Histo-Anatomic Aspects on *Zea Mays* L. Influenced by Hemp Shives Polyphenolic Extract

Corneliu TANASE*, Sanda COȘARCĂ, Silvia OROIAN

Faculty of Pharmacy, University of Medicine and Pharmacy, Gheorghe Marinescu, 38, 540139, Tîrgu Mureș, Mureș, Romania

* corresponding author: tanase.corneliu@yahoo.com

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ABSTRACT

The plant kingdom is an inexhaustible source of bioactive compounds with antioxidant character. It is known that, natural polyphenols are essential compounds in stimulating plant growth and development. The aim of this study was to identify the internal structure changes in maize (*Zea mays* L.) seedlings, under the influence of hemp shives polyphenolic extract. Hemp shives polyphenolic extract was characterized in terms of the total content of polyphenols and individual content by HPLC. Germination tests were carried out going through a standard procedure. In order to perform the histo-anatomical characterization, the vegetal material was fixed and preserved in alcohol 70%, following to be cut by hand microtome. The sections obtained were stained using double staining reagents: green - iodine and ruthenium red. It was observed, compared with the control, that hemp shives polyphenolic extract determines a good development of the root hairs and increase leading bundles number and central vessels metaxylem. Hemp shives polyphenolic extract in lower concentrations has positive effects on developed process of *Zea mays* L. plants that can be attributed to bioregulation properties.

Keywords: *hemp shives, histo-anatomic, polyphenolic extract, Zea mays* L.

INTRODUCTION

Maize is ranked third after wheat and rice among the most important cereal crops. Maize is essential for human and live-stocks consumption as a major source of carbohydrates, oil or as a protein source. Maize is widely grown as cereal crop in many developing countries including Romania (Jans *et al.*, 2010).

The hemp shives are wastes resulted after bast fibers separation of the *Cannabis sativa* plant species that is grown specifically for the industrial uses of its derived products. It is one of the fastest growing plants. It can be refined into a variety of commercial items including paper, textiles, clothing, biodegradable plastics, paint, insulation, biofuel, food, and animal feed (Keller, 2013). The outside of the hemp stalk is covered with epidermis, underneath it there are bast fibres whereas in the centre the stem there is a wood-pulp core (shive), the proportion of which in the

hemp is between 60 to 80% of the total mass of the stem. The main chemical components of the shive like in spruce wood are cellulose (34-48%), hemicellulose (21-37%) and lignin (16-28%). The difference in chemical composition is determined by the variety, the soil type, the climate conditions (Jaraboa *et al.*, 2012; Troëdec *et al.*, 2009; Troëdec *et al.*, 2011).

Phenolic compounds are secondary metabolites. They are one of the most numerous and widely distributed group of aromatic compounds in the plant kingdom, with over 8000 phenolic structures currently known, of which more than 6000 are the flavonoids (Tsao, 2010; Knezevic *et al.*, 2012). Polyphenols are natural compounds with aromatic structures containing one or more aromatic rings with or without the vicinity of a heterocycle and which are grafted with hydroxyl, carboxyl, methoxyl and carbonyl functional groups. According to the basic chemical structure,

polyphenols can be classified into more different classes (Garcia Salas *et al.*, 2010). However, two main groups of polyphenols can be identified: flavonoids and nonflavonoids.

Phenolic compounds are considered the most abundant constituents of plants and processed foods; some compounds are extremely widespread while others are specific of certain plant families or found only in some plant organs or at certain development stages. Main sources of phenolic compounds are fruits and vegetables, seeds, cereals, berries, beverages (wine, tea and juices), olive and aromatic plants (Sikora *et al.*, 2008). In last years many studies focused a special attention to the presence of these compounds in agricultural and industrial wastes, wood and non-wood forest resources (Moure *et al.*, 2001; Ignat *et al.*, 2009, 2011; Stinga *et al.*, 2009 a and b; Tanase *et al.*, 2013 a and b, 2014a, 2016; Volf and Popa, 2004; Volf *et al.*, 2006). Natural polyphenols have a broad spectrum of regulating action in plants, tissues and organs, interfering in the metabolic processes (Biesalski *et al.*, 2009; Kris-Etherton *et al.*, 2002; Tomas-Barberan *et al.*, 2000; Tringali, 2001; Zhang *et al.*, 2009). The stimulation or inhibition abilities of natural polyphenols in plant growth and development are closely correlated with the concentrations of these compounds. Thus, in some cases the presence of these compounds in low concentrations can have a beneficial effect on plant's development and in other cases, when concentrations are higher there is an inhibition phenomenon (Popa *et al.*, 2007). Increasing interest in the replacement of synthetic growth regulators (Sairam, 1994) has led to a search into natural sources of regulator agents.

The study of the polyphenolic compounds effect on the level of living systems, presents a special scientific interest. Through, it has been established that these compounds have many effects on living systems, such as: antioxidant, immunostimulating, antimutagen, antibacterial, bioregulator. In previous work (Tanase *et al.*, 2016) we found that the aqueous extract obtained from the spruce bark determines a good development of root hair, vascular bundles and metaxylem vessels. It was observed that at higher concentration of the extract root hairs are more reduced and the process of lignification is more intense compared to the control. Therefore, it was found that bioactive compounds existing

in hemp shives aqueous extracts can modulate the bioaccumulation process of lead and copper ions in *Brassica napus*. At low concentrations in soil the presence of hemp shives stimulates lead and copper bioaccumulation, promoting the translocation of heavy metals to the aerial part of the plant. In this case the polyphenolic compounds from hemp shives increase the solubilisation of heavy metals and improve the phytoextraction process (Tanase *et al.*, 2014b). The present research is concerned with the use of the wastes from the industrial hemp, *Cannabis sativa* L., to obtain polyphenolic extract and to identify the internal structure changes in maize (*Zea mays* L.) seedlings, under the influence of hemp shives polyphenolic extract.

MATERIALS AND METHODS

The hemp shives used in this study are wastes resulted after bast fibers separation. The vegetal material was randomly collected, while the species was identified and authenticated. The vegetal material was shade-dried at room temperature for two weeks, a reference sample (blank) being kept for comparison. For chemical characterization, 20 grams of ground dried material with particle size of 0.5-1mm were extracted consecutively for three times with 125 mL distilled water on a water bath, for 45 minutes, at 80°C, and finally, the extracts were cumulated to a final volume of 500 mL using distilled water. Dry matter content in the extracts was determined by evaporation of 25 mL extract on water bath and drying at 105°C, until a constant mass is reached. After that the crucible containing the dry matter was placed into a furnace at 600°C, in order to establish the mineral and organic matter content. Folin Ciocalteu (FC) method was used to determine the total polyphenolic content (TPC) in aqueous extract and the concentrations were expressed as the number of equivalents of gallic acid (GAE) (Bao *et al.* 2005; Ignat *et al.* 2011). For the HPLC analysis, the cumulated aqueous extracts were concentrated under vacuum to 10 mL. These results as well as the extraction method were published in our previous work (Tanase *et al.*, 2014b).

Maize seeds were purchased from Unisem Company, Romania. Germination tests were carried out going through a standard procedure, using increments of 10 Petri dishes for each solution studied. On a filter paper were placed

every five maize seeds, carefully selected without present any major damage. For starters, the vegetal material has undergone a presterilization process, which consisted of submerged seed in absolute ethanol for 10 seconds, following the sterilization in the presence of sodium hypochlorite 10% for 20-30 minutes (Cachiça *et al.*, 2004). The volume of hemp shives polyphenolic solution added was 10 mL / dishes meanwhile for the reference samples distilled water was used. Petri dishes thus prepared were incubated in the dark in a thermostat set at 28 ° C. After a period of seven days Petri dishes were taken out and the roots, stems and leaves separated for histo-anatomic analysis. In order to perform the histo-anatomical characterization, the vegetal material was fixed and preserved in alcohol 70%, following to be cut by hand microtome with botanical razor (Tanase *et al.*, 2016). First, were performed cross sections, using botanical razor, hand microtome and marrow elderberry through the vegetative organs of plants and seedlings. Obtained sections were collected on a watch glass with water. The sections obtained were stained using double staining reagents: green - iodine and ruthenium red. After removing water from the glass plate, the sections were stained for several tens of seconds (with a few drops of methylene blue) or 1 minute (with a few drops of iodine green). After removing the dye, the sections were washed with 70% ethanol and then once with tap water and were then stained for several tens of seconds ruthenium red and subsequently washed twice with tap water. Subsequently stained sections were mounted between slide and slide in a few drops of water and analyzed with a microscope Novex (Holland) and photographed with Nikon Coolpix L22 (Tanase *et al.*, 2016).

All the results are expressed as mean $\pm$ standard error where $n = 3$. Comparison of the means was performed by the Fisher least significant difference (LSD) test ($p \leq 0.05$) after ANOVA analysis using program PAST 2.17c. Sampling and chemical analyses were examined in triplicate, in order to decrease the experimental errors and to increase the experimental reproducibility.

RESULTS AND DISCUSSION

The characterization of hemp shives extract was made in a past work (Tanase *et al.*, 2014b). Thus, the total polyphenols content of hemp shives aqueous extract was 164 mg GAE/100g. The chromatographic analysis revealed: catechins (5.21 g/100g plant material), caffeic acid (9.68 g/100g), P - coumaric acid and ferulic acid in reduced quantites.

To highlight the effect of hemp shives polyphenolic extract, some histo-anatomical aspects of the internal structure of maize seedlings were analysed. Thus, in this paper, histo-anatomical aspects, regarding the internal structure of primary roots, adventitious roots, mesocotyl, epicotyl and leaves will be presented.

At the root level (Fig. 1. b), the root hairs are larger and many compared to the control (Fig. 1. a). Exoderma is only sketched, having larger cells compared to the endoderm. In cortical parenchyma are numerous aeriferous cavities with different size and radial elongated. Endodermis cells present the primary stage (thickening Caspary) and few of them show the inner wall thickened. The number of conductive vascular bundles is higher (8), all surrounded by a unsclerified and unligified parenchym. Medullary parenchym is noticeably lower than the control.

Fig. 1. The internal structure of the main root, a. general aspect (control), b. general aspect (P2), c. detail-P1 (pith, central cylinder, cortex, rizodermis)

Fig. 2. The internal structure of adventitious roots, a. general aspect (control), b. general aspect (P2), c. central cylinder (P2)

Fig. 3. The internal structure of mesocotyl, a. general aspect (control), b. general aspect (P2), c. central cylinder (P2)

At a higher concentration (233 mg GAE/L) is observed (Fig. 1.c) that endodermis is in the transition stage from primary to tertiary type. The number of xylem bundles, as well as the phloem bundles is smaller comparing with control (14 xylem, 14 phloem). The number of central vessels of metaxylem is 6, all surrounded by a layer of lignified parenchyma cells. Lignification process was noticed to be more intense, both in xylem vessels and in the central vessels metaxylem parenchyma. On some sections was observed the formation of lateral roots endogenously.

Adventitious roots (Fig. 2. b) present frequent and long root hairs, with only sketched exoderma consists of cellulose walls. The endodermis is in the primary type. Aeriferous cavities in the cortical parenchyma are large and often many times next to each other separated only by a series of radial parenchyma cells with a moderate sclerified pericycle. The number of vascular bundles is higher comparing with control (14) and central metaxylem vessels (5) are arranged in a circle (Fig. 2. b, c). At a higher concentration (233 mg / L) root hairs are very rare or absent and exoderma is

without suber. The cortical parenchyma includes many aeriferous cavities and endodermis is in the primary type same like P2 variant. The number of vascular bundles is higher compared to the control (13 xylem, 13 phloem) as that of the central vessels metaxylem (6) each surrounded by a layer of lignified parenchyma cells.

The coleoptile (Fig. 4.b, c) has mesophyll from the two poles, much thicker than the sides, which is often reduced to only two epidermis. The aeriferous cavities of mesophyll missing. The two vascular bundles (Fig. 4. b, c) are typically collateral closed (the phloem being partially surrounded by xylem vessels) and are less developed compared to the control (Fig. 4.a). At a higher concentrations (P1), the thickness of coleoptyle at the two poles of the section is slightly greater than the sides. The external epidermis cells are visible radial elongated and are much larger than the interior epidermis. Aeriferous cavities of mesophyll are missing and the two driving vascular bundles are structurally similar to those of P2 version, always the phloem is more abundant than xylem. In case of leaf (fig. 5. b and c) groups of bulphorm cells on

Fig. 4. The internal structure of coleoptile, a. general aspect (control), b. general aspect (P2), c. vascular bundle (P2)

Fig. 5. The internal structure of foliar blade, a. general aspect (control), b. the internal structure of foliar blade - general aspect (P2), c. the internal structure of foliar blade - vascular bundles (P2)

upper epidermis can be observed.

The cells from mesophylic hypoderm are slightly elongated radial on both sides of the leaf lamina. To the edge of the limb, mesophylic is reduced to 2-3 layers of cells and to the edge are visible only two epidermis. At a higher concentration hypodermic mesophylic has only slightly elongated cells radiate.

CONCLUSIONS

Analyzing the obtained results we can appreciate that the aqueous extract obtained from the hemp shives determines a good development of root hair, vascular bundles and metaxylem vessels. Addition of the aqueous extract obtained from hemp shives in the growth of maize seedlings leads to a increase in the number of vascular bundles. Their number decreases with increasing concentration of extract applied. In the adventitious roots are less frequent hairs root and exoderma is unsuberified. The mesocotyl has xylem vessels smaller than that of the control. At higher concentration is tertiary type of endoderm.

In the coleoptile xylem is less developed compared to the control.

Hampe shives polyphenolic extract in lower concentrations has positive effects that can be attributed to their antioxidant properties. When the extracts with high concentrations of polyphenols were used, it was found inhibitory effect on the growth and development of plant systems. The results recorded in this study confirm some of the previously hypotheses and open research in the field.

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