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COMPOSITION OF ROOT EXUDATES OF *Miscanthus* × *Giganteus* GREEF ET DEU

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Abstract

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Rate of root exudation and identification of selected compounds in root exudates of *Miscanthus* × *Giganteus* in the autumnal period of growth was performed. Total organic carbon of root exudates was formed from 7.8% by carbohydrates and from 1.5% by proteins. Aspartic acid, arginine, alanine and glutamic acid were exuded with the highest rate of all amino acids. This work brings new basic knowledge which can be used for phytoremediations.

root exudates, *Miscanthus* × *Giganteus,* proteins, carbohydrates, amino acids, carbon, phytoremediation

Miscanthus × *Giganteus* Greef et Deu is a perennial, rhizomatous C_4-grass originating from Southeast Asia. This species is sterile and probably natural hybrid involving *M.* × *sacchariflorus* (diploid) and *M. sinensis* (tetraploid) with a triploid chromosome, impossible of producing seeds (Greef and Deuter, 1993; Linde-Laursen, 1993). Its propagation is therefore performed by micropropagation or by rhizome cutting. *M.* × *Giganteus* Greef et Deu was firstly recognized in 1935 in Japan, from where it was introduced to Denmark, and it was named *Miscanthus sinensis* "*Giganteus*" hort. (Greef and Deuter, 1993; Greef et al., 1997). Canopy of *M.* × *Giganteus* can reach a height of 4 m, estimated life time of a plantation is 20–25 years, and maximum dry biomass yield was close to 40 Mg.ha^{-1} (Lewandowski et al., 2003a; Miguez et al., 2008). *M.* × *Giganteus* belongs to group of *Miscanthus* species cultivated across Europe as a potential biofuel; according to biomass yield and its quality for combustion *M.* × *Giganteus* appears to be the most suitable for midlatitudes (approximately 30° to 60°) in Europe (Lewandowski et al., 2003b; Collura et al., 2006; Michel et al., 2006 etc.). Except this, *M.* × *Giganteus* is suitable for the production of raw material for paper industry, composts etc. (Greef and Deuter, 1993; Stander, 1989). *M.* × *Giganteus* has been intensively studied for different purposes (Arduini et al., 2006; Lewandowski et al., 2003a). Knowledge about root exudation and qualitative composition of root exudates of this plant is missing.

Exudation from plant roots is of great significance for carbon and nitrogen cycling in soil. Sugars, organic acids and amino acids are commonly the most abundant compounds in water-soluble root exudates (Aulakh et al., 2001). Nitrogen compounds released from roots into the soil provide N for rhizosphere microflora (Grayston et al., 1998) and for neighboring plants (Høgh-Jensen and Schjoerring, 2001; Jones et al., 2005). Amino acids, which are readily degradable by soil microorganisms, are usually the third (or second) most abundant compounds in water-soluble root exudates and have been the subject of many studies (e.g. Mench, 1985; Merbach et al., 1999; Gransee and Wittenmayer, 2000). Exudation from plant roots is very important for phytoremediation of polluted sites. Plant root exudates are generally hypothesised to increase the population numbers and activity of soil microorganisms that degrade various organic contaminants via cometabolism or metabolism that is linked to growth on other substrates and may increase contaminants bioavailability (Haby and Crowley, 1996; Miya and Firestone, 2001; Henry et al., 2007). Root exudates can also increase mobility or bind heavy

metals in the rhizosphere and can be used as a natural chelating agens to enhance phytoextraction (Mench and Martin, 1991; Dousset et al., 2001; Kim et al., 2010). A review on root exudation of amino acids related to plants of different type of photosynthesis is presented in work of Rejšek et al. (2009).

In this study we have attempted to determine exudation rate from roots of *Miscanthus* × *Giganteus* cultivated in Botanic Gardens and Arboretum of Mendel University in Brno, the Czech Republic for total organic carbon, total carbohydrates, proteins and amino acids.

MATERIAL AND METHODS

M. × *Giganteus* was taken from its cultivation in Botanic Gardens and Arboretum of Mendel University of Agriculture and Forestry in Brno, the Czech Republic (N 49°12'54.240", E 16°36'41.989", 235.19 meters a. s. l). The cultivation of this plant was there established more than 15 years ago on plot of 291.41 m^2 when aboveground biomass has been harvested every year in period of February – March. Rhizomes with roots were digged out from cultivation of *M.* × *Giganteus* in autumnal period of growth. After careful washing in tap water were roots clipped off rhizomes and carefully washed in demineralized water. Due to presence of rhizomes was impossible to collect exudates of naturally cultivated plants without root clipping off. Root exudates were collected in demineralized water for a period of 2 hours at room temperature. Immediately after collection, each sample of exudates solution was successively filtered through a filter paper and a 0.45 µm membrane filter to remove root detritus and microbial cell debris.

Total proteins were assessed according to Bradford (1976) using bovine serum albumin (BSA) as the standard. For recalculation to carbon of proteins, TOC (total organic carbon) of BSA was assessed to be about 50 wt % (Wen et al., 1999). Total carbohydrates were determined using the anthrone colourimetric assay (Brink et al., 1960). TOC was determined by the wet digestion method when an aliquot of 8 ml was mixed with 15 ml of mixture (100 ml of 66.7 mM $K_2Cr_2O_7$ + 500 ml concentrated H_2SO_4 + 200 ml concentrated H_3PO_4) and kept at 125 °C for 45 min. The samples were cooled and titrated with 0.05N Fe $(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in H_2SO_4.

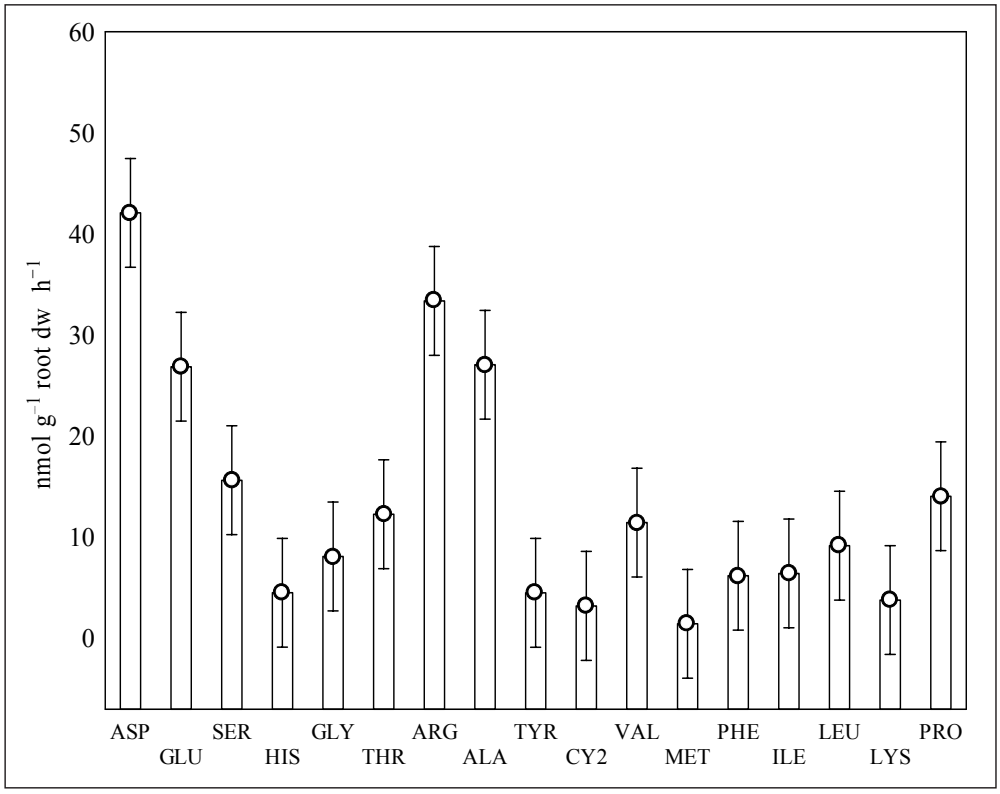
After concentration of part of the solution with root exudates to powder by freeze-drying were samples analyzed for amino acids using the HP 1100 liquid chromatograph (Hewlett Packard, Wilmington, DE, USA) with a FLD HP 1100 fluorometric detector (operating at 450 nm (Ex = 340 nm)) and a Zorbax Exlipse AAA Rapid Resolution column (4.6 × 150 mm, 3.5 µm particle size, Agilent Technologies, USA). A linear gradient mobile phase profile, consisting of 40 mM Na_2HPO_4, pH 7.8 (solvent A) and ACN/MeOH/water at 45:45:10 (v/v) (solvent B), was applied at a flow rate of 2.0 ml/min. The procedure and gradients produced were as follow: 0% B (1.9 min), 0–57% B (16.2 min), 57–100% B (0.7 min), 100% B (3.5 min), 100–0% B (0.9 min) and 0% B (2.8 min). The column was equilibrated for 5 min under initial conditions prior to injection of each sample. Column temperature was 40 °C. Amino acids from soil extracts were then determined using pre-column derivatization with *o*-phthalaldehyde (OPA) (Formánek et al., 2005).

RESULTS AND DISCUSSION

As found in this work, the average rate of TOC exudation from roots of *M.* × *Giganteus* was 0.22 $mmol.g^{-1}$ root $dw.h^{-1}$. Of TOC was 7.8 ± 1.65% given by carbon of total carbohydrates, and 1.5 ± 0.09% by carbon of proteins (mean ± SE, n = 4). Compared to the other works, recalculation of data of Aulakh et al. (2001) showed exudation rate of TOC by rice cultivars roots in demineralised water around 0.02–0.05 $mmol.g^{-1}$ root $dw.h^{-1}$ and 62–83% of TOC was formed by total carbohydrates. Exudation rate of total carbohydrates from roots of Sudangrass was found to be 0.01–0.04 mmol $C.g^{-1}$ root $dw.h^{-1}$ (recalculated from Schwab et at., 1983). Rape (*Brasica napus* L.) root exudates of stressed and control plants contained 2–5% of C as carbohydrates and 7–28% as amino acids (Svenningsson et al., 1990). Henry et al. (2007) reported TOC exudation rate from differently treated crested wheatgrass (*Agropyron cristatum*) upto approx. 0.06 $mmol.plant^{-1}.day^{-1}$. Total carbohydrates formed 9.6–46.8% of total C of root exudates of *Nicotiana tabacum* L., *Nicotiana rustica* L. and *Zea mays* L. (recalculated from Mench and Martin, 1991). Heim et al. (2001) reported exudation rate of TOC by roots of differently treated Norway spruce seedlings in a 2 day period in range 20–40 $\mu mol.g^{-1}$ fw. Total carbohydrates in this study formed 3.7–12% of TOC what is in accordance with results obtained in this study.

Total proteins and sugars in root exudates of herbaceous plants were found to decrease with plant age (Hamlen et al., 1972). According to Vančura (1988) root exudates of plants contained 4.1–11.8% peptides and proteins together. Vančura and Hanzlíková (1972) reported that proteins alone correspond to approx. 3–8% of dry mass of root exudates. Proteins formed 0.9–1.3% of total C of root exudates of *N. tabacum* L., *N. rustica* L. and *Zea mays* L. (recalculated from Mench and Martin, 1991).

From the results of this study is evident that aspartic acid, arginine, alanine and glutamic acid are the most abundant amino acids in root exudates of *M.* × *Giganteus* (see Fig. 1). Each of these amino acids forms > 10% of total amino acids of root exudates. These results broaden a range of C_4-plants including corn (*Zea mays* L.), millet (*Panicum miliaceum* L.), Sudan grass (*Sorghum vulgare* Pers.) or blue grama grass (*Bouteloua gracilis* (Kunth) Lag. ex Griffiths) where root exudation of amino acids was determined. Based on the knowledge available for these plants we can state that alanine, glutamine, aspartic acid, glycine, serine, glutamic acid, proline, lysine, γ-aminobutyric acid, valine, arginine and cystine are the most abundant amino acids in root exudates of



1: *Rate of individual amino acids exudation by roots of Miscanthus × Giganteus. All values represent means ± SE (n = 4)*

C_4 plants (see review of Rejšek et al., 2009). Aspartic acid, arginine, alanine and glutamic acid which were dominant amino acids in root exudates of *Miscanthus* × *Giganteus* are also in accordance with the dominant amino acids found in root exudates of different C_3-plants. In case of various C_3-plants the most abundant amino acids were found to be serine, glycine, glutamic acid, alanine, tyrosine, arginine, proline, cystine and aspartate (Rejšek et al., 2009). Exudation rate of amino acids found in this study is within the range determined for amino acid efflux by different research works. For example, Kraffczyk et al. (1984) determined that the net rate of efflux of amino acids was 4.7 nmol.g^{-1} root dw.h^{-1} and Jones and Darrah (1994) calculated that the actual efflux of amino acids was 8540 nmol.g^{-1}.h^{-1}. Paynel et al. (2001) determined amino acid exudation rate in range 3–25 nmol.plant^{-1}.day^{-1}. In our study was exudation rate of total amino acids 230.3 nmol.g^{-1} root dw.h^{-1} what is 98 nmol C.g^{-1} root dw.h^{-1}.

SUMMARY

The objective of this work was to determine exudation rate of total organic carbon, carbohydrates, proteins and amino acids from roots of *Miscanthus* × *Giganteus*. *M.* × *Giganteus* Greef et Deu is a perennial, rhizomatous C_4-grass originating from Southeast Asia and is studied within Europe for different purposes including the production of biofuels, composts, paper industry, phytoremediations etc. This species is a sterile and probably natural hybrid involving *M.* × *sacchariflorus* (diploid) and *M. sinensis* (tetraploid) with a triploid chromosome, impossible of producing seeds. The performation of this study was due to need of broadening the basic knowledge on *M.* × *Giganteus* and obtaining information for purposes of phytoremediations. The study was performed on roots clipped off the rhizomes of plants cultivated for more than 15 years in Botanic Gardens and Arboretum of Mendel University of Agriculture and Forestry in Brno, the Czech Republic. Collection of exudates was performed in demineralized water for a period of 2 h at room temperature. Individual compounds, including total organic carbon, total carbohydrates, proteins and amino acids were determined either before or after concentration of solution to powder by freeze-drying; using commonly used methods (wet digestion, anthrone colorimetric assay, Bradford method or HPLC). Results of this study showed that carbon was exuded from roots of *M.* × *Giganteus* in average rate of 0.22 mmol.g^{-1} root dw.h^{-1} and from this amount was 7.8 % formed by carbohydrates and from 1.5 % by proteins. Of 17 measured amino acids (aspartic acid, glutamic acid, serine, histidine, glycine, threonine, alanine, tyrosine, valine, methionine, pheny-

lalanine, isoleucine, leucine, proline, arginine, cystine and lysine), aspartic acid, arginine, alanine and glutamic acid were exuded with the highest rate.

SOUHRN

Složení kořenových exsudátů *Miscanthus* × *Giganteus* Greef et Deu

V této práci bylo studováno složení kořenových exsudátů rostliny *Miscanthus* × *Giganteus* a rychlost kořenové exsudace. *Miscanthus* × *Giganteus* Greef et Deu je vytrvalá, rhizomatózní rostlina (C_4-tráva), pocházející z jihovýchodní Asie. Tato rostlina je v rámci Evropy intenzivně studována z důvodu jejího využití pro výrobu biopaliv, kompostů, v papírenském průmyslu či fytoremediacích atd. Druh *M.* × *Giganteus* představuje sterilní a pravděpodobně přírodní hybrid vzniklý křížením *M.* × *sacchariflorus* (diploid) a *M. sinensis* (tetraploid), s triploidním chromozomem, postrádající schopnost produkce semen. Tato studie byla iniciována z důvodu rozšíření základních poznatků o *M.* × *Giganteus*, které mohou být následně využívány pro účely testování účinnosti dekontaminace půd, znečištěných organickými polutanty. Rhizomy s kořeny *M.* × *Giganteus* byly získány z více než patnáctiletého porostu, který se nachází v Botanické zahradě a arboretu Mendelovy zemědělské a lesnické univerzity v Brně. Exsudáty z odstříhaných kořenů byly sbírány v demineralizované vodě po dobu 2 hodin, při pokojové teplotě. Analýza jednotlivých složek kořenových exsudátů (celkový organický uhlík, celkové cukry, bílkoviny a jednotlivé aminokyseliny) byla provedena standardně používanými metodami (spalování za vlhka, reakce s anthronovým reagentem, metodou podle Bradforda či HPLC). Výsledky studie ukázaly, že rychlost exsudace celkového organického uhlíku z odstříhnutých kořenů *M.* × *Giganteus* dosahovala v průměru 0,22 $mmol.g^{-1}$ suchých kořenů za hodinu. Celkové cukry tvořily 7,8 % a bílkoviny 1,5 % celkového organického uhlíku. Z celkového počtu 17 aminokyselin (kyselina asparagová a glutamová, serin, histidin, glycin, threonin, alanin, tyrosin, valin, methionin, fenylalanin, isoleucin, leucin, prolin, arginin, cystin a lysin) byla nejvyšší rychlost kořenové exsudace stanovena v případě kyseliny asparagové, argininu, alaninu a kyseliny glutamové.

kořenové exsudáty, *Miscanthus* × *Giganteus*, proteiny, cukry, aminokyseliny, uhlík, fytoremediace

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