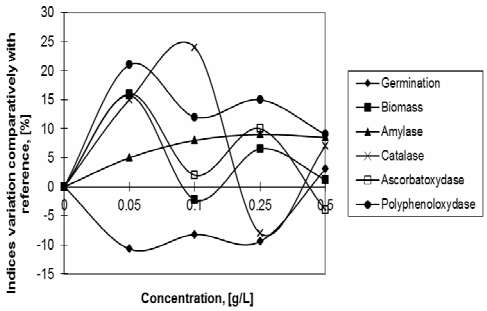

Figure 3: Influence of flax lignin addition on different biosynthesis indices

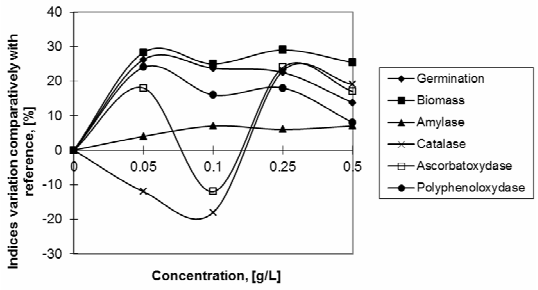

Figure 4: Influence of flax nitrolignin addition on different biosynthesis indices

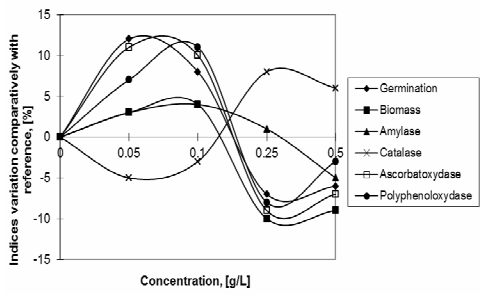

Figure 5: Influence of spruce bark polyphenols addition on different biosynthesis indices

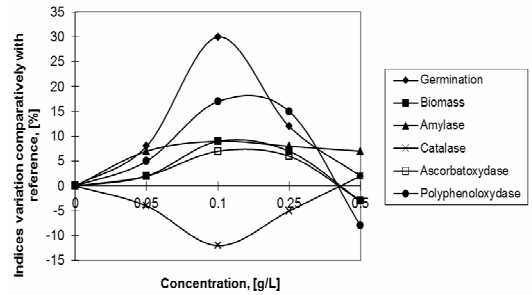

Figure 6: Influence of nitrated spruce bark polyphenols addition on different biosynthesis indices

If lignin and nitrolignin have no great impact upon germination, this is not the case when considering biomass accumulation (Fig. 2). It seems that all the tested compounds act as plant growth hormones in a dose dependent manner, the increase of biomass being between 20-40% compared with the reference, at a concentration ranging between 0.05-0.1 g/L.

The influence of flax lignin and polyphenols upon the activity of enzymatic systems seems to be more intricate. The data presented in Figures 3-6 were computed as the difference between

sample and reference for each studied parameter of the biosynthesis processes.

Plotting the experimental data revealed a curious evolution of the biosynthesis parameters. In Figures 3-6, the sinusoidal character of the evolution curves may be easily remarked. The modification of the relative values of the activity for some enzymes, compared to the reference, by transition from the positive to the negative plane, is perfectly correlated with the inversion of the evolution for the tested biological media.

Thus, for a concentration of the biologically active compound ranging between 0.05 and 0.1 g/L, the increase of the germination capacity, along with biomass accumulation, is related with increased activities of amylase, ascorbatoxidase and polyphenol oxidase, on the average by 15-25%, in comparison with the reference, with a decreased activity of catalase.

The inflexion points situated between the 0.1 and 0.25 g/L concentration domain emphasize the inversion of the observed parameters, which means an inhibition of amylase, ascorbatoxidase and polyphenol oxidase, decreasing the germination capacity and biomass accumulation, all of these being accompanied by a higher catalase activity.

These findings fit well the molecular logic. At low concentrations, polyphenols act as signal molecules for the embryo to exit from the dormant state. Moreover, upon the action of the tested products, the increase in amylase activity determines a more intense mobilization of the reserve substances necessary for the development of the embryo.

The increased activities of ascorbatoxidase and polyphenol oxidase reflect the modification of redox state for intracellular media with intensification of cellular respiration processes. The stimulation of anabolic pathways is translated into the biosynthesis of proteins, distinguished by biomass accumulation.

The higher activity of ascorbatoxidase is a measure of the intensification of cellular respiration, the oxidation of ascorbic acid producing a high quantity of H_2O_2. The decrease in catalase activity denotes that hydrogen peroxide is decomposed by the polyphenol oxidase/flavon-O-diphenol system. Thus, hydrogen peroxide oxidizes polyphenols to the corresponding di-quinoidic forms under the catalytic action of polyphenol oxidase, the latter being reduced by ascorbic acid. The resulted dehydroascorbic acid is reduced to ascorbic acid under the action of some dehydrase.

CONCLUSION

The data presented in this paper establish that there are sufficient arguments to conclude that lignin and polyphenolic compounds extracted from spruce bark, along with their nitro derivatives, show biological activities with an influence upon the development of *Phaseolus vulgaris* L. platules. Thus, in a dose dependent manner, at moderate concentration of biologically active compounds ranging between 0.05 and 0.1 g/L, the germination capacity increased by 10-20% and biomass accumulation reached a maximum peak of 20-40%. These products are directly involved in plant metabolism, influencing the activities of some key enzymes involved in glucidic metabolism and cellular respiration, and are perfectly correlated with the evolution of biological media. This research work contributes to a better understanding of the intricate mechanism by which polyphenols greatly influence metabolic pathways during seed germination and plant development.

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