Electrically induced changes in amaranth seed enzymatic activity and their effect on bioactive compounds content after germination

César Ozuna¹ · Abel Cerón-García¹ · Ma. Elena Sosa-Morales¹ ·
Julián Andrés Gómez Salazar¹ · Ma. Fabiola León-Galván¹ ·
Ma. del Rosario Abraham-Juárez¹

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Abstract Electric treatment applied to seeds and sprouts can change their phytochemical composition. However, only a handful of studies have investigated the effects of treating seeds with electric current prior to their germination on the enzymatic antioxidant system of their sprouts. The aim of this study was to determine the changes in bioactive compounds and the enzymatic antioxidant activities in seeds and amaranth sprouts under direct electric current (DC) treatments. Amaranth seeds were treated with DC at 500 mA for different periods of time (0, 2, 5, 10 and 30 min) and let sprout (85% RH, 25 ± 2 °C) for 6 days. Significant changes were found in the antioxidant enzymatic activities and in the total content of flavonoids (15.44 ± 0.56 mg RE/gDW) and phenolic compounds (35.87 ± 0.17 mg GAE/gDW) in 6-day-old sprouts from DC-treated seeds in comparison to sprouts from non-treated seeds. The results suggested that DC treatment for short period (5 min) can induce quantitative changes to the enzymatic antioxidant system of amaranth sprouts, thus representing a relatively cost-effective method for enhancing health-improving properties of sprouts.

Keywords Amaranth · Sprouts · Direct electric current treatment · Phytochemicals

Introduction

Amaranth (*Amaranthus hypochondriacus*) is a pseudo-cereal which belongs to the dicotyledonous class of the *Amaranthaceae* family and was one of the staple foods in pre-Hispanic America (Caselato-Sousa and Amaya-Farfán 2012). Nowadays, amaranth seeds are an important component in the diet of many Latin American countries, mainly by their processing in the form of flour, as an ingredient in bakery products, in beverages or in the form of traditional snack bar made with puffed amaranth seeds. Techno-functional properties of *Amaranthaceae* germplasm has been deeply studied (Kaur et al. 2010; Singh et al. 2014). Although consuming amaranth in the form of sprouts is not as common, its popularity is currently increasing among people who want to maintain healthy lifestyle (Paško et al. 2009).

The germination process or sprouting has been suggested to be an inexpensive and effective way to improve the nutraceutical quality of cereals, pseudo-cereals and legumes (Hübner and Arendt 2013). Moreover, sprouts are fast-growing and have low production cost (Silva et al. 2013). During germination, the content and the bioactivity of compounds with nutraceutical potential, the activity of several enzymes related to the plant secondary metabolism and the enzymatic antioxidant system dynamically change and may be strongly affected by the germination conditions (Randhir et al. 2009). The main components of the complex antioxidant system in plants are enzymes, such as superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6) and ascorbate peroxidase (APX, EC 1.11.1.11). SOD enzyme, the first defense line of the cell, avoids oxidative cellular damage by partitioning superoxide radicals into either molecular oxygen or H_2O_2 . In turn, CAT and APX take care of reducing any H_2O_2 possibly

✉ Abel Cerón-García
abel.ceron@ugto.mx

¹ Departamento de Alimentos, División de Ciencias de la Vida, Campus Irapuato-Salamanca, Universidad de Guanajuato, Carretera Irapuato-Silao km 9, Ex Hacienda El Copal, C.P. 36500 Irapuato, Guanajuato, Mexico

generated by SOD (Vattem et al. 2005). On the other hand, phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) is the first line enzyme in biosynthesis of secondary metabolites (Tomás-Barberán and Espin 2001). This enzyme plays an important role in the germination process of many seeds.

Seed sprouting increases the contents of compounds with antioxidant activity, mainly polyphenols and flavonoids. Both antioxidant compounds, as active ingredients of nutritional and functional foods, may provide protection against oxidative cellular damage by either inhibiting or delaying the oxidative process and thus benefit their potential consumers (Alvarez-Jubete et al. 2010; Paško et al. 2009). These are some of the reasons why the consumption of sprouts can be very important in reducing the effects of human diseases associated with oxidative stress (Silva et al. 2013).

One of the methods that have been studied to enhance the secondary metabolism in plants is the use of electricity, which is able to produce a controllable stress condition. The use of electricity has been evaluated in different modalities, such as high electrical fields (Zhang and Hashinaga 1997), pulsed electrical fields (PEF) (Odriozola-Serrano et al. 2007) or intermittent direct electric current (DC) (Dannehl et al. 2012), but mainly in fruits and vegetables. Very few studies have applied electricity only to seeds in order to see an improvement during their germination. Leong et al. (2016) investigated the influence of PEF treatment on the antioxidant metabolism of wheatgrass sprouts. In that study, the electric field application (2 kV/cm) provoked a significant improvement on the antioxidant system of wheatgrass sprouts.

Many phytochemicals (phenolic compounds, flavonoids and peptides) have been reported in amaranth (Caselato-Sousa and Amaya-Farfán 2012) and in other pseudo-cereals (Paško et al. 2009; Repo-Carrasco-Valencia et al. 2010). However, these bioactive compounds are normally analyzed only in seeds or in whole plants, not in sprouts (Kraujalis et al. 2013). There are no studies that compare the bioactive compounds and the enzymatic antioxidant system between seeds and sprouts, nor DC treatments in amaranth. However, DC treatments of amaranth seeds might improve the bioactive compound content in their sprouts. Therefore, the aim of this work was to determine the changes in the enzymatic antioxidant activities, total anthocyanins, total flavonoid and total phenolic compound in amaranth sprouts from DC-treated seeds.

Materials and methods

Materials

All chemical reagents were provided by Sigma Aldrich (St. Louis, MO, USA). Amaranth seeds were harvested in August 2014 in Puebla, Mexico. Prior to the treatment, seeds were washed and sanitized by immersion (2% NaClO, 10 min). Seeds were abundantly washed with distilled water until a neutral pH (7.0) was reached, measured directly in the washing solution using an immersion pH-meter (VWR, Pennsylvania, USA). The rinsed seeds were dried in a convection oven (Thermo, Massachusetts, USA) at 35 °C for 30 min.

Direct electric current treatment

A total of 5 filter-paper envelopes was filled with 20 g of dried amaranth seeds each and were submerged into electrically conductive solution (2% NaCl). A laboratory power supply (Labnet, New Jersey, USA) at a constant 500 mA was used to apply direct electric current (DC) treatment to the seeds, varying in treatment time for each batch (0, 2, 5, 10 and 30 min; nomenclature was defined as T0, T1, T2, T3 and T4, respectively). The 500 mA electric intensity had been chosen as optimal, according to bioactive levels detected, after previously evaluating conditions from low (100 mA) to high (1000 mA) intensity levels. After each DC treatment, seeds were again thoroughly washed with distilled water until a neutral pH (7.0) was reached.

Germination process

Amaranth germination was carried out in a commercial sprouter (Easygreen®, Nevada, USA) with automatic spraying of water four times a day for 15 min throughout a built-in micro-sprinkler. For each DC treatment, 3 batches of 5 g of seeds on plastic trays (8 × 3 × 30 cm) were placed into the sprouter (25 ± 2 °C) and let sprout under 16/8 h light/darkness conditions (16 W white light lamp, Osram; Toluca, Mexico). Relative humidity was monitored inside the sprouter with a digital hygrometer (VWR, Pennsylvania, USA). After 6 days, the sprouts (2.5 ± 0.33 cm of length) were freeze-dried (Labconco, Missouri, USA) and stored at − 20 °C until the analysis. Non-germinated neither electrically induced amaranth seeds were used as controls.

Bioactive compound

Total anthocyanins determination by differential pH

A 1 g sample of freeze-dried amaranth sprouts (FDAS) was ground with 10 mL of cold acetone. Then, the mixture was vortexed for 30 min, avoiding light and centrifuged (Hermle, Germany) at $20,000\times g$ for 15 min at 4 °C. Then, 1 mL of the supernatant was carefully mixed with 1 mL of 0.1 M sodium borate at pH 1.0 and 4.5. Absorbances were measured at 515 and 700 nm for each sample (Thermo, Massachusetts, USA). Total anthocyanin content (TA) was reported in mg of cyanidin 3-glucose equivalents/g of dry weight (C-3-glc E/gDW) (Paško et al. 2009).

Total flavonoids

A 1 g sample of FDAS was ground with 10 mL of 90% methanol solution. Then, the mixture was put inside borosilicate tubes with caps and the closed tubes were allowed to boil for 1 h in a hot-water bath (80–85 °C). Once the boiling time finished, the tubes were placed into ice water for 20 min. Samples were clarified by centrifugation ($10,000\times g$, 15 min) and the supernatants (0.25 mL) were added to 2.25 mL of the reaction mixture (90% methanol solution, 10% Aluminum chloride, 1 M potassium acetate and distilled water). The samples were vortexed and their absorbances were measured at 415 nm (Thermo, Massachusetts, USA). Total flavonoid (TF) content was determined by rutin standard curve and reported as mg of rutin equivalents/g of dry weight (mg RE/gDW) (Khanam et al. 2012).

Total phenolic compounds

A 1 g sample of FDAS was mixed with 2.5 mL of acidified acetone (50:49.5:0.5 (v/v/v) distilled water, acetone and acetic acid) and vortexed (1 min). Then, the mixture was slowly stirred (30 min) in the darkness and clarified by centrifugation ($10,000\times g$, 15 min). The phenolic extracts (0.25 mL) were mixed with 2.5 mL of reaction mixture that contained 100 mL stock solution of 2% of sodium carbonate, 80 mg of sodium–potassium tartrate and 2 mL of 0.5% cupric sulfate solution. Finally, 0.25 mL Folin–Ciocalteu reagent was added to the samples, they were lightly vortexed and let react for 1 h in the darkness. Absorbances were measured at 765 nm (Thermo, Waltham, MA, USA). Total phenolic compounds (TP) were determined by gallic acid standard curve and reported as mg of gallic acid equivalents/g of dry weight (mgGAE/gDW) (Slinkard and Singleton 1977).

Enzymatic activity determination

Phenylalanine ammonia-lyase activity (PAL)

A 1 g sample of FDAS was homogenized (1000 rpm, 1 min) in 25 mL of cold acetone, using an Ultra-Turrax (IKA, Staufen, Germany) and the mixture was vacuum-filtered. The sample was then left at room temperature (1 h) so that the remaining acetone would evaporate. The resulting tissue was mixed with extraction buffer (0.1 M sodium borate pH 8.8 with 5 mM β -mercaptoethanol, 2 mM ethylene diamine tetracetic acid and 1% polyvinyl pyrrolidone) and stirred slowly (1 h, 4 °C). PAL activity was measured by the absorbance change at 290 nm as the amount of cinnamate produced during 1 h at 35 °C (Martínez-Téllez and Lafuente 1997). PAL activity was expressed in μmol of trans-cinnamic acid/mg of protein, using the molar extinction coefficient ($\epsilon = 17,400 \text{ mM/cm}$) for trans-cinnamic acid.

Polyphenol oxidase activity (PPO)

A 1 g sample of FDAS was homogenized with 25 mL of 50 mM sodium phosphate dibasic buffer pH 6.5, 2% polyvinyl pyrrolidone and 0.2 mL of Triton X-100, using an Ultra-Turrax (IKA, Staufen, Germany) for 1 min at 1000 rpm and the mixture was centrifuged ($2300\times g$, 20 min, 4 °C). The supernatant was collected and stored at -20°C until the analysis. For polyphenol oxidase (EC. 1.14.18.1) activity determination, the enzyme kinetic was carried out by measuring absorbance changes (420 nm) every 5 s for 3 min at 30 °C. PPO activity was expressed as $\mu\text{mol/mg}$ of protein per min (Rocha and Morais 2002).

Superoxide dismutase activity (SOD)

Enzyme extracts were obtained according to Cerón-García et al. (2011). For each sample, 1 mL of the reaction mixture (0.1 M sodium phosphate dibasic pH 7.8, 0.01 M L-Methionine, 0.025 M Triton X-100, 11 μM EDTA, 5.7 μM nitroblue tetrazolium chloride (NBT), 1.3 μM riboflavin) was added to aliquot equal to 50 μg of the enzyme extract and the reaction mixtures were illuminated with 30 W white light lamps (General Electric, Connecticut, USA) for 10 min. For correct background absorbance values, non-illuminated mixtures were used. SOD activity determination, which was carried out at 560 nm (Thermo, Massachusetts, USA). Changes in SOD activity were expressed in units/mg of protein, where one unit was defined as the amount of enzyme capable of reducing NBT by 50% (Wu et al. 2008).

Catalase activity (CAT)

Enzymatic activity of CAT (Aebi 1984) was determined by following the consumption of H_2O_2 at 240 nm for 3 min at 30 °C. Enzyme extracts were obtained according to Cerón-García et al. (2011). CAT activity was calculated as the H_2O_2 molar extinction coefficient (28 mM/cm), which was expressed in mmol of reduced H_2O_2 /mg of protein per min.

Ascorbate peroxidase activity (APX)

The enzymatic activity of APX was determined by monitoring absorbance changes at 290 nm for 3 min at 25 °C (Nakano and Asada 1987). Enzyme extracts were obtained according to Cerón-García et al. (2011). The APX activity was expressed in mmol of oxidized ascorbate/mg of protein per min and the ascorbate molar extinction coefficient (2.8 mM/cm) was used to calculate this enzymatic activity.

Total protein

For all enzyme extracts, total protein was measured using the Bio-Rad® Protein Assay reagent and bovine serum albumin as a standard.

Statistical analysis

All experimental was repeated twice and then, each measure was done by triplicate. The data were analyzed by analysis of variance (ANOVA) with Tukey–Kramer multiple comparison test using a NCSS statistical software (NCSS, LLC; Utah, USA). The value $p \leq 0.05$ was considered statistically significant.

Results and discussion

Bioactive compounds

Total anthocyanin content

Anthocyanins are responsible for conferring the characteristic red color to amaranth sprouts. In general, the germination process enhanced the total anthocyanin (TA) content in amaranth seeds (Fig. 1). In fact, 6-day-old amaranth sprouts from non-treated seeds (T0) were found to contain about 200% of TA compared to the seeds (1.16 ± 0.02 and 0.47 ± 0.01 mg C-3-glc E/gDW, respectively). Paško et al. (2009) reported similar values for the content of TA in non-treated *Amaranthus cruentus* seeds and 6-day-old sprouts (roughly 1.0 and 1.5 mg C-3-glc E/gDW, respectively) which represented an increment of about 50% as an effect of mere sprouting.

As for the application of DC treatments to the amaranth seeds prior to germination, T1 and T2 did not show significant differences ($p > 0.05$) in TA content in comparison to non-treated seeds in 6-day-old amaranth sprouts (Fig. 1). On the other hand, higher DC treatment times (T3 and T4) resulted in a significant decrease of TA in 6-day-old amaranth sprouts ($p \leq 0.05$).

Although DC treatments did not enhance the TA content in amaranth sprouts in comparison to sprouts from non-treated seeds, intermittent DC treatments may enhance the TA content when applied to growing plants (Dannehl et al. 2009). In this study was applied intermittent DC (600 and 1000 mA, 1 h per day) to radish tubers during their growth period. They observed a 28% increase in the TA content in the treated plants in comparison to controls.

Total flavonoid content

It is evident from Fig. 2a that the sprouting process drastically increased the total flavonoid (TF) content. TF content in amaranth seed was 0.78 ± 0.13 mg RE/gDW. Amaranth sprouted for 6 days showed an increase nearly 20 times in TF content as compared to unsprouted amaranth seeds (15.44 ± 0.56 mg RE/gDW). Perales-Sánchez et al. (2014) also reported an increase, although only threefold, in TF content after 3-day of sprouting amaranth. However, Paško et al. (2008) reported 8% decrease in TF content in amaranth sprouts produced under the same conditions as in the present work (0.620 mg RE/gDW) in comparison to non-treated amaranth seeds (0.676 mg RE/gDW). On the other hand, 200-fold increase in TF content was found by Ren and Sun (2014) in 7-day-old buckwheat sprouts compared to buckwheat flour (79.9 ± 3.15 and 0.4 ± 0.03 mg RE/gDW, respectively).

DC treatments did not enhance the TF content in amaranth sprouts in comparison to sprouts from non-treated seeds ($p > 0.05$). A slight decrease in the amount of this metabolite was observed with increasing DC treatment time but this decrease was significant ($p \leq 0.05$) for T3 and T4 (Fig. 2a). In other words, DC treatment of up to 5 min does not adversely affect the amount of TF for 6-day-old amaranth sprouts. However, research on the use of DC treatments in seeds in order to enhance the TF content in their sprouts is still very scarce, particularly for anthocyanins.

Total phenolic content

The sprouting process increased the total phenolic compound (TP) content between 5 and 7 times the value found in amaranth seeds, irrespectively of the application of DC

Fig. 1 Influence of direct electric current treatment in total anthocyanins content at 0 (□ T0), 2 (▨ T1), 5 (▤ T2), 10 (▥ T3) and 30 min (■ T4) of 6-day-old amaranth sprouts. Segmented line refers to amaranth seed anthocyanins level. Values are mean $\pm$ standard error, $n = 3$. Different lowercase letters indicate significant differences ($p \leq 0.05$)

treatment (Fig. 2b). Between 28.50 ± 0.15 and 35.87 ± 0.17 mg GAE/gDW of TP were found in the sprouts, compared to only 5.30 ± 0.03 mg GAE/gDW that was detected in the amaranth seeds. However, other researchers reported the TP content in amaranth sprouts without any previous DC treatment to be lower than 10 mg GAE/gDW (Alvarez-Jubete et al. 2010; Paško et al. 2009; Perales-Sánchez et al. 2014).

In comparison to sprouts from non-treated seeds, the application of DC treatments to the seeds significantly enhanced ($p \leq 0.05$) TP content in the sprouts for treated for 5 min (T2, 35.87 ± 0.17 mg GAE/gDW). As can be seen in Fig. 2b, longer treatment times (T3 and T4) yielded the same amount of TP as for sprouts from non-treated seeds. On the other hand, shorter treatment time (T1) caused a significant decrease in the amount of TP in the sprouts ($p \leq 0.05$).

Besides DC treatments, other abiotic treatments may increase the amount of TP in other kinds of sprouts. Randhir et al. (2009) reported significant increase in TP content in fenugreek sprouts up to 35% by priming the seeds with peptides and phytochemicals that elicit the pentose phosphate pathway. Similarly, Świeca et al. (2014) successfully improved the TP content in lentil sprouts, which increased by 19 and 14% in comparison to the control, by applying phenylalanine and phenylalanine + UV-B light treatment of seeds, respectively, prior to germination. However, research on the use of DC treatments in seeds in order to enhance the TP content in their sprouts has not been conducted earlier.

Enzymatic activities

Phenylalanine ammonia-lyase activity

In plants, PAL is the first line enzyme in biosynthesis of secondary metabolites (Tomás-Barberán and Espin 2001) which include phytochemicals such as anthocyanins, flavonoids, and polyphenols. For this reason, it is desirable for PAL enzyme to be enhanced in amaranth sprouts. In the present work, the 6-day-long germination process of seeds that were not treated by DC increased the PAL enzymatic activity in amaranth sprouts by more than 200% in comparison to amaranth seeds (Fig. 3). Ren and Sun (2014) reported similar increases in PAL activity in buckwheat sprouts after a 9-day-long period of germination without any DC treatment. Moreover, the authors observed a positive linear relationship between PAL activity and phytochemicals like flavonoids and phenols in buckwheat sprouts.

As for the amaranth seeds treated by DC after 6-day of sprouting showed a significant higher in PAL activity, both in comparison to the seeds and the amaranth sprouts germinated without DC treatment. Amaranth sprouts PAL activity presented a bimodal trend (Fig. 3) similar to UV-B irradiated mung bean sprouts reported by Wang et al. (2017). The DC treatment for 5 and 30 min (Fig. 3) increased PAL activity by more than 300% in comparison to the seeds. Thus, a 500 mA DC treatment for 5 min was the most suitable option for enhancing PAL activity in amaranth sprouts since it yielded comparable results to longer and 30 min DC treatment. Lentil sprouts that were under abiotic elicitor treatment (UV light and phenylalanine) prior to germination process show enhanced PAL

Fig. 2 Influence of direct electric current treatment in (a) total flavonoids and (b) total phenolic content at 0 (□ T0), 2 (▨ T1), 5 (▤ T2), 10 (▥ T3) and 30 min (■ T4) of 6-day-old amaranth sprouts. Segmented line refers to amaranth seed bioactive compound levels. Values are mean $\pm$ standard error, $n = 3$. Different lowercase letters indicate significant differences ($p \leq 0.05$)

activity without changes in stress markers like as PPO, POD, and CAT enzymatic activities (Świeca et al. 2014).

Polyphenol oxidase activity

The effects of PPO in biosystems are rather complex. This enzyme plays the main role in the germination process during early developmental stages of plants. On the other hand, PPO also causes quality loss in many foodstuffs since it is responsible for phenolic compound oxidation (Vadivel and Biesalski 2011). Thus, the aim of treatments that modify the levels of PPO in sprouts is to find an equilibrium, which would allow a proper germination process to take place, but it would not adversely affect the content of total polyphenols in the product. During the oxidation of phenolic compounds mediated by PPO activity, reactive oxygen species like hydrogen peroxide are produced, so these oxidation products must be controlled by the plant

antioxidant enzymatic system (Tomás-Barberán and Espin 2001).

In the present work, PPO enzymatic activity in amaranth seeds (15.18 $\mu\text{mol}/\text{mg}$ of protein per min before germination) was enhanced by the 6-day germination process by about 300% when no DC treatment was applied (Fig. 4). However, in amaranth sprouts germinated after DC treatment, this increase reached even higher values (up to 100%), especially for the 2 min treatment (T1, Fig. 4) in comparison to the amaranth sprouts germinated without DC treatment ($p \leq 0.05$). On the other hand, PPO activities in sprouts from amaranth seeds treated by DC for 5 and 30 min (T2 and T4, respectively, Fig. 4) were not statistically different from the PPO activity in the control sprouts ($p > 0.05$).

These results clearly show that an application of short time DC treatment (T1, Fig. 4) to the seeds enhances the PPO activity in amaranth sprouts. This high enzymatic

Fig. 3 Influence of direct electric current treatments in phenylalanine ammonia lyase activity at 0 (□ T0), 2 (▨ T1), 5 (▤ T2), 10 (▥ T3) and 30 min (■ T4) of 6-day-old amaranth sprouts. Segmented line refers to amaranth seed PAL activity level. Values are mean $\pm$ standard error, $n = 3$. Different lowercase letters indicate significant differences ($p \leq 0.05$)

Fig. 4 Influence of direct electric current treatment in polyphenol oxidase activity at 0 (□ T0), 2 (▨ T1), 5 (▤ T2), 10 (▥ T3) and 30 min (■ T4) of 6-day-old amaranth sprouts. Segmented line refers to amaranth seed PPO activity level. Values are mean $\pm$ standard error, $n = 3$. Different lowercase letters indicate significant differences ($p \leq 0.05$)

activity value correlates with the lowest TP level in amaranth sprouts (T1, Fig. 2a). PPO activity is recognized as an enzymatic marker of plant stress metabolism with high enzymatic activity levels during germination (Vadivel and Biesalski 2011). If the DC treatment is capable of creating a stable stress condition in amaranth sprouts, it could be an excellent approach for enhancing metabolic pathways for foodstuffs rich in bioactive compounds.

Antioxidant enzymatic system

In 6-days-old sprouts from non-treated amaranth seeds, SOD activity remained roughly the same as in the seeds (Fig. 5a). However, the enzymatic activities of both CAT

and APX sharply increased in sprouts compared to seeds (Fig. 5b, c) due to the fact that CAT and APX enzymatic activity levels in amaranth seeds prior to germination were minimal in comparison to their levels in 6-day-old sprouts. Similar results for dormant cereal seeds were reported by De Tullio and Arrigoni (2003). In fact, two fundamental activities involving APX during seed germination are the re-starting of ascorbate biosynthesis together with APX activity and the H_2O_2 control mediated by APX enzymatic activity (De Tullio and Arrigoni 2003).

Randhir et al. (2009) reported that 4-days-old *Mucuna pruriens* sprouts showed an increase in the CAT activity, but not in the SOD activity. These results are consistent

Fig. 5 Influence of direct electric current treatment in (a) superoxide dismutase, (b) catalase, and (c) ascorbate peroxidase activity at 0 (□ T0), 2 (▨ T1), 5 (▤ T2), 10 (▥ T3) and 30 min (■ T4) of 6-day-old amaranth sprouts. Segmented line refers to amaranth seed antioxidant enzymatic activity levels. Values are mean $\pm$ standard error, $n = 3$. Different lowercase letters indicate significant differences ($p \leq 0.05$)

with those obtained for amaranth sprouts in the present work.

About the effects of the different DC treatments on antioxidant enzymatic activity levels in amaranth sprouts,

these were either positive or the levels remained the same as in sprouts from non-treated seeds. In any case, the applied DC treatments did not cause any overall adverse

effects on the antioxidant enzymatic system in the amaranth sprouts.

The SOD enzymatic activity in 6-day-old sprouts from DC treatments (Fig. 5a) shows no significant differences between all treatment times ($p > 0.05$). In fact, there was no difference ($p > 0.05$) between sprouts from treated and non-treated seeds. Therefore, neither germination nor DC treatments caused significant changes in this enzymatic activity ($p > 0.05$). Similarly, the CAT enzymatic activity in 6-day-old sprouts shows no significant differences ($p > 0.05$) for the different DC treatment (Fig. 5b). However, all DC treatments yielded significantly higher CAT activities compared to both sprouts from non-treated amaranth seeds and the seeds themselves ($p \leq 0.05$). The CAT activity in non-treated amaranth sprouts reached the levels of 0.27 mmol of reduced H_2O_2 /mg protein per min, while the maximum level induced by DC treatments was between 0.42 and 0.57 mmol of reduced H_2O_2 /mg protein per min. Thus, even a minimal 2 min DC treatment enhances this enzymatic activity in amaranth sprouts.

In the case of the APX activity, statistically significant differences were found between all four DC treatments ($p \leq 0.05$), as well as between these and the non-treated sprouts (Fig. 5c). In general, shorter DC treatments (2 and 5 min; T1 and T2, respectively) cause an increase in APX activity while longer DC treatments (10 and 30 min, T3 and T4, respectively) cause its sharp decrease. The best treatment for APX enhancement in amaranth sprouts was the 5-min, 500 mA DC treatment. This particular treatment is also the best for the antioxidant enzymatic system taken as a whole (SOD, CAT, and APX).

There are not many studies that investigated the effects of electrically-treated seeds on the enzymatic antioxidant system of the sprouts. Leong et al. (2016) found a significant ($p \leq 0.05$) increase in the activities of antioxidant enzymes (SOD, CAT, and APX) in sprouts from appropriately hydrated wheatgrass seeds treated with PEF at 2 kV/cm in comparison to non-treated seeds.

The enhanced secondary metabolites in amaranth seem to have been produced by the DC treatments applied to the seeds and the germination itself. Biochemically, these responses could be attributed to the increased membrane permeability for Ca^{2+} provoked by the DC treatments applied. These changes at membrane level create a rapid influx of Ca^{2+} through cation channels. In fact, changes in Ca^{2+} levels in DC-treated garden cress were reported by Dannehl et al. (2012). During stress conditions, like DC treatments, the membrane permeability changes produce the phosphorylated PAL mediated by calcium-dependent protein kinases (CDPK). In the antioxidant enzymatic system of amaranth sprouts from DC-treated seeds, CDPK might have also increased the reactive oxygen species or other secondary messengers that acts as signal molecules

and induce the expression of plant defense genes which encode enzymes related to plant defense such as PAL in *Arabidopsis* (Desikan et al. 1998). In fact, SOD, CAT, and APX activities in amaranth seeds treated with and without DC were enhanced through germination in this work.

Conclusion

The application of DC treatments on amaranth seeds can induce significant changes in enzymatic activity and bioactive compounds after germination. Treatment of 500 mA DC for 5 min induced the highest increase in PAL activity as measured for 6-day amaranth sprouts, as well as for the antioxidant enzymatic system taken as a whole (SOD, CAT, and APX). Moreover, the highest amounts of polyphenols were found for sprouts for this treatment time and this seems to be directly linked to the PPO enzymatic activity. For all these reasons, this makes the T2 (5-min, 500 mA) treatment the best DC condition out of the ones studied in this work, as a relatively simple, quick and inexpensive method for bioactive compound enhancement in edible amaranth sprouts.

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