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Research article

Effects of cadmium-resistant fungi *Aspergillus aculeatus* on metabolic profiles of bermudagrass [*Cynodondactylon* (L.)Pers.] under Cd stress

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Plants' tolerance to heavy metal stress may be induced by the exploitation of microbes. The objectives of this study were to investigate the effect of cadmium (Cd)-resistant fungus, *Aspergillus aculeatus*, on tolerance to Cd and alteration of metabolites in bermudagrass under Cd stress, and identify the predominant metabolites associated with Cd tolerance. Two genotypes of bermudagrass with contrasting Cd tolerance (Cd-sensitive 'WB92' and Cd-tolerant 'WB242') were exposed to 0, 50, 150 and 250 mg kg^{-1} Cd for 21 days. Physiological responses of bermudagrass to Cd stress were evaluated based on the relative growth rate (RGR) and normalized relative transpiration rate (NRT). Plants inoculated with *A. aculeatus* exhibited higher RGR and NRT under Cd stress than those of non-inoculated plants, regardless of genotypes. A total of 32 Cd-responsive metabolites in leaves and 21 in roots were identified in the two genotypes, including organic acids, amino acids, sugars, and fatty acids and others. Interestingly, under Cd stress, the leaves of inoculated 'WB92' accumulated less citric acid, aspartic acid, glutamic acid, sucrose, galactose, but more sorbose and glucose, while inoculated 'WB242' leaves had less citric acid, malic acid, sucrose, sorbose, but more fructose and glucose, compared to non-inoculated plants. In 'WB92' roots, the *A. aculeatus* reduced mannose content, but increased trehalose and citric acid content, while in 'WB242', it decreased sucrose, but enhanced citric acid content, compared to Cd regime. The results of this study suggest that *A. aculeatus* may induce accumulation of different metabolites associated with Cd tolerance in bermudagrass.

1. Introduction

Heavy metals such as Cd, copper, lead and zinc enter the environment via disposal of acidified soils or urban sewage sludge, utilization of agricultural phosphate fertilizers and industrial activities, which poses considerable threat to agriculture and forestry (Barceló and Poschenrieder, 1990; Khan, 2005). Among the mentioned heavy metal pollutants, Cd, one of the most dangerous and toxic environmental pollutant, is an important abiotic stress affecting the growth of plants (Xu et al., 2012).

It has been postulated that higher plants are more sensitive to Cd stress than microorganisms. Soil Cd phytotoxicity has been associated with exposure to 1 mg kg^{-1} total Cd and higher total Cd in soils might impose phytotoxic effects on higher plants. Therefore, the presence of Cd, even at minimum quantities causes phytotoxicity to higher plants (Peterson and Alloway, 1979; Wagner, 1993; Yan et al., 2016). For microorganisms, the vast range of Cd concentration, from 20 to over 1000 mg kg^{-1}, in contaminated areas suggests a corresponding range of adaptability and sensitivity of microorganisms to Cd (Babich and Stotzky, 1977). Although Cd is a nonessential trace element in the process of plant growth; it can displace essential elements thus inhibiting plant growth (Ahsan et al., 2012). Previous research has demonstrated that Cd can restrain growth and transpiration of plants, inhibit chlorophyll synthesis and impair photosynthesis (Xie et al., 2014a, 2014b). In addition, Cd toxicity can also trigger a series of plant

Abbreviations: IAA, indole-3-acetic acid; Cd, cadmium; F, *Aspergillus aculeatus*; GC-MS, gas chromatography-mass spectrometry; GSH, glutathione; RGR, relative growth rate; ROS, reactive oxygen species; HCA, hierarchical clustering analysis; H_2O_2, hydrogen peroxide; NRT, normalized relative transpiration; OH^-, hydroxyl radical; O_{2^-}, superoxide radical; PCA, principal component analysis; PGRP, plant growth-promoting rhizobacteria.

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physiological and biochemical changes by inhibiting nutrient absorption and generating oxidative stress (Belkhadi et al., 2010). Previous studies have demonstrated that Cd could induce the production of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-) and superoxide radical (O_{2^-}), leading to serious damage to primary cellular components, which causes root necrosis and retarded growth (Foyer et al., 1994; Schützendübel et al., 2001). Fortunately, plants have evolved a series of complex antioxidant enzymes to remove different types of ROS in response to oxidative damage arising from Cd toxicity (Srivastava et al., 2004).Furthermore, heavy-metal pollution significantly influences the growth and metabolism of microorganisms by limiting biochemical activity and altering community structure (Kandeler et al., 2000). Previous studies indicated that heavy-metal pollution decreased growth rate of bacteria and reduced bacterial diversity in heavy-metal contaminated regions (Pennanen et al., 1996; Gans et al., 2005). In other studies, it was reported that Cd toxicity also inhibits growth and chlorophyll synthesis of microalgae (Awad and Chu, 2005; Lamai et al., 2005). To sum up, heavy-metal soil contamination to a certain extent negatively affects plants, growth and microbial community.

Plants respond to Cd stress in a variety of ways including a series of stress-inducible reactions and defense mechanisms (Zhang et al., 2005). One of the vital mechanisms that plants use to cope with heavy-metal toxicity is the accumulation of an array of metabolites such as amino acids, organic acids, and sugars which may serve as osmoprotectants and antioxidants (Sharma and Dietz, 2006; Shulaev et al., 2008). Previous study indicated that the Cd-induced production of citric acid displayed a remarkable role in iron translocation and xylem transport (Durrett et al., 2007). The involvement of citric acid in Cd hyper-accumulation was observed in *Solanum nigrum*, a Cd-hyper accumulator species (Sun et al., 2006). Ernst (1975) first reported that plants' zinc-tolerance ability is closely related to high malic acid concentration. In addition, plants could accumulate specific amino acids, such as proline, in response to heavy metals exposure (Xie et al., 2014a). Previous studies have demonstrated that plants could accumulate proline in response to heavy-metal stress, and that it enhanced plants tolerance to heavy metals by regulating cell osmotic potential and detoxifying the reactive oxygen free radicals (Chen et al., 2003; Xu et al., 2009). The increase of amino acid and citric acid in *S. nigrum* under Cd condition suggested that the plants may tolerate metal toxicity and improve stress tolerance by accumulating certain metabolites (Xu et al., 2012). Thus, Cd toxicity remarkably increased the accumulation of several amino acids and organic acids related to plants, adaptation to this kind of stress, and application of exogenous organic acids may have the potential to improve Cd extraction in *S. nigrum* (Sun et al., 2006; Gao et al., 2010). These results suggest that the differential metabolic responses to Cd stress may be associated with the differences in Cd tolerance among plant species.

Heavy-metal contamination of land is a critical environmental problem, especially Cd contamination of soils. Hence, reclaiming the Cd-contaminated soil is urgent for environmental conservation (Abou-Shanab et al., 2006). Several remediation strategies for Cd-contaminated soil are feasible, such as chemical engineering, mechanical removal, microbial remediation and phytoremediation, i.e., the use of plant systems to remove Cd from the soils. Particularly, phytoremediation is an environment-friendly and cost-effective approach that is widely employed compared to the other methods. However, phytoremediation also has some important shortcomings such as being quite slow, limited contaminant uptake and phytotoxicity (Shi and Cai, 2009; Weyens et al., 2010). Recently, microbial remediation has attracted considerable attention due to its high potential of providing an economical and efficient method. This is because microorganisms can minimize the toxicity of heavy metals through absorption and fixation of the heavy-metal ions (Singh et al., 2008). Although heavy metals affect soil microorganisms by decreasing their biochemical activity and altering their community structure, it can result in the establishment of resistant microbial populations. Even individual microorganisms can thrive in metal-contaminated area due to their remarkable heavy-metal absorption capacity. These microorganisms with metal tolerance play a significant role in bioremediation of metal-contaminated soil (Kapoor et al., 1999; Kandeler et al., 2000). In addition, the secretions of microorganisms, such as indole-3-acetic acid (IAA) and siderophores, are able to stimulate the growth of plant. On the other hand, nutrition provided by plant roots can enhance microbiological activity. Viable phytoremediation can be accomplished by microbial removal of heavy metals and plant colonization of contaminated soils (Khan, 2005). Thus, enhancing the interaction between plants and microorganisms can improve the biomass production of plants, which has been considered as an effective remediation approach for improving the efficiency of heavy-metal removal (Gao et al., 2010).

Bermudagrass [*Cynodon dactylon* (L.)Pers.] is a widespread perennial warm-season turfgrass because of its extensive rooting system, superior regeneration ability and superior stress tolerance (Wang et al., 2010). Previous researchers indicated that bermudagrass is a dominant species in heavy-metal-contaminated soils (Archer and Caldwell, 2004; Madejón et al., 2006). Besides, the well-developed rhizome of bermudagrass can effectively prevent the loss of Cd through formation of compact ground covers, thus it has a potential to be applied for phytoremediation of Cd contaminated soils (Xie et al., 2014b). Several studies have reported that microorganisms could reduce heavy-metal toxicity to plants and influence their accumulation and transportation (Dell'Amico et al., 2008; Wang et al., 2009). Generally speaking, most fungi appear to be more resistant to toxic heavy metals than bacteria and in remediation of heavy metal contaminated soils, the utilization of fungi, such as *Aspergillus niger* and *Piriformospora indica*, has caused a widespread concern (Kapoor et al., 1999; Kumar, 2013). It has previously been shown that fungal activity was less affected by heavy metals compared with bacterial (Rajapaksha et al., 2004), which was in accordance with the previous studies indicating that fungi appeared to be less sensitive to heavy metals than bacteria (Khan and Scullion, 2002; Müller et al., 2001). Xie et al. (2014b) isolated the Cd-resistant fungi from Cd-contaminated soils and identified it as *A. aculeatus*. The previous investigation demonstrated that the *A. aculeatus* had remarkable resistance to Cd, and could be resistant to 200 mg L^{-1} Cd concentration. In this particular research, the fungus was a pure culture and had no host specificity. What's more, the *A. aculeatus* facilitated plant growth under Cd stress and alleviated Cd toxic effects by improving turf quality, growth rate, chlorophyll content and photosynthesis (Xie et al., 2014a, 2014b; Yan et al., 2016). The previous study indicated that the *A. aculeatus* carries out a heavy-metal tolerance mechanism which highly enhanced Cd immobilization in bermudagrass roots thereby suppressing the transportation of Cd to shoots (Xie et al., 2014b). In addition, the *A. aculeatus* was capable of solubilizing natural forms of phosphorus thereby promoting plant absorption and utilization of phosphorus (Narsian and Patel, 2000). Hence, these features of the fungi make it suitable for phytoremediation in Cd-contaminated soils.

Hence, the objectives of this research were to: (1) characterize the effect of exogenous Cd-resistant fungi on the growth status of Cd-sensitive and Cd-tolerant bermudagrass under Cd stress; (2) examine the effect of exogenous Cd-resistant fungi on metabolites in leaves and roots of two genotypes of bermudagrass under Cd stress.