VII CONGRESO IBÉRICO DE AGROINGENIERÍA Y CIENCIAS HORTÍCOLAS
Madrid, 26-29 Agosto 2013

Effect of fresh spent coffee grounds on the oxidative stress and antioxidant response in lettuce plants

Teresa Gomes[1], José Alberto Pereira[1], Elsa Ramalhosa[1], Susana Casal[2] & Paula Baptista[1]

[1]Mountain Research Centre (CIMO), School of Agriculture – Polytechnic Institute of Bragança, *Campus* Sta Apolónia, Apartado 1172, 5301-855 Bragança, Portugal. Email: pbaptista@ipb.pt
[2]REQUIMTE/Laboratory of Bromatology and Hydrology, Faculty of Pharmacy, Porto University, Rua Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal

Abstract

Spent coffee grounds (SCG) are widely used in domestic cultures, apparently as fertilizer and due to its repellent effect. However, scientific evidences about their effect on plants remain unknown. The high residual amounts of caffeine (about 0.2%) in SCG, together with tannins and chlorogenic acids, might impose some toxicity to the plants.
The aim of this study was to evaluate the oxidative stress response of lettuce plants to fresh SCG. The roots of one month-old lettuces plantlets cv. "Wonder of four seasons" were immersed in aqueous solutions with different fresh SCG concentrations [0%, 5%, 10% and 15% (w/v)]. During the first 24 hours of elicitation, the roots and leaves were periodically harvested and the levels of hydrogen peroxide (H_2O_2), as well as the activities of superoxide dismutase (SOD) and catalase (CAT), were determined.
The results indicated that fresh SCG induce oxidative stress in lettuce plants. An increase of reactive oxygen species (ROS), namely on H_2O_2, was more evident within the first 12 h of elicitation with increasing fresh SCG concentrations. This pattern was coincident with an increase of SOD activity, but not of CAT. Oxidative stress induction by fresh SCG was observed in both roots and leaves, being more noticed in the former. Altogether, the results show that the fresh SCG modify the equilibrium between production and scavenging of ROS, causing oxidative stress in lettuce plants, especially at the root level.

Keywords: *Lactuca sativa*, reactive oxygen species, catalase, superoxide dismutase.

Efeito da borra de café fresca na indução de stresse oxidativo e na resposta antioxidante em plantas de alface

Resumo

A borra de café tem vindo a ser bastante utilizada em culturas domésticas, aparentemente como fertilizante e repelente. No entanto, permanecem desconhecidas as evidências científicas acerca do seu efeito nas plantas. O alto teor em cafeína (cerca de 0,2%), taninos e ácidos clorogénicos na borra de café, pode causar alguma toxicidade nas plantas.
No presente estudo pretendeu-se avaliar o efeito da borra de café fresca na indução de resposta de stresse oxidativo em plântulas de alface. Para tal, as raízes de plântulas de alface cv. "Maravilha das quatro estações" foram mergulhadas numa solução aquosa contendo diferentes concentrações de borra de café fresca [0%, 5%, 10% e 15% (m/v)]. Durante as primeiras 24 horas de eliciação, as raízes e as folhas foram colhidas periodicamente e os níveis de peróxido de hidrogénio (H_2O_2), bem como as atividades da superóxido dismutase (SOD) e catalase (CAT) foram determinadas.
Os resultados obtidos indicaram que a borra de café fresca induziu uma resposta de stresse oxidativo nas plântulas de alface. Foi observado um aumento de espécies reativas de oxigénio (ROS), nomeadamente de H_2O_2, nas primeiras 12 horas de eliciação com o aumento da concentração de borra de café. Este padrão foi coincidente com um aumento da atividade da SOD, mas não da CAT. O stresse induzido pela borra fresca foi observado tanto nas raízes como nas folhas das plântulas de alface, sendo, contudo, mais notório no primeiro órgão. No total, os resultados mostraram que a borra de café fresca alterou o equilíbrio entre a produção e a eliminação de ROS, causando, assim, um stresse oxidativo nas plântulas de alface, especialmente ao nível da raiz.

Palavras-chave: *Lactuca sativa*, espécies reativas de oxigénio, catalase, superóxido dismutase.

Introduction

Spent coffee ground (SCG) is a solid residue obtained from the treatment of coffee powder with hot water to prepare coffee beverages. Several references indicate beneficial effects of SCG uses in the domestic agriculture; however, scientific evidences about its effectiveness or safety remain unknown. In fact, bioactive compounds presents in the SCG, such as caffeine (about 0.2%) (Cruz et al., 2012) and chlorogenic acids might impose some toxicity to the soil microorganisms and plants.

Plants frequently encounter biotic and abiotic stresses that adversely affect growth, development, or productivity. These stresses trigger a wide range of plant responses, being one of the most rapid reactions the enhanced production of reactive oxygen species (ROS), such as superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) (Atkinson and Urwin, 2012). Such ROS are extremely reactive and unstable chemical species, and can react with vital biomolecules, such as proteins, lipids, carbohydrates and nucleic acids (Foyer and Noctor, 2005).

Although plants have enzymatic and non-enzymatic mechanisms to detoxify ROS, under stress conditions, ROS generation often exceeds the overall cellular antioxidant potential leading to stress-dependent adverse effects on plant growth (Garg and Manchanda, 2009). Antioxidants (ROS scavengers) include enzymes, such as catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase and glutathione reductase, as well as non-enzyme molecules, such as ascorbate, glutathione, carotenoids, and anthocyanins (Gill and Tuteja, 2010).

The aim of this work was to evaluate the possibility of fresh SCG to induce oxidative stress in lettuce plants. This was studied in lettuce plantlets at different time points up to 24 hours after exposition to different concentrations of fresh SCG.

Material and methods

Fresh SCG were collected from various coffee establishments in the Bragança city (Portugal), serving espresso coffee on a regular basis. Roots of one month-old of *Lactuca sativa* L. cv. "Wonder of four seasons" were immersed in aqueous solutions with different fresh SCG concentrations [0%, 5%, 10% and 15% (w/v)]. After 0.5, 1, 2, 3, 5, 12 and 24 hours post-elicitation, both roots and leaves were recovered, grounded to a fine powder in liquid nitrogen and stored at -80 ºC, until analysis.

Superoxide dismutase (SOD, EC 1.11.1.5) and catalase (CAT, EC 1.11.1.6) were assayed according to Baptista et al. (2009). Hydrogen peroxide (H_2O_2) levels were measured according to Loreto and Velikova (2001). All parameters were determined on leaves and roots for each elicitation time and its respective controls.

Data are expressed as percentage of variation between elicited plants and the respective control of three independent experiments displaying the respective SE bars. The significance of differences among means was tested by analysis of variance (ANOVA), using SPSS v.20 software (SPSS Inc.), in which the averages were compared using Tukey test ($p \leq 0.05$).

Results and discussion

Hydrogen peroxide formation

To ascertain the involvement of reactive oxygen species during the first hours of *L. sativa* exposure to fresh SCG, the quantification of H_2O_2 was performed on both leaves and roots of SCG-elicited lettuces plants. As depicted in Figure 1, the fresh SCG increased significantly the H_2O_2 levels of both leaves and roots of lettuce plants when compared to the control, in all concentrations tested. This increase was particularly noticed within the roots. In both roots and leaves of SCG-elicited plants were also observed variation on H_2O_2 levels along the experimental period. The levels of H_2O_2 on leaves were increased gradually over the experimental period, reaching significant maximum values after 3 and 5

hours post-elicitation at concentrations of 15% and 10%, respectively. Afterwards, H_2O_2 levels decreased until 24h after fresh SCG addition but still remained significantly higher as compared to control. The leaves of lettuce plants elicited with SCG at low concentration (5%) showed less variation on H_2O_2 contents, being possible to notice three slightly peaks production at 1, 3 and 12h post-inoculation. Roots of lettuce plants exposed to fresh SCG at concentrations of 5% and 15% displayed two significant production peaks of H_2O_2. The first peak occurred 1h after SCG addition and, after a decrease in H_2O_2 levels until 3h of elicitation, it was observed the second peak at 5h after SCG addition. In roots of lettuce plants exposed to fresh SCG at concentrations of 10% an additional significant peak of H_2O_2 production at 3h post-elicitation was observed. In general, high SCG concentration (10 and 15%) lead to a more strong variation in the production of H_2O_2, being however noticed some exceptions. As far as we know this is the first time where there is direct evidence for the ROS production by plants in response to fresh SCG. The promotion in ROS production by SCG was probably due to toxic compounds, such as caffeine, tannins and chlorogenic acids (Batish et al., 2008), often found in high amounts in fresh SCG (Cruz et al., 2012).

Activity of ROS-scavenging enzymes

In order to maintain ROS at physiological levels, plant cells possess both enzymatic and non-enzymatic defense systems (Gill and Tuteja, 2010). The most important enzymatic antioxidants include SOD, that catalyzes the disproportion of superoxide ($O_2^{\bullet-}$) to H_2O_2, and CAT that can convert H_2O_2 to H_2O (Gill and Tuteja, 2010). The results showed that SCG didn't cause statistically significant changes on CAT activity in comparison with control (data not shown). Therefore, the increase H_2O_2 levels observed on roots and leaves of SCG-elicited lettuce plants was probably due to the inhibition and/or not sufficiently increased of CAT, as well as of other enzymatic and non-enzymatic components responsible for H_2O_2 detoxification.

By contrast, SOD activity on both roots and leaves were significantly increased by SCG when compared to control (Fig. 1). This increase was particularly notice on lettuce roots and in treatments with high SCG concentration (10 and 15%). In addition, changes on SOD activity was observed along the experimental period. In the leaves of lettuces elicited with SCG at concentrations of 10 and 15% were noticed two significant peaks of SOD activity, at 2h and 5h after elicitation. At the lowest SCG concentration (5%) was only observed a significant increase on leaves SOD activity as compared to control after 2h of elicitation. Two significant enhanced synthesis of SOD has also been observed in the roots: at 2h and 5h after elicitation in treatments with SCG concentrations of 5 and 10%, and at 2h and 12h after elicitation in the treatment with SCG concentrations of 15%. The pattern of SOD activity and of H_2O_2 production on both roots and leaves was, in general, not exactly coincident. This data suggest that, although the increase in SOD activity may account the enhanced formation of H_2O_2, an alternative pathway for the production of this ROS might be additionally present. As mentioned above the phytotoxic compounds present in SCG could itself generate ROS, either by direct involvement in radical production or by inhibition of biosynthetic pathways.

Comparison between roots and leaves

The results from H_2O_2 and activity of ROS-scavenging enzyme assays strongly suggest that roots and leaves of lettuce plants differed greatly in their responses to oxidative stress when exposed to fresh SCG. The higher SOD activity associated to the unchanged CAT activity observed on the roots comparatively to the leaves, was resulted in a greatest enhanced formation of H_2O_2 and of its accumulation in the first plant organ (Fig. 1). This result is indicative of higher oxidative stress in roots than inleaves. Thus, it would be expected that root cells suffered more severe injury due to SCG exposition than leaves. The roots are in contact with SCG; hence, it is expected that the deleterious effects caused by the phytotoxic compounds present in this organic residue would be firstly and mostly perceive by the roots. The distinct oxidative responses between roots and leaves of lettuces might additionally be explained by the marked differences found among the types of cellular and

organelle metabolism existing in those organs (Cavalcanti et al., 2007). Previous studies have also reported differences on oxidative responses of roots and leaves of plants during salt (Cavalcanti et al., 2007) and heavy metal (Zhang et al., 2007) stresses.

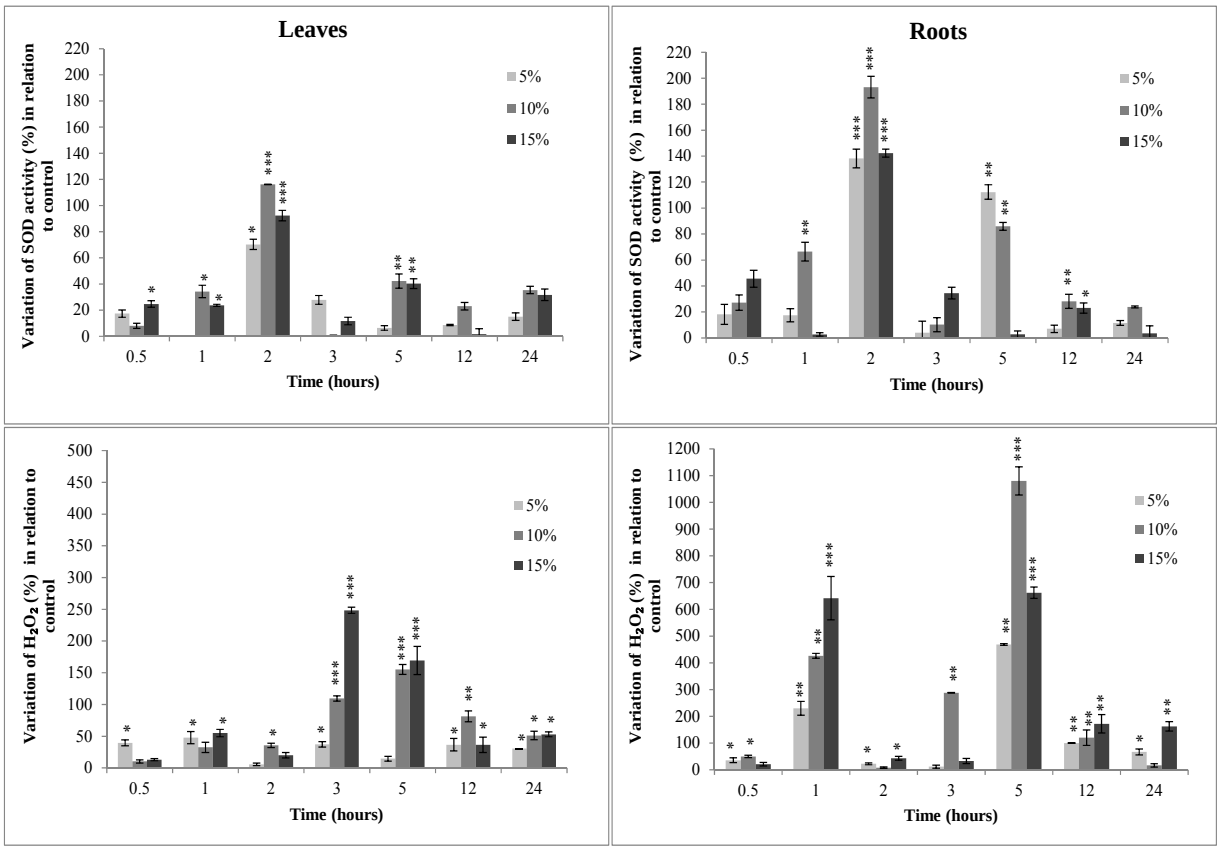

Figure 1. Relative changes in relation to the control (%) of SOD activity and H_2O_2 levels in the roots and leaves of lettuce plants exposed over 24 hours to fresh spent coffee grounds at different concentrations (5-15%, w/v). Each value is expressed as mean ± SE (n=3). Asterisks indicate values that differ significantly from controls on each hour at * p<0.05; ** p<0.01; *** p<0.001.

Conclusions

In the present study, it was observed that fresh SCG impose precocious oxidative stress through H_2O_2 accumulation in lettuce plants, which was probably related to its composition on phytotoxic compounds. The increase and accumulation of H_2O_2 in lettuce plants is due, among other things, to the increase of SOD activity (which generates H_2O_2) associated to the unchanged CAT activity (inducing H_2O_2 accumulation). The activity of SOD, as well as the levels of H_2O_2, was much higher in roots than in leaves, which show that the deleterious effect caused by SCG was greater in the first plant organ. An understanding of the mechanisms of plant defense response to SCG exposition is crucial to ensure that the incorporation of this organic residue directly into the soil does not present any risks to the crops.

Acknowledgments

Authors are grateful to Fundação para a Ciência e Tecnologia (FCT) for financial support (Project PTDC/AGR-AAM/102447/2008).

References

Atkinson, N. J., and Urwin, P. E. (2012). The interaction of plant biotic and abiotic stresses: from genes to the field. *Journal of Experimental Botany* **63**, 3523–3543

Baptista, P., Ferreira, S., Soares, E., Coelho, V., and Bastos, M. D. (2009). Tolerance and Stress Response of *Macrolepiota procera* to Nickel. *Journal of Agricultural and Food Chemistry* **57,** 7145-7152

Batish, D. R., Singh, H. P., Kaur, M., Kohli, R. K., and Yadav, S. S. (2008). Caffeine affects adventitious rooting and causes biochemical changes in the hypocotyl cuttings of mung bean (*Phaseolus aureus* Roxb.). *Acta Physiologiae Plantarum* **30,** 401-405

Cavalcanti, F. R., Lima, J. P., Ferreira-Silva, S. L., Viégas, R. A., and Silveira, J. A. (2007). Roots and leaves display contrasting oxidative response during salt stress and recovery in cowpea. *Journal of Plant Physiology* **164**, 591-600

Cruz, R., Cardoso, M. M., Fernandes, L., Oliveira, M., Mendes, E., Baptista, P., Morais, S., Casal, S. (2012). Espresso coffee residues: a valuable source of unextracted compounds. *Journal of Agricultural and Food Chemistry* **60**, 7777−7784

Foyer, C. H., and Noctor, G. (2005). Redox homeostis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. *Plant Cell* **17**, 1866–1875

Garg, N., and Manchanda, G. (2009). ROS generation in plants: Boon or bane?. *Plant Biosystems* **143**, 81 – 96

Gill, S. S., and Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry* **48**, 909-930

Loreto, F., and Velikova, V. (2001). Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. *Plant Physiology* **127**, 1781–1787.

Zhang, F. Q., Wang, Y. S., Lou, Z. P., and Dong, J. D. (2007). Effect of heavy metal stress on antioxidative enzymes and lipid peroxidation in leaves and roots of two mangrove plant seedlings (*Kandelia candel* and *Bruguiera gymnorrhiza*). *Chemosphere* **67**, 44–50.