South African Journal of Botany 106 (2016) 35–40

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Effects of gibberellic acid on the process of organic reserve mobilization in barley grains germinated in the presence of cadmium and molybdenum

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ARTICLE INFO

Article history:
Received 31 October 2015
Received in revised form 5 April 2016
Accepted 13 May 2016
Available online 4 June 2016

Edited by AK Cowan

Keywords:
Barley
Enzymatic activity
Gibberellic acid (GA_3)
Heavy metal
Reserve mobilization

ABSTRACT

Soil contamination by heavy metals such as cadmium (Cd), molybdenum (Mo), lead, zinc and others as a result of industrial and agricultural practices, is a widespread problem in many countries across the world. Despite the fact that Mo is an essential nutrient required by plants in small concentrations, the exposure of crops, including metabolic and enzymatic activities during seed germination, to high concentrations of these metals can have adverse effects on their growth and performance. The current study assesses not only the deleterious effects of Cd and Mo contamination on barley grain germination but also the ability of gibberellic acid (GA_3) to alleviate these negative effects. Stress generated by Cd and Mo contamination engendered the accumulation of total soluble proteins and a reduction of free amino acids in the endosperm followed by a decline of soluble proteins in seedling roots. This shows that protein reserves were not successfully mobilized in the endosperm of Cd or Mo-treated seeds, thus inhibiting protein synthesis in the roots. A reduction of soluble sugar content in the endosperm followed by a decrease in the activities of hydrolytic enzymes (α- and β-amylase, acid and alkaline phosphatase) also unveiled inhibited starch degradation caused by these heavy metals. However, the addition of 0.5 μM GA_3 to the germination medium significantly alleviated the inhibitory effect of Cd and Mo on the activity of the four hydrolytic enzymes and concomitantly increased the sugar and amino acid content of the endosperm. Thus, GA_3 treatment partially restored the mobilization of protein and starch reserves from the endosperm to seedling roots during germination. Alleviation of the phytotoxic effects of heavy metal pollution by GA_3 in barley shows that the major effect of Cd and Mo toxicity is in suppressing the production of GA_3 or inhibiting its activity in the aleurone tissue of the seed. In the future, barley improvement programs can use this information to devise strategies to enhance plant growth and production output in soils infected by heavy metals.

1. Introduction

Industrial activity and anthropogenic lifestyles have resulted in a progressive increase in water and soil pollution by heavy metals, including cadmium (Cd) and molybdenum (Mo), which can enter the food chain as a result of their uptake by plants and pose risks to human health (Marichali et al., 2014). Several studies have shown that various heavy metals are essential for plant growth and development. Yet, excessive concentrations can cause deleterious effects on physiological and biochemical parameters of plants such as photosynthesis and mineral nutrition while also significantly decreasing growth and biomass accumulation (Gangwar et al., 2014). Many plant species have the capacity to absorb and accumulate contaminants such as lead, Cd, chromium, and arsenic although survival depends on the balance between the rate at which metal ions are taken up and the efficiency with which they are detoxified within the plant (Hajar et al., 2014). Usually, germination and early seedling growth stages are key steps in a plant's life and are more sensitive to metal toxicity than fully developed plants, especially when some defense mechanisms have not yet been fully developed (He et al., 2014). Kalai et al. (2013) studied the effect of heavy metals on barley (*Hordeum vulgare* L.) seed germination, and reported that the exposure of seeds to high concentrations of Cd (100 μM) and Cu (500 μM) for 2 days led to a decrease in the growth of radicals and shoots, a decline in the activities of α-amylase, acid phosphatase and alkaline phosphatase in the endosperm, the accumulation of soluble sugar

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http://dx.doi.org/10.1016/j.sajb.2016.05.007
0254-6299/

in the endosperm and a significant accumulation of proline, essentially in the radicles. Similarly, Gubrelay et al. (2013) found that treatment of barley seeds with 10, 20 and 30 mM Cd had a toxic effect on germination percentage, germination rate and seedling growth while Cd also inhibited root and shoot growth with the negative effect increasing significantly as Cd concentration increased. However, no research has ever been conducted on barley germination in which heavy metals and gibberellic acid (GA_3) or other phytohormones were used.

During the germination of a cereal grain, the aleurone layer is a secretory tissue that produces enzymes to hydrolyze the starchy endosperm (Aoki et al., 2014). The synthesis and secretion of hydrolytic enzymes are induced by gibberellins synthesized in embryos and that diffuse to the starchy endosperm where they catalyze the degradation of starch, protein, cell wall components and other storage compounds (Murray et al., 2006). GA_3 has an important role in seed germination: it stimulates the synthesis and translation of mRNA specific for α-amylase, a hydrolytic enzyme responsible for the digestion of reserves within the seed (Muralikrishna and Nirmala, 2005). After it has been synthesized, α-amylase diffuses into the endosperm and produces sugars that are necessary for embryo growth (O'Brien et al., 2010).

Barley belongs to the oldest and economically most important cereals (Bolechova et al., 2015). Hence, in approximately 75% of the world, barley production is used for animal feed, 20% is malted for use in alcoholic and non-alcoholic beverages while 5% is employed as an ingredient in food products (El Halal et al., 2015). In Tunisia, barley is an important cereal crop where it is mainly used for human and animal nutrition and has been the topic of ample research investigations (Bettaieb Ben Kaâb and Attias, 1992; Bettaieb Ben Kaâb et al., 2005; Bchini et al., 2010; Kalai et al., 2013).

Contamination of agricultural soils by heavy metals has become a concern of scientific interest because the uptake of heavy metals by crops affects food quality and security (Qi et al., 2015). Tunisia is one of the largest phosphate producers in the world (production was 8 million tons in 2007) and this industry produces emissions rich in trace elements, including Cd, zinc, chromium and strontium, that can contaminate environmental pools such as air, water, plants and agricultural soils (Galfati et al., 2011). A scientific understanding of Cd and Mo toxicity in barley can be very useful to devise strategies for alleviating its negative effects on crop performance.

The two key objectives of this study were as follows: (1) to investigate the effects of Cd and Mo on the organic reserves of barley grains after 96 h of germination and on the mobilization of these reserves by assaying amylase and phosphatase enzymes given their important role in starch metabolism in developing as well as germinating seeds; and (2) to study the effectiveness of GA_3 in reversing the inhibitory effects of Cd and Mo on organic reserve mobilization during barley seed germination.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals and reagents were purchased from Sigma Aldrich (St. Louis, MI, USA) and were of the highest purity available.

2.2. Plant material

All experiments were conducted on seeds of a widely adapted barley cultivar 'Manel', which is an important part of the cereal production system in Tunisia. 'Manel' was developed by the National Agricultural Research Institute of Tunis (INRAT) in 1996 and is known for its high yield potential and disease resistance, especially in sub-humid areas (Deghais et al., 1999). The experiments were conducted in November 2014 at the laboratory of the Biology Department, Faculty of Sciences of Tunis, Tunisia, as part of a research project on 'Plant Nutrition, Nitrogenous Metabolism and Proteins of Stress during heavy metal stress' (UR/13ES-29).

2.3. Germination conditions and heavy metal treatment

'Manel' seeds (300 in total) were surface disinfected with 2% sodium hypochlorite for 10 min, and then rinsed thoroughly with distilled water. For each treatment, three replicates of 30 seeds each were placed on two layers of Whatman™ filter paper (0.5 mm; General Electric Co. Healthcare Life Sciences, Buckinghamshire, UK) in 15-cm diameter glass Petri dishes to which distilled water (control), 150 μM $CdCl_2$ or 100 μM $(NH_4)_2MoO_4$ were added. One set each of control and heavy metal-treated seeds (i.e., GA_3 + Cd and GA_3 + Mo) were allowed to germinate for 96 h in the dark at 25 °C in the presence of a 0.5 μM solution of GA_3 while another set of seeds received no GA_3 treatment. The 0.5 μM GA_3 concentration was selected based on its ability to maximize the germination rate of barley seeds at all time points up to 96 h without inhibiting germination (Table 1). Furthermore, an analysis of variance (ANOVA) for germination (%) as a function of GA_3 concentration and incubation time (H) (Table 2a and b) was achieved by using the Proc GLM (general model) implemented in the statistical software SAS version 9.1. That analysis showed that germination (%) varied significantly as a function of incubation period and GA_3 concentration. Thus, based on this ANOVA analysis, GA_3 = 0.5 μM and 96 h incubation time were selected for the remainder of the experiments.

Afterward, the seed integument and plumule of the embryonic axis of each germinated seed were excised, and the seeds were dissected using a razor blade into radicles and endosperm samples. The bulked radicle and endosperm samples of each replicate were then frozen by liquid nitrogen separately, before grinding by a mortar into a powder, and then stored at −80 °C in liquid nitrogen for up to 3 days to analyze enzymatic activities. To determine the total soluble sugar content, the radicles and endosperm samples were vacuum dried (Memmert, Schwabach, Germany) at 70 °C for at least 24 h before grinding. All experimental treatments were replicated three times.

2.4. Biochemical analyses

2.4.1. Soluble sugar assay

Total soluble sugars were assayed in the endosperm following the method of Yemm and Willis (1954). Soluble sugars were extracted after macerating 25 mg of dry endosperm powder in 5 ml of 80% ethanol in a test tube and then stirring in a water bath at 70 °C for 30 min. The homogenate was centrifuged at 6000×*g* for 15 min at 4 °C and 25 μl of supernatant for each sample was added to 5 ml of anthrone. After shaking, the test tubes, they were placed into a preheated water bath at 100 °C for 10 min then cooled in the dark for 30 min to prevent the oxidation of sugars. Total soluble sugar concentration was estimated colorimetrically at 640 nm using a spectrophotometer (Lambda 25, Perkin Elmer, Norwalk, CT, USA) and expressed in mg/g dry mass (DM) based on a glucose standard curve generated using the same protocol.

2.4.2. Total soluble proteins assay

Total soluble proteins were assayed following the Bradford and Marion (1976) method using bovine serum albumin as the protein

Table 1
Germination (%) of barley seeds as a function of time (h) and as a function of different GA_3 concentrations (μM).

GA_3 treatment (μM)	Germination (%) at 24 h	Germination (%) aat 48 h	Germination (%) at 72 h	Germination (%) at 96 h
0 (control)	80 ± 4.5	88 ± 2.2	88 ± 2.2	92 ± 2.2
0.1	84 ± 2.2	88 ± 6.7	90 ± 2.2	94 ± 2.2
0.2	84 ± 7.8	90 ± 2.2	94 ± 2.2	94 ± 3.9
0.3	86 ± 2.2	92 ± 2.2	94 ± 2.2	96 ± 3.9
0.4	86 ± 3.9	94 ± 2.2	96 ± 2.2	98 ± 2.2
0.5	92 ± 2.2	96 ± 2.2	96 ± 3.9	98 ± 2.2

Percentage values based on three replications.