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Pre-germination treatment with hydrogen peroxide as a controlled elicitation strategy to improve chemical properties of hydroponic barley fodder

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Abstract. Barley (*Hordeum vulgare* L.) is one of the most used species for hydroponic green fodder. The chemical properties of fodders may be enhanced through use of various strategies during crop production, including stress applications. In this context, hydrogen peroxide (H_2O_2) is used as a stress factor in controlled elicitation, a technique used to increase secondary metabolites in food. The aim of this research was to evaluate the possibility of using controlled elicitation with H_2O_2 for enhancing the chemical properties of hydroponic barley fodder. H_2O_2 was applied to the seeds as a pre-germination treatment at three concentrations: 50, 100 and 150 mM. Morphological changes, enzymatic defence-related activities (superoxide dismutase, catalase, chalcone isomerase, phenylalanine ammonia-lyase), total phenolics content, and antioxidant capacity were evaluated. Significant increases in total phenolics content, phenylalanine ammonia-lyase activity and ABTS antioxidant capacity were obtained when seeds were treated with 50 mM H_2O_2. Plant growth was promoted with 100 mM and 150 mM H_2O_2 treatments, and germination of seeds with 100 mM H_2O_2. It was established that controlled elicitation using H_2O_2 increased some morphological and biochemical variables of hydroponic barley fodder related to food properties.

Keywords: controlled elicitation, enzymatic activities, phytochemicals, antioxidant capacity, chemical properties, fodder, secondary metabolites, nutritive value.

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Introduction

Barley (*Hordeum vulgare* L.) is an important food and fodder resource globally, along with the other cereals maize, rice and wheat (Ghaffari *et al.* 2016). Barley is abundant in metabolites such as carbohydrates, protein and lipids, as well as minerals, dietary fibre, vitamins B and E and high levels of antioxidants (Hussain *et al.* 2019). This grain has important levels of dietary fibre and phytochemical compounds, among them flavonoids, folates, phenolic acids, lignans, tocols, phytosterols and folates (Idehen *et al.* 2017). Among these, the phenolic compounds, β-glucans, arabinoxylans are recognised for their positive role in human health (Sher *et al.* 2017).

Barley hydroponic green fodder is considered a sustainable feed option that could reduce feeding cost and increase product quality. With hydroponic technology, 8 kg of green fodder can be obtained from 1 kg of seed in a week (Abouelezz *et al.* 2019). The process used in hydroponic green fodder production increases protein content and availability owing to the sprouting grains converting complex proteins into amino acids and other compounds; the vitamin content is also enhanced as well as the availability of some minerals such as iron and phosphorus (Abouelezz *et al.* 2019).

Application of exogenous stress could influence plant growth, yield and stress tolerance; moreover, it has been proven to raise the production of specialised metabolites through secondary metabolism (Kaburagi *et al.* 2015; Ding *et al.* 2017). However, these effects depend on time of exposure and dose of stress application (Gorelick and Bernstein 2014; Vázquez-Hernández *et al.* 2019*b*). This phenomenon, called 'hormesis', refers to the application of a stress factor to an organism, generating beneficial effects (eustress) at low dose and toxic ones (distress) at high dose (Kacienė *et al.* 2015; Vargas-Hernandez *et al.* 2017). For this reason, it is desirable to test a broad spectrum of dosages of a stress factor, in order to place those dosages on the hormetic curve and thus determine whether a specified dosage is beneficial for our purposes (Vázquez-Hernández *et al.* 2019*b*).

This knowledge can be applied as a controlled elicitation technique to increase the production of specialised metabolites in crops (Garcia-Mier *et al.* 2014; Biczak *et al.* 2016). Controlled elicitation uses stress factors at specific dosages and application times to increase bioactive contents without negative effects on plant behaviour. For example, exogenous applications of salicylic acid to kidney beans improved seedling growth and total phenolics content (Limón *et al.* 2014). There are also examples of soybean seeds given pre-germination treatment with salicylic acid and broccoli seeds given chitosan, which improved seedling growth and the nutritional properties (Anaya *et al.* 2018). In the case of barley, exogenous applications of hydrogen peroxide (H_2O_2) at 0.25 M salt concentration had positive effects on seed germination index and vigour index but decreased biomass production (Kilic and Kahraman 2016). In addition, applications of ozone (O_3) gas at 180 and 360 μg m^{-3} enhanced antioxidative enzymes and diminished dry biomass of shoots (Kacienė *et al.* 2015).

The controlled elicitation technique has been useful for increasing the content of secondary metabolites in several species including barley (Kacienė *et al.* 2015; Holub *et al.* 2019; Vazquez-Hernandez *et al.* 2019*a*; Parola-Contreras *et al.* 2020). In the case of the sprouts and fodders, this technique can be useful for enhancing the chemical properties, and so may be an appropriate option for human food and animal feed as was found for common beans (Mendoza-Sánchez *et al.* 2016), soybean (Anaya *et al.* 2018) and barley (Kacienė *et al.* 2015; Kilic and Kahraman 2016).

With the aim of finding a favourable strategy to provide animals with foods of high beneficial bioactive content and antioxidant properties, this research evaluated the effect of controlled elicitation, using H_2O_2, on the chemical properties of hydroponic barley fodder. To our knowledge, this is the first time that H_2O_2 has been applied as a pre-germination elicitor treatment for barley seeds.

Materials and methods

Plant material, growth conditions and stress treatments

Seeds of barley var. Cantabria were given pre-germination treatment for 24 h in pots that contained H_2O_2 (Golden Bell, India) at concentrations of 50 mM, 100 mM and 150 mM, with distilled water as the control. Seeds were drained and put in trays 54 cm by 35 cm by 7 cm, and these trays were placed in a fodder growth chamber for 11 days. At 24, 48 and 72 h after sowing, a sample was taken of 100 seeds per try and the germination percentage was determined. Conditions during the experiment were: temperature 25°C, photoperiod 14 h light/10 h dark, and relative humidity 80%. Seedlings were watered (no nutrients) six times per day for 2 min each time. On Day 11 after sowing, plant morphological variables were determined: stem height (SH, mm), measured with a graduated ruler; root length (RL, mm), measured with a graduated ruler; plant fresh weight (PW, g), determined with a balance (Etekcity, Anaheim, CA, USA). Fodder samples (leaves and roots) were collected on Day 11 and immediately frozen in liquid nitrogen and stored at −80°C for analysis.

Determination of antioxidant activity

Antioxidant activity in each treatment was evaluated through the 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$) and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) tests.

DPPH$^{\bullet}$ radical scavenging activity (DRSA)

The DRSA was determined according to the method of Chen *et al.* (2012), with some modifications. An aliquot (0.5 mL) of sample solution (600 μg mL^{-1}) was added to 0.5 mL DPPH$^{\bullet}$ radical (0.1 mM) in methanol solution. The mixture was shaken vigorously and incubated for 30 min in the dark at room temperature. The absorbance was measured at 515 nm. Methanol, instead of DPPH$^{\bullet}$, was used for the blank, and deionised water, instead of sample, was used for the control. DRSA was calculated by the following Eqn 1:

$$\text{DRSA}\ (\%) = \left[1 - \left(A_{\text{sample}} - \frac{A_{\text{blank}}}{A_{\text{control}}}\right)\right] \times 100 \qquad (1)$$

$ABTS^{\bullet+}$ radical scavenging activity (ARSA)

The ARSA was determined according to the method of Re *et al.* (1999), with some modifications. The $ABTS^{\bullet+}$ radical cation was generated by mixing ABTS stock solution (7 mM) with potassium persulfate (2.45 mM), and the resulting mixture was rested in the dark at room temperature for 12 h before use. The $ABTS^{\bullet+}$ radical solution was diluted in phosphate buffered saline (0.15 M, pH 7.4) at ~1:35 to obtain an absorbance of 0.70 ± 0.02 at 734 nm. This diluted solution (~3 mL) was mixed with an aliquot (150 μL) of sample solution (600 μg mL^{-1}). The absorbance was read from 1 and up to 6 min after the initial mixing, under conditions of darkness. Phosphate buffered saline, instead of $ABTS^{\bullet+}$, was used for the blank, and deionised water, instead of the sample, was used for the control. ARSA was calculated by the following Eqn 2:

$$\text{ARSA}\ (\%) = \left[1 - \left(A_{\text{sample}} - \frac{A_{\text{blank}}}{A_{\text{control}}}\right)\right] \times 100 \qquad (2)$$

Enzyme activity assays

Sample preparation of enzymatic assays

Barley fodder dried samples (0.5 g) were homogenised with cold extraction buffer (1 mL) and the slurry centrifuged at 10188*g* for 20 min at 4°C. The supernatants were used to determine enzyme activities. Protein concentration in enzymatic extracts was determined according to the method reported by Bradford (1976), using bovine serum albumin as a standard for protein concentration (Sigma-Aldrich, St. Louis, MO, USA).

Superoxide dismutase (SOD) activity assay

The activity of SOD (EC 1.15.1.1) was assessed by the inhibition of photochemical reduction of nitro blue tetrazolium (NBT) according to the method described by Hayat *et al.* (2018). The reaction was performed by adding 1.5 mL 50 mM potassium phosphate buffer (pH 7.8), 0.3 mL 0.1 mM EDTA, 0.3 mL 0.13 M methionine, 0.3 mL 0.02 mM riboflavin, 0.05 mL enzymatic extract and 0.25 mL distilled water. The mixture was exposed to fluorescent light (86.86 μmol

m^{-2} s^{-1}) for 20 min. Subsequently, the solution absorbance was measured at 560 nm; 1 U SOD inhibits reduction of NBT by 50% at pH 7.8 and 25°C. Data are expressed as U mg^{-1} protein.

Catalase (CAT) activity assay

The activity of CAT (EC 1.11.1.6) was spectrophotometrically assessed by measuring the rate of H_2O_2 decrease at 240 nm according to the method described by Afiyanti and Chen (2014). The reaction was performed by adding 0.95 mL 50 mM potassium phosphate buffer (pH 8.0), 0.05 mL enzymatic extract and 0.1 mL 100 mM H_2O_2. The change in absorbance at 240 nm was measured for 1 min and used to determine the rate of decomposition of H_2O_2 by CAT; 1 U CAT decomposes 1 µmol H_2O_2 min^{-1} at pH 8.0 and 25°C. Data are expressed as U mg^{-1} protein (Afiyanti and Chen 2014).

Phenylalanine ammonia-lyase (PAL) activity

The activity of PAL (EC 4.3.1.5) was spectrophotometrically assessed according to the method described by Toscano *et al*. (2018). L-Phenylalanine was used as the substrate, and the released cinnamic acid was quantified by absorbance at 290 nm. The reaction was performed by adding 2.3 mL 0.1 M borate buffer (pH 8.8 containing 10 mM L-phenylalanine) and 0.2 mL enzymatic extract. The mixture was incubated at 40°C for 1 h and the reaction was stopped by the addition of 0.5 mL 5 N HCl. Subsequently, the solution absorbance was measured at 290 nm; 1 U PAL releases 1 µmol cinnamic acid min^{-1} at pH 8.8 and 40°C. Data are expressed as U mg^{-1} protein.

Chalcone isomerase (CHI)

The activity of CHI was measured by using the methods proposed by Dixon *et al*. (1982) and Mol *et al*. (1985). The reaction was performed by adding 2.5 µL substrate solution (1.0 mg naringenin chalcone mL^{-1} ethanol), 2.5 mL sulfate potassium buffer and 2.5 µL enzymatic extract (300 g sample and 2.0 mL extraction buffer). The extraction buffer consisted of 0.1 M sodium phosphate, pH 8, and 1.4 mM 2-mercaptonol. The absorbance was determined on the spectrophotometer at 400 nm during six time intervals (15 s, 30 s, 60 s, 120 s, 240 s and 280 s).

Total phenolic compounds

The hydroponic green fodder from each treatment was sampled and ground in liquid nitrogen. Ground samples of fodder were macerated with methanol in a 1:10 ratio (w/v). The suspensions were collocated in a sonicator (Bransonic M2800-CPX-HE; Emerson, Ferguson, MO, USA) for 30 min and centrifuged at 5590*g* for 15 min at 4°C. The resultant supernatants were frozen at 4°C in vials in dark conditions until use.

Total phenols were determined spectrophotometrically by the Folin–Ciocalteu method as reported by Vergara-Castañeda *et al.* (2010), using gallic acid as a standard. Briefly, 140 µL extract was mixed with 460 µL distilled water and 250 µL Folin–Ciocalteu (1 N) reagent. After 5 min, 1250 µL 20% (w/v) sodium carbonate was added. The mixture was shaken in the vortex and incubated for 2 h in darkness. Absorbance was measured at 760 nm. Results are expressed as mg equivalent gallic acid (EGA) g^{-1} fresh weight.

Statistical analyses

A randomised completely block experimental design was used to evaluate the effect of the pre-germination treatment of seed on morphological changes, antioxidant activity, phenolic compounds and enzymatic activity. The arrangement of the experiment was four treatments with three blocks and three experimental units per treatment. Data was subjected to analysis of variance (ANOVA) and Tukey test ($\alpha = 0.05$), using the Statgraphics Centurion XVI statistical software (StatPoint Technologies, Bedford, MA, USA).

Results

Germination percentage

Germination percentages on trays at 24, 48 and 72 h after sowing are presented in Fig. 1. After 24 h, the 100 mM H_2O_2 treatment showed significantly higher ($P < 0.05$) germination percentage than the other treatments. After 48 h, the 100 mM H_2O_2 treatment gave the highest germination percentage, although not significantly different ($P > 0.05$) from the 50 mM H_2O_2 treatment. Finally, at 72 h, the 100 mM H_2O_2 treatment again gave the highest germination percentage, although not significantly different from 150 mM H_2O_2.

Morphological responses

Leaf height was significantly greater ($P < 0.05$) in the 150 mM H_2O_2 treatment than the control and 50 mM H_2O_2 treatments; the 100 mM H_2O_2 treatment was intermediate and not significantly different ($P > 0.05$) from any other treatment (Fig. 2*a*). Plant weight was significantly greater ($P < 0.05$) in the 150 mM H_2O_2 treatment than in the remaining treatments (Fig. 2*b*). Pre-germination treatment with H_2O_2 had no significant effect ($P > 0.05$) on root length (data not presented).

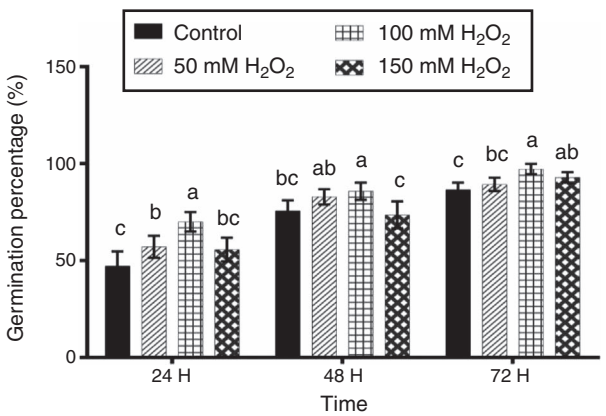

Fig. 1. Germination percentage of barley seeds at 24, 48 and 72 h after three pre-germination H_2O_2 treatments. For each time, means with the same letter are not significantly different according to ANOVA and Tukey test ($\alpha = 0.05$). Error bars are: 24 h ± 3.6165, 48 h ± 2.8389, 72 h ± 1.8581.

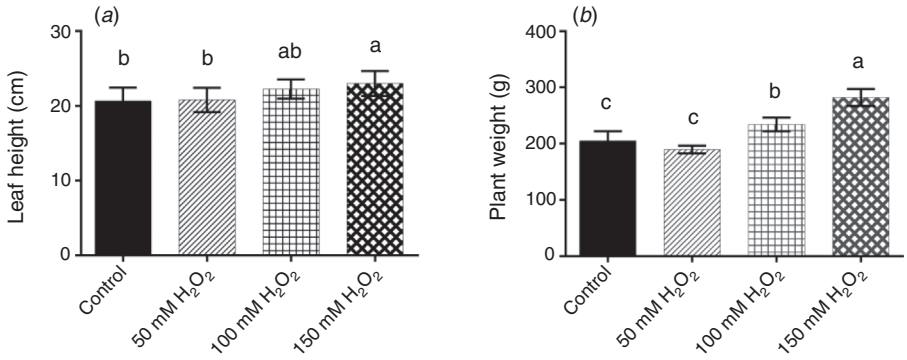

Fig. 2. Effect of three pre-germination dosages of H_2O_2 on plant morphology of hydroponic barley green fodder: (*a*) leaf height, (*b*) plant weight. For each parameter, means with the same letter are not significantly different according to ANOVA and Tukey test ($\alpha = 0.05$). Error bars are: *a* ± 0.4588, *b* ± 15.2577.

Antioxidant activity

Natural antioxidants act as free radical scavengers and reduction agents. These compounds express a wide variety of biological effects in barley (Madhujith *et al.* 2006), and the DPPH and ABTS antioxidant activity can be associated with phenols content. With regard to ARSA (Fig. 3), the 50 mM H_2O_2 treatment had the highest antioxidant capacity, although not significantly different ($P > 0.05$) from the 100 mM treatment. The 150 mM H_2O_2 treatment showed significantly lower ($P < 0.05$) antioxidant capacity than the other two H_2O_2 treatments. The control was intermediate between 100 mM and 150 mM treatments. Pre-germination treatment with H_2O_2 had no significant effect ($P > 0.05$) on DRSA.

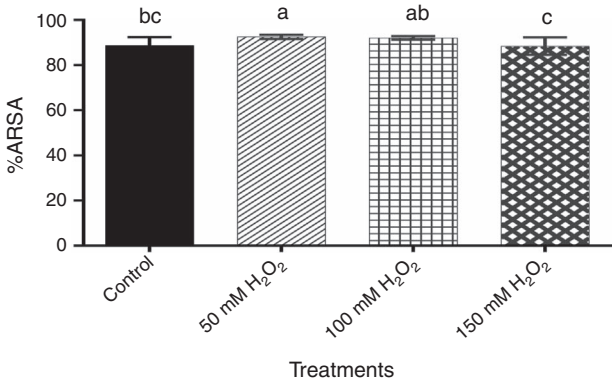

Fig. 3. Anti-radical activity of methanolic extracts from hydroponic barley green fodder after three pre-germination treatments of H_2O_2. ARSA, ABTS radical scavenging activity. Means with the same letter are not significantly different according to ANOVA and Tukey test ($\alpha = 0.05$). Error bars are ± 0.9202.

Enzymatic activity assays

Enzymatic activity is associated with the activation of defence response to diverse stresses in plants. Some enzymes such as POD (peroxidase), SOD, CAT, PAL and CHI could increase their activity as a response to application of an elicitor (Mejía-Teniente *et al.* 2013). Pre-germination treatment of barley seed with H_2O_2 induced variation in SOD, CAT and PAL activities (Fig. 4), whereas CHI activity was not affected. Stress application triggers the activation of NADPH oxidase and O_2^-, which is quickly converted by SOD into H_2O_2, followed by the activation of another reactive oxygen species (ROS)-scavenging enzyme such CAT and POD (Tamás *et al.* 2010). SOD activity was significantly lower ($P < 0.05$) in the 50 mM H_2O_2 treatment than the 100 mM H_2O_2 and control treatments; the 150 mM H_2O_2 treatment was intermediate. CAT activity was significantly higher ($P < 0.05$) in the 100 mM H_2O_2 treatment than all other treatments, which did not differ ($P > 0.05$) from each other. Finally, PAL activity was highest ($P < 0.05$) in the 50 mM H_2O_2 treatment, followed by 100 mM H_2O_2 treatment, then the 150 mM H_2O_2 and control treatments.

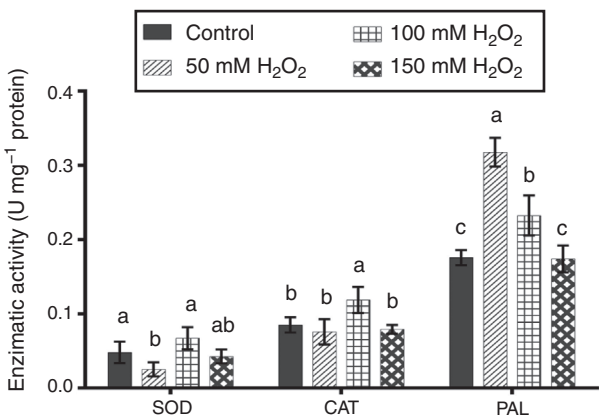

Fig. 4. Enzymatic activity measured in hydroponic barley green fodder after three pre-germination treatments of H_2O_2. SOD, Superoxide dismutase; CAT, catalase; PAL, phenylalanine ammonia-lyase. For each enzyme, means with the same letter are not significantly different according to ANOVA and Tukey test ($\alpha = 0.05$). Error bars are: SOD ± 0.011, CAT ± 0.010, PAL ± 0.012.

Total phenolic compounds

For total phenols, the data showed significant ($P < 0.05$) differences among pre-germination treatments (Fig. 5),

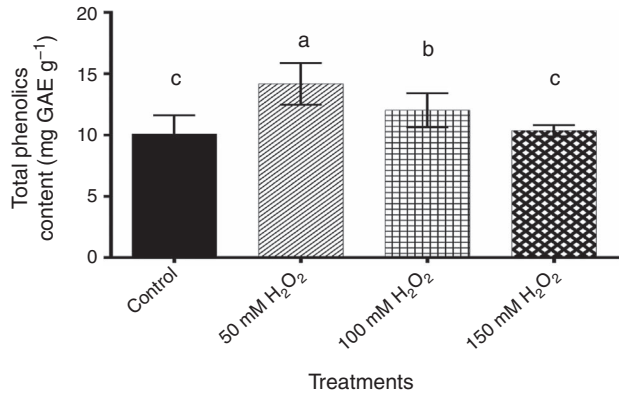

Fig. 5. Total phenolics content in hydroponic barley green fodder, after three pre-germination treatments of H_2O_2. Means with the same letter are not significantly different according to ANOVA and Tukey test ($\alpha = 0.05$). Error bars are $\pm$ 0.2598.

being highest in the 50 mM H_2O_2 treatment, followed by 100 mM H_2O_2 treatment, then the 150 mM H_2O_2 treatment, which was statistically similar to the control.

Discussion

Hydrogen peroxide can enhance environmental stress tolerance; it reduces the negative effects of stress over germination and improves growth parameters, activating the plant immune system in barley (Hussain *et al.* 2019). Therefore, barley could respond positively to pre-germination treatment with H_2O_2. Furthermore, H_2O_2 has a growth promoting effect because the pretreatment of the seeds relieves latency (Bahin *et al.* 2011). Significant difference in the length of barley acrospires is found with of exogenous application of H_2O_2, which is highly correlated with the regulation of growth (Baldus *et al.* 2020). According to those authors, it can be inferred that H_2O_2 is naturally accumulated during the process of germination, inhibiting gibberellic acid (GA) catabolism and simultaneously promoting its biosynthesis. The germination process seems to be a consequence of growth and elongation of cells overcoming dormancy.

Likewise, H_2O_2 is a powerful molecule signalling transduction related to external stimuli (Petrov and Van Breusegem 2012). Fodder, as feed for cattle, produced after pre-germination treatment with H_2O_2 can help to improve the quality of the products such as meat and milk (Chen *et al.* 2017). For example, the use of H_2O_2 increases the shelf life of cow milk (Ivanova *et al.* 2019). In the same sense, H_2O_2 is added to activate the natural enzyme lactoperoxidase, which has antimicrobial activity and increases the shelf life of pasteurised milk; this treatment is allowed in only a few countries, but it can be used in the case of cheese production (Lima *et al.* 2020). Moreover, the antioxidant properties in the milk protein reduce ROS such as hydroxyl radicals, peroxide radicals and superoxide radicals (Khan *et al.* 2019).

Our results show that the antioxidant capacity that featured in the 50 mM H_2O_2 treatment is dependent on the production of phenolic compounds, as reinforced by PAL activity; the 50 mM H_2O_2 treatment showed significantly higher PAL activity and total phenols than other treatments. The ABTS antioxidant capacity and total phenols were the result of PAL enzymatic activity. Besides, it is known that the total phenolic compound activity in plants is responsible for the antioxidant activity (Ren and Sun 2014).

On the other hand, the antioxidant activity induced with the 50 mM H_2O_2 treatment in general caused a reduction in plant growth promotion relative to 100 mM and 150 mM H_2O_2 treatments and in germination of seeds relative to the 100 mM H_2O_2 treatment. The induction of phenolic production in some way redirected metabolism to this activity, to the detriment of growth. Greater antioxidant plant capacity related to secondary metabolite production, in this case phenolic compounds, has certain effects on plant growth (Jamwal *et al.* 2018).

Natural forages with high antioxidant content reduce oxidative stress mechanisms that are behind pathologies such as cardiac, digestive and respiratory diseases, bringing benefits to health and meat production of livestock (Vizzari *et al.* 2021).

Activity of SOD was highest in the 100 mM H_2O_2 treatment, although it was probably due to the stress action; there was not a high induction of phenol synthesis, and so, no adverse effect on plant growth, as also seen with 150 mM H_2O_2. SOD and CAT are enzymes that respectively increase and decrease the quantity of H_2O_2 in plants, and they are related to antioxidant capacity (Gupta *et al.* 2018). CAT is the key enzyme in the catalytic scavenging of H_2O_2 to oxygen and water, and usually, an increase of the CAT activity improves the antioxidant defence in plants; however, plant growth can be negatively affected (Gupta *et al.* 2018).

The treatment with 100 mM H_2O_2 significantly increased the germination rate of barley seeds. H_2O_2 in low concentrations promotes an improvement in the germination process through the breaking of seed dormancy (Çavuşoğlu and Kabar 2010). Those authors found in barley that treatment with 30 mM H_2O_2 accelerated germination at 20°C to 84% on the second day, and that it maintained growth parameters at 25°C, such as radicle length (1.03-fold) and fresh weight (1.04-fold). In that study, the same dose of H_2O_2 ameliorated effects of 0.30 M NaCl at 20°C, improving germination 7.11-fold on the second day, radicle length 1.34-fold and fresh weight 1.15-fold.

Kilic and Kahraman (2016) reported the highest vigour index, germination index, coleoptile length and fresh weight when barley received pre-germination treatment with 0.3 µM H_2O_2. In addition, H_2O_2 pre-germination treatment had positive effects on sizes and count of photosynthetic apertures of leaves and on plant growth. Exogenous H_2O_2 applications can enhance root growth, aiding development of a vigorous root system, as well as reducing germination time and plant development under stress conditions (Cesur and Tabur 2011).

In the present study, the reduction of $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radicals occurred through methanolic extract of H_2O_2 treatments that presented antioxidant activity. The transfer of electrons existing in the phenolic compounds counteracts the free radicals. From the results, ARSA could be regulated

by the composition of the phenols present in the extracts. The degree of antioxidant activity is correlated with the number of hydroxyl groups; this also is connected with biological activity such as avoidance of a few types of cancer, cardiovascular diseases and antioxidative injuries in the organism (Holub *et al.* 2019). The stress treatments affected the enzymatic activities, which in turn caused a chain reaction of the phenylpropanoid pathway (Fig. 4). The increase in PAL can directly affect the number of phenolic compounds throughout the development of barley.

The antioxidant enzyme system is involved in ROS scavenging. Plant development has a balance of antioxidants and pro-oxidants; a minimum change in this balance triggers the cellular response, including SOD, CAT, POD and POX (guaiacol peroxidase) (Caverzan *et al.* 2016). SOD is the most important enzyme against ROS, the main function being to eliminate the superoxide radical (O_2^-) by conversion into H_2O_2 and O_2 (Kaya *et al.* 2015). Elevated ROS synthesis is a component of stresses including oxidative stress (H_2O_2) as a result of biotic and abiotic stress factors (Tamás *et al.* 2010).

Tamás *et al.* (2010) showed that the response of barley root *in vitro* to 10 mM H_2O_2 was an increased synthesis of O_2^-, activation of SOD and elevated H_2O_2 formation. Triggering O_2^- occurred possibly through the activation of NADPH oxidase. By contrast, our experiment resulted in decreased SOD activity in barley with pre-germination treatment; this response could be due increased CAT activity, ameliorating excessive H_2O_2 in the fodder, and avoiding SOD activity.

Furthermore, increased CAT activity could positively influence seed germination and seedling growth stages (Kilic and Kahraman 2016). A direct relationship was found between morphological growth and enzyme activity. PAL activity in *Capsicum annuum* L. showed significant difference 5 min post-application of 18 mM H_2O_2, after 1 day at 14 mM H_2O_2; however, at the fifth day, major PAL activity was registered with the 6 mM H_2O_2 treatment (Mejía-Teniente *et al.* 2013).

Conclusions

Based on the above, it can be concluded that the pre-germination treatment of barley significantly increased morphological growth and triggered the adaptive process. In addition, barley seeds treated at 50 mM H_2O_2 showed enhanced biochemical mechanisms such as PAL activity, total phenol content and ABTS antioxidant activity as seedlings. Higher activities of antioxidant defence processes correlated with the phenolic compounds and antioxidative activity under stress conditions. Pre-germination application in barley hydroponic green fodder may be an option to enhance the food and feed properties for humans and livestock. Finally, it was established that controlled elicitation using H_2O_2 increased some morphological and biochemical variables of hydroponic barley fodder related to food properties.

Conflict of interest

The authors declare that they have no conflict of interests.

Data availability statement

The data that support this study are available online as supplementary material.

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