NÍCOLAS OLIVEIRA DE ARAÚJO

DIFFERENTIAL RESPONSE OF SWEET POTATO CULTIVARS TO COLD STORAGE

Dissertation presented to the Universidade Federal de Viçosa as part of the requirements of the Plant Physiology Graduate Program to obtain the tittle of *Magister Scientiae*.

Adviser: Fernando Luiz Finger

Co-adviser: Fernanda Ferreira de Araujo

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ABSTRACT

ARAÚJO, Nícolas Oliveira de, M.Sc., Universidade Federal de Viçosa, July, 2019. **Differential response of sweet potato cultivars to cold storage.** Adviser: Fernando Luiz Finger. Co-adviser: Fernanda Ferreira de Araujo.

Cold-induced sweetening (CIS) and chilling injury symptoms are the main physiological disorders that compromise the quality of sweet potato roots stored at suboptimal temperatures. The manifestation of chilling-induced physiological disorders depends on the temperature, time of exposure to the stress condition and chilling sensitivity level of the genotype. Therefore, the objective of this work was to describe differences in chilling sensitivity among sweet potato cultivar roots; evaluate the involvement of invertases and sucrose synthase in CIS; describe the impact of CIS on non-enzymatic darkening of sweet potato chips; and to evaluate the importance of the enzymatic and non-enzymatic antioxidant system in the presence or not of chilling injury symptoms in sweet potato roots. Sweet potato roots of the cultivars BRS Rubissol, BRS Cuia and Beauregard were stored at 6 or 13 °C for up to 60 days. The roots of the cultivar Beauregard showed higher chilling injury tolerance when compared to the cultivars BRS Rubissol and BRS Cuia. The CIS was shown at 6 °C due to the accumulation of non-reducing sugars, and the low invertase activity in the cultivars BRS Rubissol and BRS Cuia. On the other hand, the CIS in the Beauregard cultivar was characterized by the high invertase activity and the relatively equivalent accumulation amidst reducing and non-reducing sugars. Sucrose synthase activity was induced only in the cold-sensitive cultivars BRS Rubissol and BRS Cuia when stored at 6 °C, probably as an energy conservation mechanism. Non-enzymatic darkening of sweet potato chips was more influenced by the non-reducing sugar content when compared to reducing sugars. In contrast to the cultivars BRS Rubissol and BRS Cuia, the roots of the Beauregard cultivar did not exhibit symptoms of chilling injury by reducing oxidative stress at 6 °C through increased catalase and ascorbate peroxidase activities, as well as by increasing the content of carotenoids.

Keywords: *Ipomoea batatas*. Invertases. Sucrose synthase. Sweetening. Chilling injury.

RESUMO

ARAÚJO, Nícolas Oliveira de, M.Sc., Universidade Federal de Viçosa, julho de 2019. **Resposta diferencial de cultivares de batata-doce ao armazenamento refrigerado**. Orientador: Fernando Luiz Finger. Coorientador: Fernanda Ferreira de Araujo.

O adoçamento induzido pelo frio (AIF) e os sintomas de injúria por frio são as principais desordens fisiológicas que comprometem a qualidade das raízes de batata-doce armazenadas em temperaturas sub-ótimas. A manifestação dos distúrbios fisiológicos induzidos pelo frio depende da temperatura, do tempo de exposição à condição de estresse e do nível de sensibilidade do genótipo ao frio. Portanto, os principais objetivos do trabalho foram descrever diferenças na sensibilidade ao frio entre raízes de cultivares de batata-doce; avaliar o envolvimento das invertases e sacarose sintase no AIF; descrever o impacto do AIF no escurecimento não-enzimático de *chips* de batata-doce; e avaliar a importância do sistema antioxidante enzimático e não-enzimático na manifestação ou não dos sintomas de injúria por frio em raízes de batata-doce. Diante do exposto, as raízes de batata-doce das cultivares BRS Rubissol, BRS Cuia e Beauregard foram armazenadas a 6 e 13 °C por 60 dias. As raízes da cultivar Beauregard exibiram maior tolerância ao frio, comparadas às raízes das cultivares BRS Rubissol e BRS Cuia. O AIF manifestou-se a 6 °C pelo acúmulo de açúcares não-redutores e pela baixa atividade das invertases nas cultivares BRS Rubissol e BRS Cuia. Por outro lado, o AIF na cultivar Beauregard caracterizou-se pela alta atividade das invertases e pelo acúmulo praticamente equivalente entre açúcares redutores e não-redutores. A atividade da sacarose sintase foi induzida pelo armazenamento a 6 °C apenas nas cultivares sensíveis BRS Rubissol e BRS Cuia. O escurecimento não-enzimático dos *chips* de batata-doce foi mais influenciado pelo teor de açúcares não-redutores, comparado aos açúcares redutores. Em contraste às cultivares BRS Rubissol e BRS Cuia, as raízes da cultivar Beauregard não exibiram sintomas de injúria por frio por reduzir o estresse oxidativo a 6 °C através dos aumentos das atividades da catalase e peroxidase do ascorbato, bem como pelo aumento do conteúdo de carotenoides.

Palavras-chave: *Ipomoea batatas*. Invertases. Sacarose sintase. Adoçamento. Injúria por frio.

SUMMARY

OVERVIEW

Sweet potato (*Ipomoea batatas*) is a crop of great economic and feeding importance. In the world scenario, the production of sweet potato has granted the species the third place in the ranking of the most important tuberous crops in the promotion of food security, behind only potato and cassava (FIRON *et al.*, 2013). At the national level, sweet potato has gained relevance for its versatility, rusticity and nutraceutical value. In the last five years, the Brazilian production of sweet potato has increased exponentially (IBGE, 2019) and the increase in the export volume recorded in 2016 has placed the crop in a prominent position among the five most exported Brazilian vegetable crops (AGROSTAT, 2018). In addition to the already consolidated fresh consumption, the sweet potato market has been expanding for industrial processing. The acceptance of industrialized forms of sweet potato by the consumer market has aroused the interest of industrial complexes in Brazil in the transformation of fresh sweet potato into chips and frozen pre-fried (CAETANO *et al.*, 2018). Therefore, the postharvest storage of this tuberous root is an essential practice to supply raw material to the processing industry throughout the year. Nevertheless, commercial-scale storage of sweet potato in Brazil is still incipient, and scientific studies focusing on the potential and optimal storage conditions of sweet potato cultivars grown in Brazil are scarce.

The shelf-life of sweet potato at room temperature is short and ranges from two to four weeks (SUGRI *et al.,* 2017; LIMA, 2018). Under these conditions, events associated with respiration, mass loss, budding, and pathogen infection are accentuated and reduce the storage potential of tuberous roots. Thus, the use of refrigeration is a tool that can extend the postharvest life of these roots (TASHTOUSH, 2000; PATHIRANA, HARRIS, MCKENZIE, 2008; SUGRI *et al.*, 2017), allowing its availability in the market throughout the year, as the incidence of sprouting, respiratory rate, and pathogenic pressure are minimized (MARWAHA *et al.*, 2010). However, although the use of the cold chain after the sweet potato roots harvesting is advantageous since it is a tropical agricultural product temperature below 13 °C may promote the onset of physiological disorders known as cold or chilling injury (JACKMAN *et al.*, 1988; SEVILLANO *et* al., 2009; JI *et al.*, 2017a).

Chilling injury is characterized as a syndrome involving various physiological events, with characteristic and recognizable symptoms in fruits and vegetables during or after storage below the critical temperature (RAISON & LYONS, 1986; VALENZUELA *et al.*, 2017). Some chilling-sensitive species share similar symptoms when stored below sub-threshold temperatures (LYONS, 1973). The most notable changes are damage to the product outer surface, characterized by translucent stains, darkened depressions, and increased sensitivity to pathogens (ZOU et al., 2014).

Previous researches have investigated the biochemical and physiological changes in sweet potato stored at temperatures below 13 °C (URITANI & YAMAKI, 1969; YAMAKI & URITANI, 1972; XIE *et al.*, 2017). The primary symptoms of sweet potato in response to chilling injury include changes in the membrane lipid conformation (YAMAKI & URITANI, 1974), resulting in reduced plasma membrane fluidity and permeability. These changes increase the production of reactive oxygen species (ROS), intensifying oxidative stress as a secondary response to chilling injury (JI *et al.*, 2017a). Increased ROS, in turn, promotes the peroxidation of unsaturated fatty acids and induces the accumulation of malondialdehyde, a metabolite that, together with the quantification of electrolyte leakage, is used as an indicator of chilling injury severity (XIE *et al.* 2017; JI et al., 2017b).

The intensity of chilling injury symptoms is believed to be dependent on the balance between ROS production and its elimination (VALENZUELA *et al.*, 2017). Induction of the antioxidant system has been considered a critical factor to reduce oxidative stress and confers chilling tolerance in rice plants (VIGHI *et al.*, 2017) and eggplant fruits (SHI et al., 2018). However, the involvement of enzymatic and non-enzymatic antioxidant components in the tolerance or sensitivity to chilling injury remains poorly understood for sensitive and chilling tolerant sweet potato cultivars.

Another issue observed in tuberous roots, including sweet potatoes, stored at suboptimal temperatures is the phenomenon known as cold-induced sweetening (LI, YANG & LU, 2018). Sweetening is a result of the imbalance between starch breakage and sucrose synthesis, followed by glucose and fructose accumulation (McKENZIE *et al.*, 2013). High levels of reducing sugars impact negatively the sweet potato chips quality due to their reaction with free amino acids through the non-enzymatic Maillard reaction, producing products of dark pigmentation not accepted in the market (BHASKAR *et al.*, 2010; McKENZIE *et al.*, 2013; WIBERLEY-BRADFORD *et al.*, 2014).

The biochemical understanding of carbohydrate metabolism during sweetening is mainly focused on potato tubers (HERMAN, KNOWLES & KNOWLES, 2017). Biochemical and molecular evidence revealed that during sweetening, the net rate of sucrose synthesis increases and part of that sucrose is transported to the vacuole, where vacuolar acid invertase converts it to glucose and fructose (WIBERLEY-BRADFORD *et al.*, 2014). However, information on carbohydrate metabolism, the role of saccharolytic enzymes in sweetening during cold-storage of sweet potatoes, and the natural variation in cultivar sensitivity to chilling injury are still scarce. Therefore, determining biochemical markers of chilling injury in sweet potato roots and understanding the relation between sucrose metabolism enzymes and sweetening can provide technical support to improve the sweet potato production chain, especially the processing industry, through the identification of more low-temperature tolerant cultivars.

OBJECTIVES

Overall objectives

Biochemically characterize the development of physiological disorders, such as cold-induced sweetening and chilling injury in roots of sweet potato cultivars stored at low temperatures.

Specific objectives

- Report whether low storage temperatures promote cold-induced sweetening in the sweet potato cultivars BRS Rubissol, BRS Cuia, and Beauregard;

- Evaluate the relationship between sucrose synthase activity, soluble acid invertase, neutral invertase, and soluble sugar content during cold-induced sweetening;

- Characterize chilling injury in three sweet potato cultivars during cold storage;

- Assess the involvement of the balance between oxidative stress and the antioxidant system on sweet potato root sensitivity or tolerance to cold stress.

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ARTIGO 1

Sucrose degradation pathways in cold-induced sweetening and its impact on the non-enzymatic darkening in sweet potato root

(Artigo nas normas da revista Food Chemistry)

Abstract

This study investigated sucrose catabolism during cold-induced sweetening (CIS) and its impact on the quality of sweet potato chips of cultivars with varied levels of tolerance to cold during storage at 6 or 13 ºC. In contrast to cultivar Beauregard, cultivars BRS Rubissol and BRS Cuia were cold-sensitive exhibiting intense symptoms of chilling injury at 6 °C. In the sensitive cvs BRS Rubissol and BRS Cuia, CIS was characterized by low accumulation of reducing sugars (RS), high non-reducing sugars content, low invertase activity and high sucrose synthase (SuSy) activity. In the tolerant cv. Beauregard, the high content of RS was due to high invertases activity. In the three cultivars, the darkening of chips was more influenced by the non-reducing sugars, instead of RS. Our results suggest that SuSy was induced by cold stress in cold-sensitive cultivars, but did not contribute to the CIS in sweet potato.

Keywords: *Ipomoea batatas*; sucrose catabolism; invertase; sucrose synthase

Introduction

Sweet potato (*Ipomea batatas*) roots destined for processing are often stored under refrigeration to reduce dry matter losses, slow down sprout growth, prevent pathogen incidence and enable their continuous supply to processing industries (Ji et al., 2018). Nevertheless, storage under temperatures as low as 6 ºC heightens the risk of roots being affected by cold-induced sweetening (CIS), a physiological disorder caused by a significant increase in glucose and fructose that implies additional expenditures for the processing industry (Sakamoto et al., 2014; Ji et al., 2018). At high temperatures, those reducing sugars interact with free amino acids in the non-enzymatic Maillard reaction, affecting the taste and color of fried sweet potato products (McKenzie et al., 2013). This reaction also generates acrylamide, a probable carcinogenic agent that is harmful to humans and whose quantity is also influenced by the reducing sugar content (Kumar et al., 2018).

Accumulation of reducing sugars during CIS is a result of the complex interaction between diverse metabolic pathways, among which those of sucrose catalyzed by invertases (Inv, EC 3.2.1.26) and sucrose synthase (SuSy, EC 2.4.1.13) stand out (Sakamoto et al., 2014). Inv are β-fructofuranosidases that catalyze the irreversible hydrolysis of sucrose to glucose and fructose (Wan et al., 2018). In higher plants, Inv are found in another three isoforms: the cell wall and vacuolar isoforms, whose optimum pH is acid (4.5-5.5), and the cytoplasmic form, which exhibits maximum activity at neutral and alkaline pH (Wan et al., 2018). In contrast to the Inv, SuSy catalyzes both sucrose synthesis and degradation reactions; however, sucrose degradation into UDP-glucose and fructose prevails under normal physiological conditions (Stein and Granot, 2019).

Besides the difference between the products of sucrose catabolism, both enzymes differ in their affinity for the substrate (Avigad, 1982) and energy expenditure required to degrade sucrose to hexoses-phosphates (Santaniello et al., 2014). The pathway catalyzed by SuSy is more favorable at high sucrose concentrations due to its low affinity for the substrate compared to invertases (Avigad, 1982). For each sucrose degraded via Inv, two ATP molecules are required to convert it to hexoses-phosphates, whereas the pathway of SuSy linked to UGPase requires only one molecule of PPi (Santaniello et al., 2014; Stein and Granot, 2019). These differences are advantageous to plants in that they ensure greater metabolic plasticity, providing a means of optimizing the partitioning of sucrose and the use of its by-products according to the current needs and physiological conditions of cells.

In sweet potato, the role of Inv and SuSy in root growth and tuberization is well established in the literature (Li et al., 2003); however, the relative contribution of Inv and SuSy to the pool of reducing sugars in sweet potato roots affected by CIS remains underinvestigated (Sakamoto et al., 2014). On the other hand, advanced information is known about the sucrose metabolism in potato tubers (*Solanum tuberosum*) and, in this species, acid-soluble invertase (AS-Inv) is mentioned as the key enzyme in the control of CIS, as it regulates the hexose/sucrose ratio (Zrenner et al., 1996). This hypothesis was confirmed by transgenic approaches whereby the silencing of the *StvacINV1* genes of AS-Inv and the overexpression of their specific inhibitors *StInvInh2A* and *StInvInh2B* prevented CIS in potato tubers (Liu et al., 2011; Liu et al., 2013).

Although the role of invertases has been extensively investigated in roots and tubers at low temperatures, the role of SuSy in CIS in sweet potato has not yet been elucidated. Considering that oxidative phosphorylation is compromised under cold stress (Blenkinsop et al., 2003; Pinhero et al., 2007), we have hypothesized that, because it requires less energy expenditure, the pathway catalyzed by SuSy is also involved in accumulation of reducing sugars in sweet potato affected by CIS. On these bases, the present study proposes to examine the relative contribution of Inv and SuSy to sucrose catabolism in sweet potato roots affected by CIS and its impact on the quality of chips made of cultivars which differ in their sensitivity to cold.

Materials and Methods

Plant Material

The propagation material of sweet potato cvs. BRS Rubissol, BRS Cuia and Beauregard was acquired from the Frutplan company (Pelotas, RS, Brazil). Plantlets 10 to 12 cm high, with 3-4 leaves, were transplanted to the experimental field at the Universidade Federal de Viçosa, in Viçosa, MG, Brazil (20º 45’ 20’’ S and 42º 52’ 40’’ W, 651 m altitude). The period between planting and harvesting was November 2017 to May 2018.

The material was harvested manually, and disease- and damage-free tuberous roots weighing between 300-800 g were selected. After curing for seven days at 30 ºC and 90% relative humidity (RH), the roots were stored at 6 or 13 ºC for 0, 20, 40 and 60 days under 90% RH.

Soluble sugars and alcohol-insoluble solids contents

Soluble sugars were extracted after grinding, homogenizing and centrifuging approximately 5 g of pulp in 80% ethanol heated at 85 ºC. The total soluble sugar (TSS) content was quantified by the phenol-sulfuric acid method (Dubois et al., 1956), whereby the reaction mixture is composed of 250 µL of the ethanol extract, 250 µL 5% phenol and 1.25 mL concentrated H_2SO_4. Readings were taken with a spectrophotometer at 490 ηm and results were expressed as % TSS. The reducing sugar (RS) content was analyzed by an adapted version of the dinitrosalicylic acid (DNS) method (Gonçalves et al. 2010), in which the assay consisted of the reaction of 500 µL of the ethanol extract and 500 µL of DNS. Readings were performed with a spectrophotometer at 540 ηm. The result was expressed as a % of RS. The non-reducing sugar (NRS) content was estimated as the difference between TSS and RS, with results expressed as a % of NRS. The alcohol insoluble solid (AIS) content was determined by the methodology described by Bonte et al. (2000), with results expressed as % of AIS.

Invertases and sucrose synthase activity assays

The soluble acid invertase activity (AS-Inv) was determined by an adaptation of the method described by Goldstein and Lampen (1975), whereas and the neutral invertase (N-Inv) and sucrose synthase (SuSy) activities were measured by the method described by Klotz et al. (2003), with some modifications. The AS-Inv protein extract was prepared by homogenizing 0.2 g of pulp in 50 mM phosphate buffer (7.0) containing 1 mM EDTA, 10 mM 2-mercaptoethanol, 5 mM $NaHSO_3$, 5 mM $MgCl_2$ and 1% PVPP. The protein extract of N-Inv and SuSy was obtained by homogenizing 0.2 g of pulp in 50 mM phosphate buffer (pH 7.2) containing 1 mM EDTA, 10 mM 2-mercaptoethanol, 10 mM $NaHSO_3$ and 1 mM $MgCl_2$. Homogenates were centrifuged at 17000 *g* for 30 min and the supernatant was used for the enzyme activity assays and protein quantification. All processes were carried out at 4 ºC and the enzyme extract protein was determined using the dye-binding method of Bradford (1976).

The AS-Inv reaction mixture consisted of the protein extract, 50 mM sucrose, distilled water and 100 mM NaOAc buffer (pH 5.0). The SuSy reaction mixture was composed of 1000 mM sucrose, 8 mM UDP and 100 mM phosphate buffer (pH 6.5). Lastly, the N-Inv medium contained 1000 mM sucrose, 8 mM distilled water and 100 mM phosphate buffer (pH 6.5). The reaction mixture of the three enzymes were incubated at 37 ºC for 14 min and fructose was quantified by the method of Nelson (Nelson, 1944). For the readings, the blanks were considered the zero point of the standard curve of fructose. For the SuSy assay, the control reaction was performed on all samples through the N-Inv activity assay in the absence of UDP. The enzyme activities were expressed in ηmol fructose mg protein^{-1} h^{-1}.

Sweet potato chip color after frying

Roots were peeled, homogenized and processed as 2-mm-thick chips. Chips were fried in refined soybean oil for 2 min, at 180 ºC, in electric fryers (Ford, Michigan, USA). Color analysis was based on the following scale of scores: 1 - chips with no surface darkening; 2 - chips with up to 25% of their surface darkening; 3 - chips with 26 to 50% of their surface darkening; 4 - chips with 51 to 75% of their surface darkening; and 5 - chips with more than 75% of their surface darkening.

Malondialdehyde content

Determined by the thiobarbituric acid method (TBA) described by Heath and Packer (1968), with some modifications. Approximately 0.2 g of pulp was macerated with 2 mL of trichloroacetic acid (TCA). After centrifugation for 15 min at 12000 *g*, 1.5 mL TCA 0.5% (w/v) in 20% TBA were added to 500 µL of the supernatant. After incubation for 20 min at 90 ºC, the samples were once again centrifuged at 3000 *g* for 4 min. Readings were taken with a spectrophotometer at 532 and 600 ηm using the absorbance coefficient of 155 mM^{-1} cm^{-1} and values were expressed as ηmol g^{-1}.

Experimental design and statistical analysis

The experiment was set up as a completely randomized design in a split-plot arrangement with five replicates. The plot consisted of 3 cultivars (BRS Rubissol, BRS Cuia and Beauregard) × 2 temperatures (6 and 13 °C), whereas the subplot was represented by the storage periods (0, 20, 40 and 60 days). Data were subjected to analysis of variance, with the temperature factor levels compared by the F test ($P \leq 0.05$), using R software version 3.4.3 (R Core Team, 2017). Pearson correlations between the variables were performed using R package '*corrplot*'.

Results

Visual alterations and MDA content in sweet potato roots under cold stress

Anecdotic evidence about the response of sweet potato cultivars to refrigerated storage suggests that cv. Beauregard is more tolerant to the cold than other cultivars. Therefore, to prove the difference in sensitivity to cold, American cv. Beauregard, widely cultivated across the globe, and Brazilian cvs. BRS Rubissol and BRS Cuia were evaluated for the incidence of chilling injury and oxidative stress by the content of malondialdehyde (MDA). Cultivar Beauregard was consistently more tolerant to the cold, exhibiting no symptoms of chilling injury or rot incidence at both the temperatures of 6 ºC and 13 ºC (Figure 1A). However, cvs. BRS Rubissol and BRS Cuia were sensitive to the temperature of 6 ºC, showing strong symptoms of chilling injury, as evidenced by browned depressions and fungal incidence observed in 100% of the roots (Figure 1A).

Storage of at 6 ºC increased oxidative stress in cvs. BRS Rubissol and BRS Cuia, as evidenced by the increased MDA content, compared to storage at 13 ºC. The MDA content of BRS Rubissol was 3.7 times higher after 60 days of storage at 6 ºC (Figure 1B), while that of cv. BRS Cuia was 2.9 times higher under the same conditions (Figure 1C). The MDA content of cv. Beauregard, on the other hand, did not differ between the temperatures, except at 60 days, when greater accumulation was observed at 6 ºC (Figure 1D).

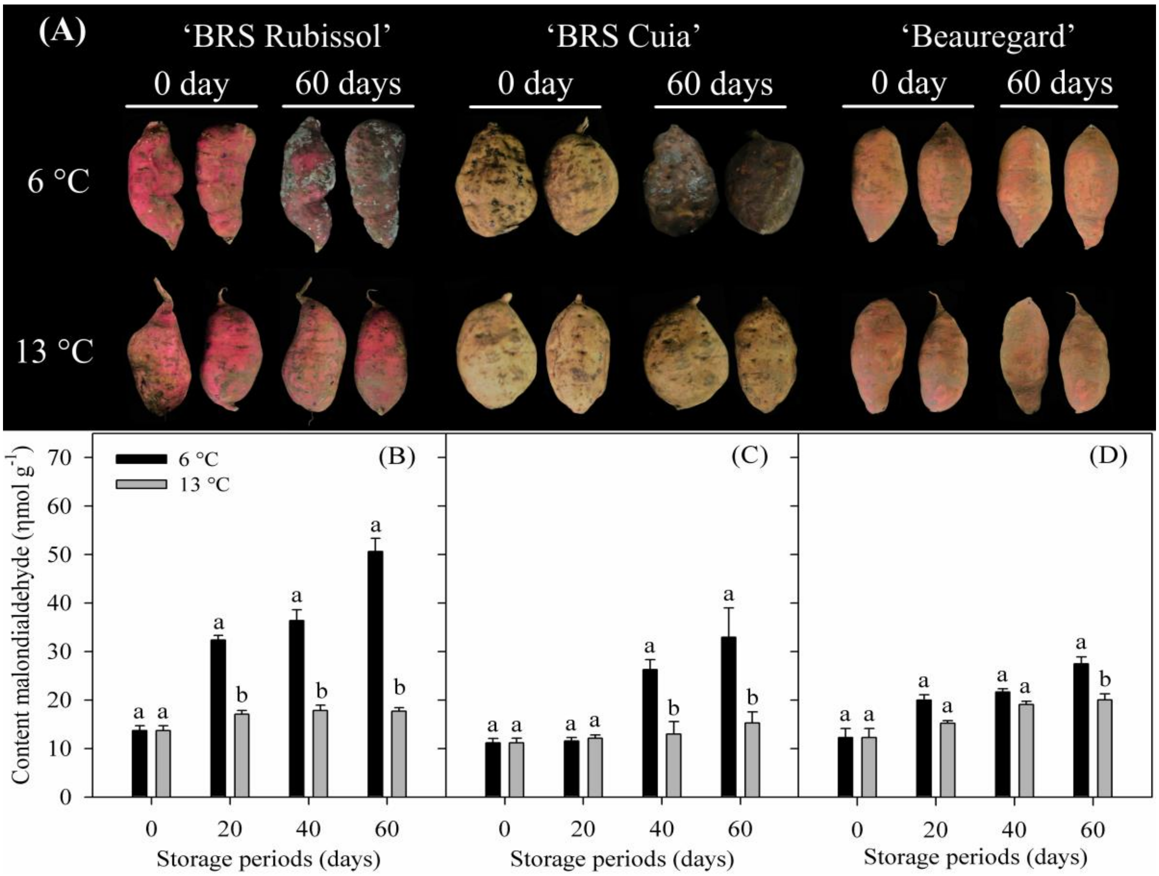

Figure 1. Effect of cold stress on the visual aspect (A) of roots of sweet potato cultivars (BRS Rubissol, BRS Cuia and Beauregard) at 0 and 60 days of storage at 6 ºC and 13 ºC. Malondialdehyde content in sweet potato cvs. BRS Rubissol (B), BRS Cuia (C) and Beauregard (D) at 0, 20, 40 and 60 days of storage at 6 ºC and 13 ºC. Different letters indicate significant differences according to the F test ($P \leq 0.05$).

Soluble sugars and alcohol-insoluble solids in sweet potato under cold stress

To understand the changes in carbohydrates during CIS, the total soluble sugar (TSS), reducing sugar (RS), non-reducing sugar (NRS) and alcohol-insoluble solid (AIS) contents were quantified (Figure 2). In the sensitive cvs. BRS Rubissol and BRS Cuia, TSS was considerably higher at 60 days of storage at 6 ºC, exceeding the value observed at 13 ºC by 2.23 and 2.92 times, respectively (Figure 2A and B). In the tolerant cv. Beauregard, the increase in TSS content was not affected by the temperatures until 40 days of storage; however, at 60 days, the TSS content of the roots at 6 ºC exceeded that achieved at 13 ºC by 1.33 times (Figure 2C).

The RS content of the sensitive cvs. BRS Rubissol and BRS Cuia was lower than 1% throughout the storage period and was practically not altered by either storage temperature (Figure 2D and E). By contrast, the RS content of the tolerant cv. Beauregard reached up to 3.1

and 3.3% during storage at 6 and 13 ºC, respectively (Figure 2F). Between 0 and 40 days, at the temperature of 13 ºC, the RS content of cv. Beauregard was up to 1.7 times higher than at 6 ºC. However, at 60 days, the RS content of this cultivar was similar between the temperatures (Figure 2F).

Sucrose, the main NRS present in sweet potato roots, rose in the three cultivars stored at 6 ºC. In cv. BRS Rubissol, the NRS content tripled in 60 days of storage at 6 ºC, with final values 2.2 times higher than those attained at 13 ºC (Figure 2G). In cv. BRS Cuia, the temperature of 6 ºC increased NRS at 60 days, exceeding the value obtained at 13 ºC in the same period by 3.9 times (Figure 2H). By contrast, the accumulation of NRS in cv. Beauregard at 6 ºC was subtler than in the other varieties, exceeding that achieved at 13 ºC by 1.7 times (Figure 2I).

Starch is the predominant content in the dry matter of sweet potato roots. In this regard, quantifying the AIS content has successfully shown to be an approximation of the starch content in tubers. In the sensitive cvs. BRS Rubissol and BRS Cuia, the AIS content at 60 days at 6 ºC decreased by 20.0 and 22.3%, respectively, vs. 6.1 and 5.6% at 13 ºC, respectively (Figures 2J and K). These results suggest that starch is the main carbon source remobilized for the synthesis of soluble sugars in those cultivars. Nevertheless, in cv. Beauregard, only at 60 days of storage did the temperature of 6 ºC intensify the reduction in AIS compared to the temperature of 13 ºC (Figure 2L), which indicates that other carbon sources may be contributing to the accumulation of soluble sugars during the storage of that cultivar.

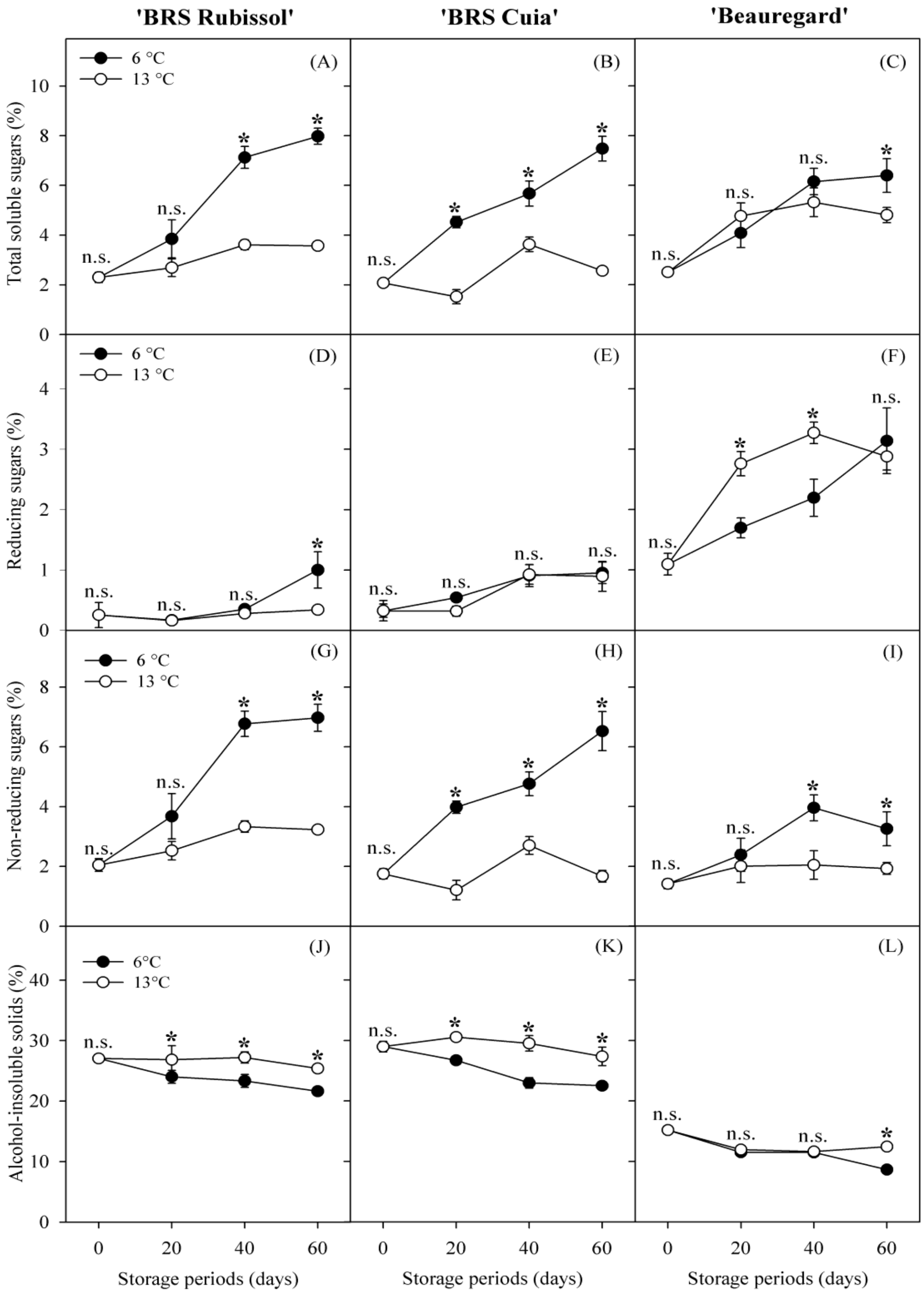

Figure 2. Total soluble sugar (A, B and C), reducing sugars (D, E and F), non-reducing sugars (G, H and I) and alcohol-insoluble solids (J, K and L) contents in roots of sweet potato cvs. BRS Rubissol, BRS Cuia and Beauregard during storage at 6 and 13 ºC. Data represent the mean ± standard error. $^{n.s.}$ and * indicate non-significant ($P > 0.05$) and significant ($P \leq 0.05$), differences, respectively, according to the F test.

Invertase and sucrose synthase activities

To investigate the effect of low temperatures on the sucrose degradation pathways, the acid-soluble invertase (AS-Inv), neutral invertase (N-Inv) and sucrose synthase (SuSy) activities were quantified (Figure 3). Throughout the storage period, at both temperatures, cvs. BRS Rubissol and BRS Cuia exhibited low invertases activity (< 12 ηmol fructose h^{-1} mg $protein^{-1}$), which explains the low accumulation of RS in those cultivars (Figure 3A and B). By contrast, the marked accumulation of RS in cv. Beauregard corroborates the high AS-Inv and N-Inv activities during storage at both temperatures (Figure 3C).

The AS-Inv activity in cv. BRS Rubissol decreased slightly at both temperatures up to 40 days of storage, whereas at 60 days the temperature of 6 ºC led to results 4.4 times higher than those seen at 13 ºC (Figure 3A). In cv. BRS Cuia, the temperatures did not influence AS-Inv, whose activity remained practically constant throughout the storage period (Figure 3B). Conversely, storage at 13 ºC for the tolerant cv. Beauregard for 20 days increased AS-Inv activity, whose value was almost 3.0 times higher than that attained in the same period at 6 ºC (Figure 3C). From 40 days, the AS-Inv activity in cv. Beauregard continued to increase, although not differences were detected between the storage temperatures (Figure 3C).

The N-Inv activity in the sensitive cvs. BRS Rubissol and BRS Cuia was not affected by the temperatures (Figure 3D and E). However, the N-Inv activity in the tolerant cv. Beauregard was responsive to the storage temperatures, exceeding the activity found in cvs. BRS Rubissol and BRS Cuia by 1000 and 670 times, respectively (Figure 3). The N-Inv activity in cv. Beauregard was markedly induced at 13 ºC, peaking at 40 days, with a 1.56 times higher value than that observed at 6 ºC (Figure 3F). At 60 days, however, this activity at 13 ºC was significantly lower than that measured at 6 ºC (Figure 3F).

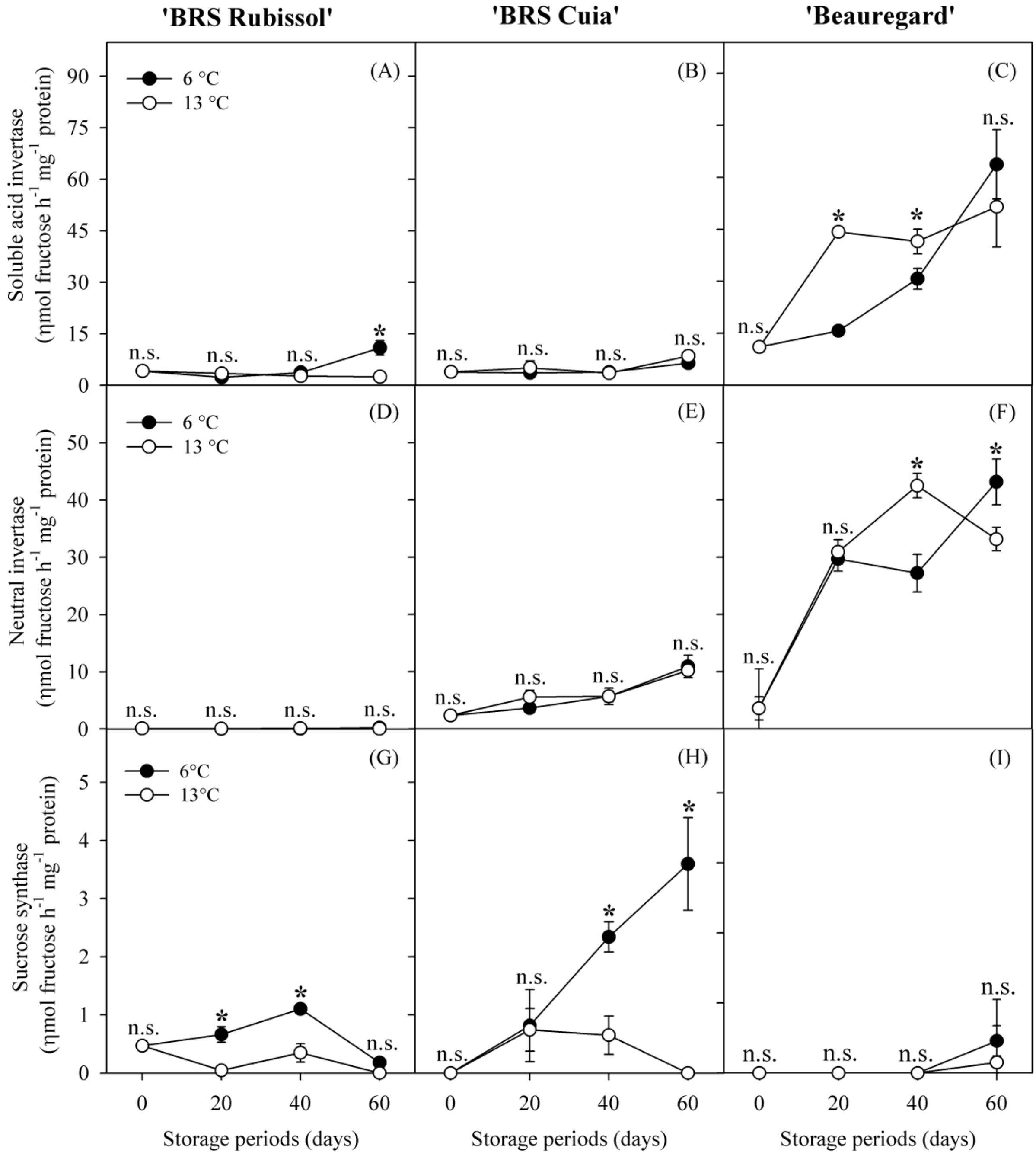

Figure 3. Acid-soluble invertase (A, B and C), neutral invertase (D, E and F) and sucrose synthase (G, H and I) activities in roots of sweet potato cvs. BRS Rubissol, BRS Cuia and Beauregard during storage at 6 and 13 ºC. Data represent the mean ± standard error. $^{\text{n.s.}}$ and * indicate non-significant ($P > 0.05$) and significant ($P \leq 0.05$) differences, respectively, according to the F test.

Non-enzymatic darkening (NED) of sweet potato chips

The degree of darkening in cv. BRS Rubissol chips increased throughout the storage period, but no significant differences were present between the temperatures of 6 and 13 ºC (Figure 4A). Compared to the other cultivars, the chips of cv. BRS Cuia exhibited a larger dark surface at zero day of storage (Figure 5), with scores twice as high as those obtained by the Rubissol and Beauregard chips in the same period. The extent of NED in the BRS Cuia and Beauregard chips increased from 20 and 40 days at 6 ºC, respectively (Figure 4C).

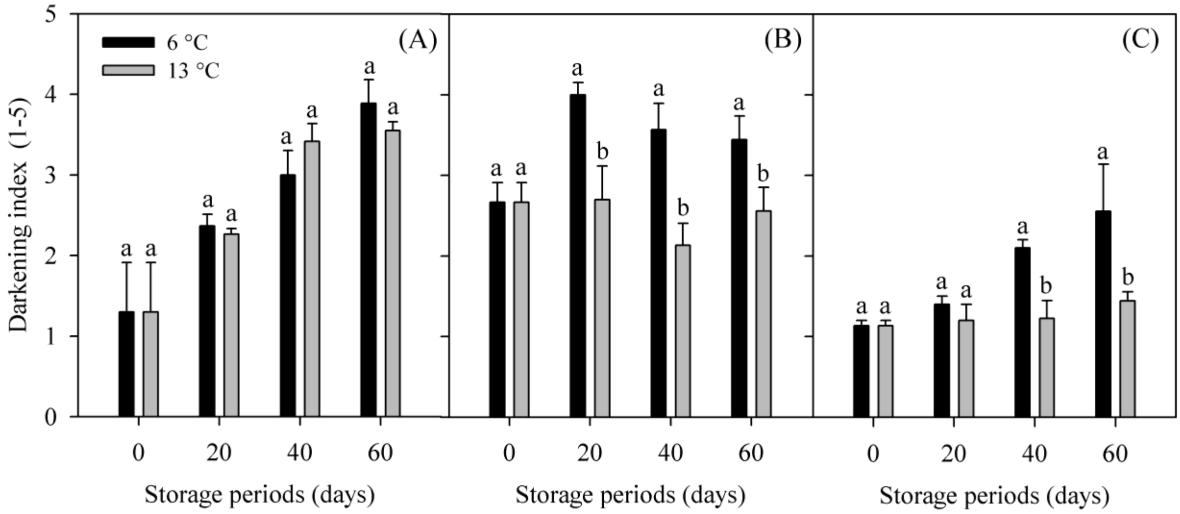

Figure 4. Darkening index in chips of sweet potato cvs. BRS Rubissol (A), BRS Cuia (B) and Beauregard (C) during storage at 6 and 13 ºC. Bars represent the mean ± standard error. $^{a\text{-}b}$ Different letters indicate significant differences between the temperatures according to the F test ($P \leq 0.05$).

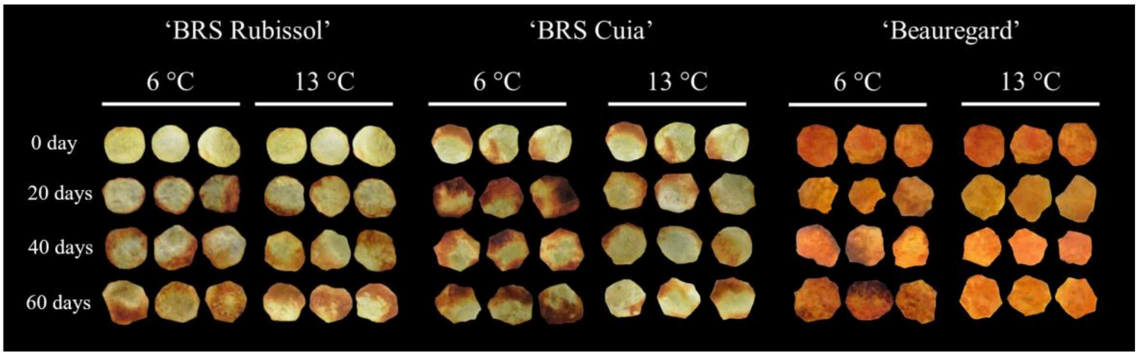

Figure 5. Visual analysis of chips of sweet potato cvs. BRS Rubissol, BRS Cuia and Beauregard during storage for 0, 20, 40 and 60 days at 6 and 13 °C.

Correlation analysis

Pearson's correlation coefficients are depicted in the heat map in Figure 6. For cv. BRS

Rubissol, the soluble sugar content was positively correlated with the darkening index and negatively correlated with the AIS content (Figure 6). The RS content of cv. BRS Cuia was correlated with the N-Inv (r = 0.49; P = 0.02) activity but not with the darkening index (r = 0.27; P = 0.2) (Figure 6). The extent of NED in the chips of cv. Beauregard was positively correlated (r= 0.68; P = 0.0) with the increase in NRS (Figure 6). The increase in RS content of cv. Beauregard was strongly correlated (r= 0.84; P =0) with the increase in N-Inv activity (Figure 6).

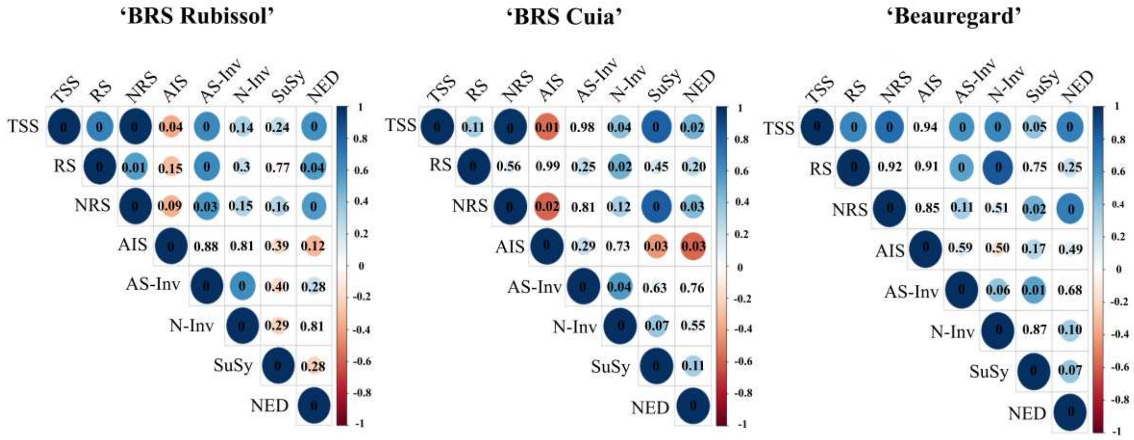

Figure 6. Heat map of Pearson's correlation between soluble carbohydrates, alcohol-insoluble solids, sucrolytic enzymes and non-enzymatic darkening in sweet potato cvs. BRS Rubissol, BRS Cuia and Beauregard. The bar colors indicate the nature of the correlation, where 1 means a perfectly positive correlation (dark blue) and -1 means a perfectly negative correlation (dark red). Strong correlations are indicated by large circles, whereas weak correlations are indicated by small circles. The numbers within the circle are the P-values.

Discussion

Sugars predominantly accumulated in CIS differ between sensitive and tolerant sweet potato genotypes

The CIS is the most noticeable response in tuberous roots stored at low temperatures. The significant correlation between degradation of AIS and intense accumulation of TSS in the cold-sensitive cvs. BRS Rubissol and BRS Cuia support the hypothesis that starch is the main carbon source mobilized for the synthesis of soluble sugars accumulated in CIS (Figure 6). By contrast, in the tolerant cv. Beauregard, the lack of differences between the temperatures in the reduction of AIS up to 40 days (Figure 2L) suggests that the starch in this genotype is little responsive to cold and that the degradation and solubilization of cell wall components may be providing carbon skeletons for the synthesis of RS and NRS (Carvajal et al., 2012).

Glucose, fructose and sucrose belong to the category of compatible solutes that accumulate at low temperatures without altering the intracellular redox potential. In the sensitive cvs. BRS Rubissol and BRS Cuia, CIS at 6 ºC was characterized by predominance of NRS, which represented up to 98 and 88% of the TSS, respectively (Figure 2G and 2H), whereas RS accumulation was not considerable in those cultivars. (Figure 2D and 2E). By contrast, the RS:NRS ratio in the tolerant cv. Beauregard remained around 1, with the RS representing up to 49 to 63% of the TSS accumulated during storage at 6 and 13 ºC, respectively (Figure 2). These results suggest that the carbohydrate metabolism in cv. Beauregard is contrasting to that of cvs. BRS Rubissol and BRS Cuia, and the distinction between the sugars predominantly accumulated by the cultivars may be associated with lesser or greater sensitivity to cold.

Many studies have reported the influence of the carbohydrate metabolism on plant tissue sensitivity to low temperatures (Tarkowski and Van den Ende, 2015). Sugars act as osmolytes, signaling molecules, and are able to mitigate cold stress by eliminating reactive oxygen species (Longo et al., 2018). Among the soluble sugars, the high relative levels of NRS are frequently associated with increased tolerance to cold, as observed in tomato seedlings (Qi and Zhou, 2011) and mandarin fruit (Holland et al., 2002). However, our results revealed a distinct behavior in relation to that reported in those species.

Sucrose accumulation in sweet potato is likely associated with low tolerance to cold, considering that cvs. BRS Rubissol and BRS Cuia, which exhibited symptoms of chilling injury, showed intense accumulation of NRS when stored at 6 ºC (Figure 1A). The high levels of RS and the maintenance of the RS:NRS ratio around 1, on the other hand, may contribute to the tolerance of cv. Beauregard to the cold, mitigating the oxidative stress indicated by the low levels of MDA (Figure 1D). This hypothesis is underpinned by results obtained with zucchini fruit (*Cucurbita pepo*), where high levels of RS were found as tolerance to cold increased (Palma et al., 2014). In grape berries, accumulation of RS was also correlated with reduced sensitivity to cold (Purvis and Grierson, 1982). However, the development of transgenic sweet potato lines possessing antisense constructions or overexpressing sense constructions of sucrose degradation pathways may better confirm this hypothesis.

CIS in sweet potato is a phenomenon independent of the SuSy activity that may or may not depend on invertase activity

The hexogenesis pathway is constituted by two enzymatic reactions: an irreversible one, catalyzed by the different invertase isoforms, and a reversible one, catalyzed by sucrose synthase (SuSy). In the sensitive cvs. BRS Rubissol and BRS Cuia, the small relative contribution of the RS to the total sugars accumulated at 6 ºC (Figure 2) is consistent with the low AS-Inv and N-Inv activities in those genotypes (Figure 3). Nevertheless, the elevated RS content of cv. Beauregard when stored at 6 and 13 ºC (Figure 2F) was a reflection of the high AS-Inv (Figure 3C) and N-Inv (Figure 3F) activities, with N-Inv predominating ($r = 0.84$; $P = 0.0$) (Figure 6).

The acid Inv activity is modulated by a post-translational modification triggered by a small group of Inv-specific inhibitor proteins (< 20 kDa) (Wan et al., 2018). Cultivars BRS Rubissol and BRS Cuia likely had higher levels of the Inv inhibitor protein, limiting the catalytic ability of the enzyme. On the other hand, the high Inv activity in cv. Beauregard is possibly a reflection of the low levels of inhibitor proteins, allowing free formation of the invertase-sucrose complex. To the present date, although no N-Inv -inhibitor proteins have been found (Ruan et al., 2010), it is possible that there is also an inhibitor that restricts the N-Inv activity in cvs. BRS Rubissol and BRS Cuia.

In sweet potato, in addition to Inv, the role of SuSy is also well-established during the development of the storage root, where the change from irreversible to reversible sucrose degradation marks the start of tuberization (Li and Zhang, 2003; Zhang et al., 2017). However, because Inv is more abundant in the post-harvest storage of tuberous roots and tubers, few studies have investigated the role of SuSy in CIS. In cvs. BRS Rubissol and BRS Cuia, SuSy was induced by the temperature of 6 ºC (Figure 3G and 3H, respectively); however, the increased activity did not lead to greater accumulation of RS (Figure 2F). By contrast, SuSy activity in cv. Beauregard was detected only at 60 days under both storage conditions, though not affected by the temperatures (Figure 3I).

The SuSy activity in cvs. BRS Rubissol and BRS Cuia is likely induced by the temperature of 6 ºC because it is advantageous as an energy recovery pathway. The pathway catalyzed by SuSy, linked to UGPase, requires less energy, as it takes only 1 mol PPi sucrose^{-1} (Stein and Granot, 2019). Because the stress caused by the cold limits ATP production by affecting oxidative phosphorylation in the mitochondria, the anaerobic pathway is induced to ensure NAD^{+} renewal and consequent ATP production (Blenkinsop et al., 2003; Pinhero et al., 2007). However, net ATP production during anaerobic respiration is low, and the increased SuSy activity may be a strategy to double the economy of ATP (Stein and Granot, 2019). This view is consistent with the results of Zeng et al. (1999), who found a reduction in Inv/SuSy activity in maize under anaerobic conditions. The increased in SuSy activity in seedlings of *Arabdopsis thaliana* under anoxic conditions (Santaniello et al., 2014) is also consistent with this hypothesis.

The contribution of Inv and SuSy to sucrose degradation may also be related to kinetic characteristics of those enzymes. Affinity for sucrose is rather distinct between Inv (K_m= 7-15 mM) and SuSy (K_m= 40-200 mM) (Avigad, 1982). Thus, the higher relative NRS content in the sensitive cvs. BRS Rubissol and BRS Cuia stored at 6 ºC may favor sucrose cleavage by SuSy, thereby reducing the ratio between the Inv and SuSy activities. Conversely, the lower relative participation of NRS in the TSS in the tolerant cv. Beauregard compared to the other genotypes might have favored the increase in Inv activity over SuSy activity, elevating the Inv/SuSy activity ratio.

The reducing sugar level was not a key factor for NED in the sweet potato chips

NED of sweet potato chips has been associated with the Maillard reaction, whereby the RS react with free amino acids, producing dark pigments with an unpalatable taste (McKenzie et al., 2013). Therefore, because the RS are involved in the production of dark components during frying, the darkening index in the chips was expected to increase with the RS levels. However, this hypothesis was partially refuted. In cv. Beauregard, the high RS content present in the roots at both temperatures did not result in intense darkening of the chips ($r = 0.24$; $P = 0.25$) compared to the other cultivars (Figure 6). In cv. BRS Cuia, the larger dark surface of the chips observed at 6 ºC showed an apparent correlation with the increase in TSS ($r = 0.47$; $P = 0.02$) and NRS ($r = 0.44$; $P = 0.03$), which supports the hypothesis that sucrose hydrolysis during frying may contribute to darkening in chips (Leszkowiat et al., 1990; McCann et al., 2010). By contrast, only in cv. BRS Rubissol were the RS associated with the darkening index (r = 0.57; $P = 0.04$).

These results reinforce the hypothesis that the RS content alone cannot be used as an index to prevent darkening in sweet potato chips, considering that other components besides the RS may influence the many stages of the Maillard reaction (Mckenzie et al., 2013). The low level of asparagine in sweet potato suggests that this amino acid may be the limiting factor to the Maillard reaction (Truong et al., 2014), and other alterations at the level of metabolites and in membrane or cell wall properties may explain the greater NED in cvs. BRS Cuia and Beauregard stored at 6 ºC compared to those stored at 13 ºC (Wiberley-Bradford & Bethke, 2018).

In addition to the Maillard reaction, NED through a reaction independent of the RS which takes place through the thermal oxidation of polyunsaturated fatty acids from frying oil (Lim et al., 2014). The low darkening index detected in cv. Beauregard may be involved with β-carotene, the predominant pigment that gives the orange color to the pulp of roots of that genotype (Vizzoto et al., 2017). The antioxidant property of this pigment likely restricts the oxidation of unsaturated fatty acids from the oil used in frying, leading to less accumulation of dark compounds in the chips of cv. Beauregard (Pokorný and Sakurai, 2002; Zamora and Hidalgo, 2008).

In summary, this study shows that CIS in sweet potato differs between cultivars sensitive and tolerant to cold, especially in the predominantly accumulated soluble sugars and in the activity of sucrolytic enzymes. Unlike cultivar Beauregard, the roots of cultivars BRS Rubissol and BRS Cuia were cold-sensitive, exhibiting intense symptoms of chilling injury at 6 °C. The CIS in sensitive cultivars BRS Rubissol and BRS Cuia manifested at 6 °C by the accumulation of non-reducing sugars, instead of reducing sugars, and low invertases activity. In the tolerant cultivar Beauregard, the high invertase activity induced the accumulation of reducing sugars in a proportion practically equivalent to the non-reducing sugars content at both temperatures of 6 and 13 °C. In the three cultivars studied, the darkening of chips was more influenced by the non-reducing sugar content, instead of reducing sugars. Sucrose synthase was induced by cold stress only in cold sensitive cultivars, but did not contribute to CIS in these genotypes. It is suggested that cultivars of cold-sensitive sweet potato activate pathway catalyzed by SuSy as a likely mechanism of energy conservation to maintain the physiological processes of these genotypes under cold stress.

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Conflict of interest

The authors declare that they have no conflict of interest.

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ARTIGO 2

Balance between oxidative stress and antioxidant system in sweet potato roots stored at low temperatures

(Artigo nas normas da revista Scientia Horticulturae)

Abstract

The balance between antioxidant metabolism and oxidative stress was investigated in roots of cold-sensitive (BRS Rubissol and BRS Cuia) and cold-tolerant (Beauregard) sweet potato cultivars stored at 6 or 13 °C for up to 60 days. We hypothesized that the absence of chilling injury symptoms in cultivar Beauregard stored at 6 °C depends from the induction capacity of the enzymatic and non-enzymatic antioxidant system. The manifestation of chilling injury in cultivars BRS Rubissol and BRS Cuia was associated with loss of membrane integrity, increased lipid peroxidation, accumulation of hydrogen peroxide (H_2O_2) and low catalase (CAT), ascorbate peroxidase (APX) activities. In these cold-sensitive cultivars, proline and total phenolics increased with the progression of chilling injury, acting as markers of cold sensitivity, along with increased activity of phenylalanine ammonia-lyase. In contrast, in the cultivar Beauregard, the increase in CAT and APX activity and carotenoid accumulation in storage at 6 °C contributed to the reduction of H2O2 content and the absence of chilling injury symptoms. Our results suggest that the balance between oxidative stress and antioxidant system is involved in the tolerance cultivar of Beauregard roots to low storage temperatures.

Keywords: *Ipomoea batatas*; antioxidant enzymes; proline; phenolic; phenylalanine ammonia-lyase.

Abbreviations:

CI: chilling injury; CII: chilling injury index; ROS: reactive oxygen species; MDA: malondialdehyde; CAT: catalase; APX: ascorbate peroxidase; POX: peroxidases; PPO: polyphenoloxidase; EDTA: ethylenediamine tetraacetic acid PVP: polyvinylpyrrolidone; PMSF: phenylmethylsufonic fluoride.

1 Introduction

The perishable nature of sweet potato roots requires the use of low temperatures (13-16 °C) for prolonged storage to delay sprouting, reduce weight loss and maintain postharvest shelf life for up to 12 months (Woolfe, 1992). However, exposure to suboptimal temperatures trigger the onset of the chilling injury (CI), considered the main limitation to maintaining sweet potato root quality under cold storage (Ji et al., 2017). The general symptoms of CI are shared among different tropical and subtropical species. In sweet potato roots this physiological disorder manifests itself mainly by discoloration of the periderm (Porter et al., 1976) and darkened depressions on the surface of the product (Li et al., 2018).

A number of biochemical and molecular approaches have been devoted to the causes of CI in sweet potato roots (Yamaki & Uritani, 1974; Ji et al., 2017; Li et al., 2018). The primary causes of CI involve changes in lipid conformation, resulting in reduced fluidity and loss of membrane semi-permeability (Shichi & Uritani, 1956). These changes trigger a cascade of secondary events, resulting in the loss of cellular homeostasis and ultimately the development of characteristic and measurable symptoms of CI (Ji et al., 2017). Overproduction of reactive oxygen species (ROS) and oxidative stress are the main secondary events of CI. Excessive low temperature-induced ROS production has been reported to be highly toxic to sweet potato cellular functions, triggering increased electrolyte leakage and synthesis of lipid peroxidation products such as malondialdehyde (MDA) (Li et al., 2018; Ji et al., 2017). These events are indicative of membrane integrity loss and are responsible for irreversible root deterioration, making them more susceptible to fungal decay (Li et al., 2018; Shichi & Uritani, 1956).

The production of ROS, such as superoxide anion (O_2^-), hydroxyl radical (OH^-) and hydrogen peroxide (H_2O_2), occur naturally and represent a normal state of plant metabolism when produced at low concentrations (Vighi et al., 2017). Under sub-critical temperatures, the ability of the plant to maintain a balance between ROS production and elimination may be crucial to prevent metabolic dysfunction and the onset of CI (Valenzuela et al., 2017). Induction of the antioxidant system has been widely reported as an important mechanism in controlling low temperature ROS levels (Shi et al., 2018). The antioxidant system includes enzymatic components, such as superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), ascorbate peroxidase (APX; EC 1.11.1.11) and peroxidases (POX), and non-enzymatic components wich are ascorbic acid, carotenoids, phenolic compounds, anthocyanins and proline synthesis (Singh et al., 2016). In potato tuber, cooperation between CAT and APX is important to reduce the detrimental effects of low storage temperatures (Mizuno et al., 1998). In rice plants (Vighi et al., 2017) and grapefruit fruits (Lado et al., 2015), the induction of the enzymatic and non-enzymatic antioxidant system reduced the ROS pool and triggered tolerance to cold stress. In eggplant, increased CAT, POX activity and phenolic content allowed the fruit to be stored at 4 °C for 9 days without manifestation of CI symptoms (Shi et al., 2018).

Metabolic adjustments in response to cold stress depend not only on plant species, but also strongly associated with the genotype (Shen et al., 1999). In our previous investigation, we observed that, unlike the Brazilian cultivars BRS Rubissol and BRS Cuia, the roots of the American cultivar Beauregard were cold tolerant, showing no symptoms of CI on storage at 6 °C. How the causes of the difference in cold sensitivity between these genotypes are unknown, we hypothesize that the differential response between these commercial cultivars is the ability to induce enzymatic and non-enzymatic antioxidant components at low temperatures. In this paper, we seek to provide evidence, using univariate and multivariate techniques that the antioxidant system is involved in the tolerance to cold in sweet potato roots.

2 Material and Methods

2.1 Plant material and storage conditions

The experiment was conducted using two Brazilian cultivars (BRS Rubissol and BRS Cuia) and one American (Beauregard). The three genotypes were cultivated in an experimental field of the Federal University of Viçosa, Viçosa, MG, Brazil l (20° 45’ 20’’S and 42° 52’ 40’’ W, 651 m altitude), following standard agronomic practices. The roots were harvested manually, cured for seven days at 30 °C and 90% relative humidity (RH), and stored in equal batches to 6 or 13 °C for up to 60 days at 92% RH. Root pulp samples were collected at 0, 20, 40 and 60 days of storage for all biochemical variables analyzed.

2.2 Chilling injury index (CII)

The CII was evaluated according to Vega-García et al. (2010), with some modifications. The criteria used to evaluate the symptoms were: SP = surface pitting and FD= fungal decay. The severity of each criterion was visually assessed as lesion level (L) using a five-point scale based on the percentage of affected tissue: 0 = 0%; 1 = ≤ 10%; 2 = 11- 30%; 3 = 31 - 60%; 4 >

60%). To assess the severity of CI, the CII was obtained from the equation:

$$\mathrm{CII} = \frac{\mathrm{LSP} + \mathrm{LFD}}{2}$$

2.3 Physiological manifestation of CI

Hydrogen peroxide (H_2O_2) quantification was done according to Velikova et al. (2000). Approximately 1 g of pulp was ground in 2 mL of 0.1% (w/v) trichloroacetic acid and centrifuged at 14000 g for 15 min. The supernatant and reaction medium consisting of 10 mM potassium phosphate buffer (pH 7.0) and 1M potassium iodide were incubated for 45 min at room temperature. The readings were taken at 390 ηm and the H_2O_2 content was calculated based on a standard curve, the values being expressed as μmol g^{-1} of fresh matter (FM).

Lipid peroxidation was measured by quantifying the malondialdehyde content (MDA), according to Heath and Packer (1968). MDA content was calculated using extinction coefficient of 155 mM^{-1} cm^{-1}, and values are expressed in nmol g^{-1} FM.

Electrolyte leakage was determined according to Lima et al. (2002), with some modifications. Five cylinders (3 mm in diameter and 1 mm in thickness) of the pulp were removed with cork borer from the equatorial region of the roots. The cylinders were previously washed and incubated in 20 ml of deionized water at room temperature. After 6 h, the electrical conductivity measurement of the solution (L_1) was made using an electrical conductivity meter. In addition, the solutions were incubated for 2 h at 90 °C and subsequently cooled to room temperature for the second reading of electrical conductivity (L_2). Electrolyte leakage was calculated by the ratio of L_1 to L_2, and the results were expressed as %.

2.4 2.4 Enzymes of the antioxidant system

The enzyme extract was prepared by macerating and homogenizing 0.2 g of pulp in 2 mL of extraction buffer composed of potassium phosphate buffer (100 mM and pH 7.0), EDTA (0.1 mM), phenylmethylsufonic fluoride (PMSF; 1 mM) and 1% (w / v) polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 14000 *g* for 15 min at 4 °C and the supernatant was used to determine catalase activity (CAT), ascorbate peroxidase (APX) and peroxidases (POX).

CAT activity was determined as described by Havir & McHale (1987), with some modifications. The reaction medium consisted of potassium phosphate buffer (50 mM and pH

7.0), H_2O_2 (12.5 mM) and enzymatic extract. Activity was monitored by decreasing absorbance at 240 ηm for 1 minute and expressed as ηmol min^{-1} mg $protein^{-1}$ considering the extinction coefficient of 36 mM^{-1} cm^{-1}. APX activity was measured according to Nakano & Asada (1981), with some modifications. The reaction medium was composed of potassium phosphate buffer (50 mM and pH 7.8), ascorbic acid (0.25 mM) and H_2O_2 (0.3 mM). The activity of enzyme was monitored by decreasing absorbance at 290 ηm for 1 min and expressed as ηmol min^{-1} mg^{-1} protein, using the molar extinction coefficient of the 2.8 mM^{-1} cm^{-1}. POX activity was quantified according to the method of Kar & Mishra (1976). The reaction medium consisted of potassium phosphate buffer (25 mM and pH 6.5), guaiacol (20 mM) and H_2O_2 (20 mM). Activity was determined by the tetraguaicol production rate at 470 ηm and was expressed in ηmol min^{-1} mg^{-1} protein,, using the molar extinction coefficient of 26,6 mM^{-1} cm^{-1}.

2.5 Polyphenoloxidase (PPO)

PPO activity was based as described by Benjamin & Montgomery (1973), with some changes. Approximately 0.2 g of pulp was homogenized in extraction solution composed of potassium phosphate buffer (0.1 M and pH 6.5) and PMSF (1 mM). The homogenate was centrifuged at 14000 *g* for 15 min at 4 °C. The reaction medium consisted of enzymatic extract, potassium phosphate buffer (0.1 M and pH 5.0) and pyrocatechol (120 mM). The readings were taken for 3 min in spectrophotometer using the wavelength of 420 ηm. Activity was expressed as ηmol min^{-1} mg^{-1} protein using molar extinction coefficient 3,450 M^{-1} cm^{-1}.

2.6 Proline and carotenoids

Proline content was determined according to the method described by Bates (1973), with modifications. Extraction was performed by homogenization of 0.1 g of pulp in 2 mL of sulfosalicylic acid (3%; m / v). The homogenate was centrifuged at 14000 *g* for 15 min at 4 °C and 1 mL of the supernatant was incubated for 1 h at 100 °C in the reaction medium, consisting of 2 mL of acidic ninhhydrin (2.5%; m / v) and 2 mL of glacial acetic acid. The reaction was paralyzed by cooling in an ice bath and the reading was done by spectrophotometer at 520 ηm. Proline content was expressed as mmol g^{-1} FM from a standard curve.

Total carotenoid content was quantified by homogenization of 1.5 g of pulp in 20 mL of cooled 80% acetone. After incubation for 12 hours at 4 °C, the extract was filtered and read on a spectrophotometer at 470, 646 and 633,2 ηm. Carotenoid content was expressed in mg 100

g^{-1} FM using the equations proposed by Wellburn (1994).

2.7 Total phenolics and phenylalanine ammonia-lyase activity (PAL)

Total soluble phenolics were extracted from 5 g of pulp homogenized in 80% ethanol. The total phenolic content was determined according to the method described by Fu et al. (2010), using gallic acid as a standard. Absorbance was measured by spectrophotometer at 760 ηm and content was expressed in mg 100 g^{-1} FM.

PAL activity was performed according to the method described by Campos et al. (2004), with some modifications. Approximately 0.2 g of pulp was homogenized in extraction solution containing borate buffer (50 mM and pH 8.5), 2-mercaptoethanol (10 mM) and PVP (1%; m/v). The homogenate was centrifuged at 14000 *g* for 15 min at 4 °C. The reaction medium containing potassium phosphate buffer (100 mM and pH 8.5), phenylalanine (100 mM), and the enzyme extract were incubated at 40 °C for 40 min. The reaction was stopped with HCl (5 N) and readings were taken by spectrophotometer at 290 ηm using trans-cinnamic acid as standard. Enzyme activity was expressed as ηmol min^{-1} mg^{-1} protein.

2.8 Protein

Protein contents of enzyme extracts were determined according to the method described by Bradford (1976), using bovine serum albumin as standard.

2.9 Experimental design and statistical analysis

The experiment was conducted in a completely randomized design in split-plot. The plots were constituted by the temperatures and genotypes, whereas the subplot by the storage periods. The analysis of variance was performed and the mean temperatures within each genotype-time combination were compared by the F test ($P \leq 0.05$) using the R software version 3.4.3 (R Core Team, 2017). The Principal Components Analysis (PCA) and the Hierarchical Cluster Analysis (HCA) were used to determine the differences between genotypes and to understand the relationship between biochemical and physiological parameters that contribute to contrasting behavior in relation to the cold sensitivity level. Both unsupervised techniques were applied to the centralized and standardized values using R package FactoMineR (Lê, Josse and Husson, 2008). From the Mahalanobis distance matrix, the dendogram was constructed using the Ward.D2 algorithm (Ward, 1963) of the hclust function. The ideal number of clusters was determined by the modal value of the 30 index results provided by the NbClust package (Charrad et al., 2014).

3 Results

3.1 Physiological manifestation of chilling

The physiological manifestation of CI in sweet potato roots of cultivars BRS Rubissol, BRS Cuia and Beauregard were evaluated at 6 °C, while 13 °C was used as a control treatment. The roots of the cv Beauregard not show CI symptoms during storage at 6 °C (Figure 1C). In contrast, the root quality of cv. BRS Rubissol and BRS Cuia suffered rapid deterioration due to intense symptoms of CI during storage at 6 °C. At 20 days at 6 °C, slight darkened depressions were visible in cvs. BRS Rubissol and BRS Cuia. From 40 days onwards, the increase of CII in BRS Rubissol (Figure 1A) was intensified by fungal infection, while the increase of CII in BRS Cuia (Figure 1B) was mainly due to widespread browning and deep bark depressions.

The physiological damage of CI was based on H_2O_2 accumulation, electrolyte leakage and MDA content. The roots of the cultivar BRS Rubissol exhibited significant accumulation of H_2O_2 when stored at 6 °C, with values 6.6 times higher than determined at 13 °C after 60 days (Figure 1D). In cv. BRS Cuia, this increase was less pronounced, exceeding that achieved at 13 °C by 1.7 times (Figure 1E). In these cold-sensitive cultivars, the tendency of H_2O_2 accumulation was accompanied by increased MDA content and electrolyte leakage during storage at 6 °C. For the cv. Beauregard the content of H_2O_2 did not differ ($P > 0.05$) between temperatures of 6 and 13 °C (Figure 1F), but MDA content (Figure 1I) and electrolyte leakage (Figure 1L) at 6 °C were higher than the values obtained at 13 °C.

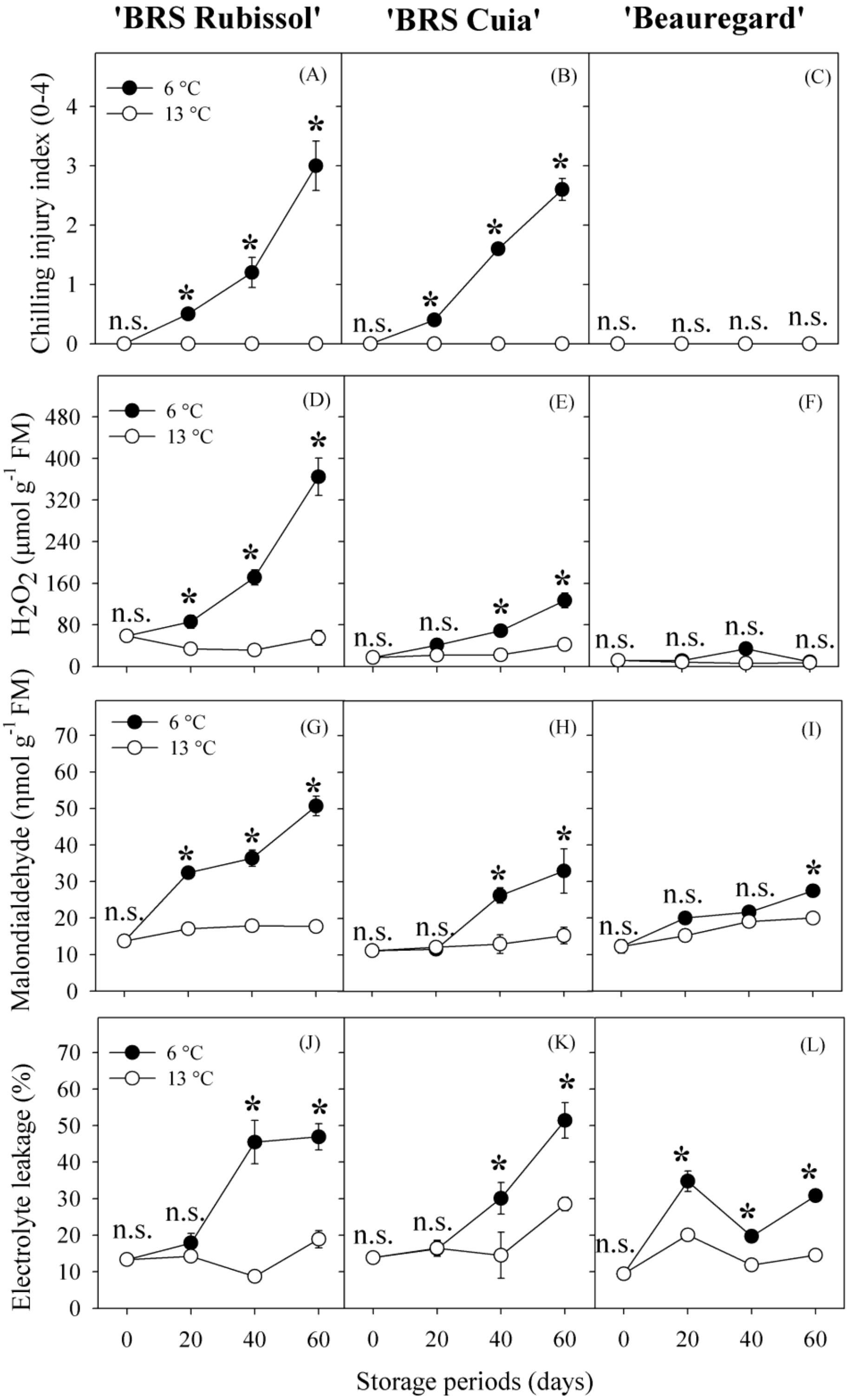

Figure 1. Chilling injury index (A, B, C), H_2O_2 (D, E, F), malondialdehyde (G, H, I) and electrolyte leakage (J, K, L) in roots of sweet potato cultivars BRS Rubissol, BRS Cuia and Beauregard stored for up to 60 days at 6 or 13 °C. Data represent the mean ± standard error (n = 5). $^{n.s.}$ and * indicate non-significant ($P > 0.05$) and significant ($P \leq 0.05$) difference by the F test.

3.2 Antioxidant system enzymes: catalase (CAT), ascrobate peroxidase (APX) and peroxidases (POX)

At the beginning of storage, cv. Beauregard exhibited high initial CAT, APX and POX activities compared to sensitive cultivars (Figure 2). The activity of antioxidant enzymes in response to low storage temperatures varied between cold-sensitive and cold-tolerant genotypes (Figure 2). CAT activity in cv. RS Rubissol (Figure 2A) and BRS Cuia (Figure 2B) was practically unaffected by storage temperatures. In contrast, CAT activity in the cold-tolerant cv. Beauregard remained higher throughout the whole storage at 6 °C compared to 13 °C, except at 40 days, where enzyme activity declined to a statistically lower value than 13 °C (Figure 2C).

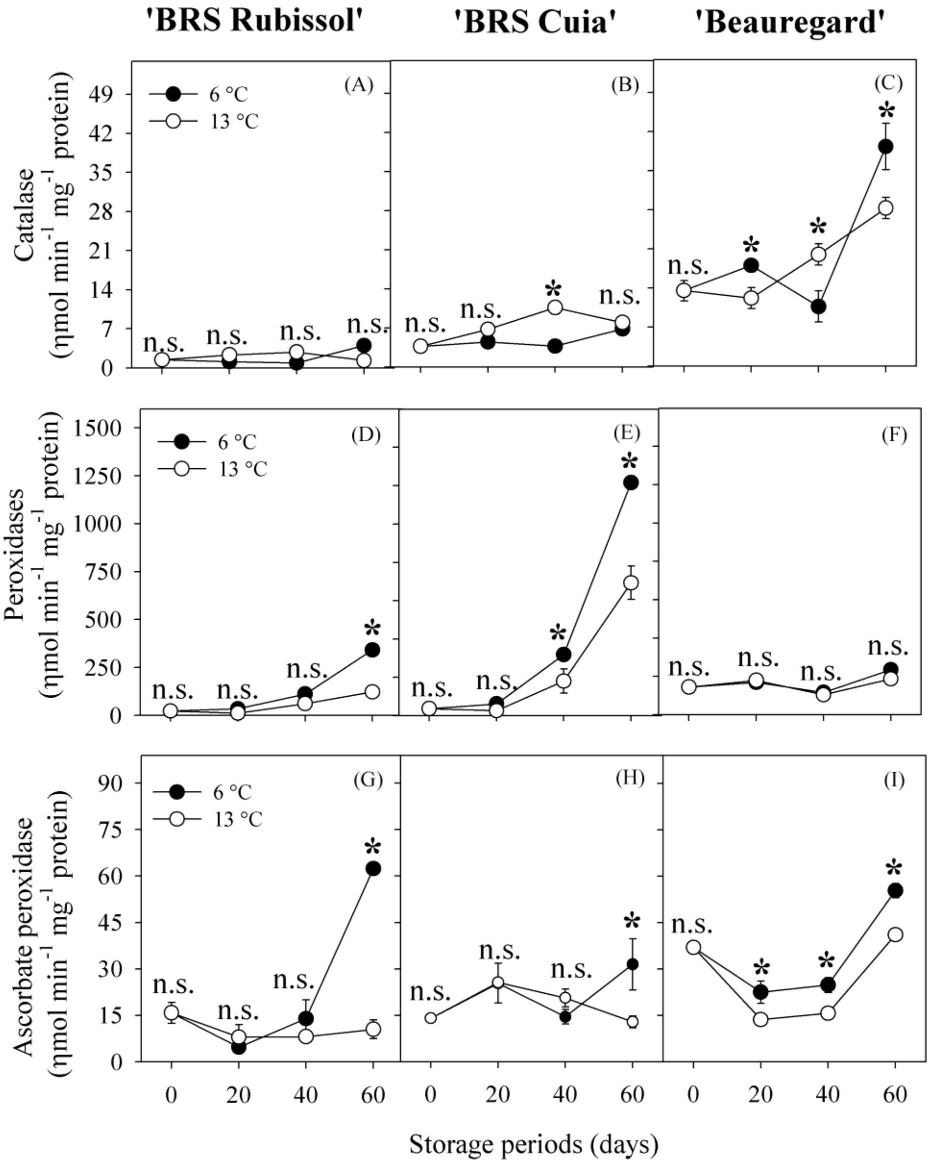

Figure 2. Catalase (A, B, C), peroxidases (D, E, F) and ascorbate peroxidase (G, H, I) activity

in roots of sweet potato cultivars BRS Rubissol, BRS Cuia and Beauregard stored for up to 60 days at 6 or 13 °C. Data represent mean ± standard error (n = 4). $^{n.s.}$ and * indicate non-significant ($P > 0.05$) and significant ($P \leq 0.05$) difference by the F test.

In cold-sensitive cultivars, the increase in POX activity in response to a temperature of 6 °C was late, occurring only at 60 days of storage for the BRS Rubissol (Figure 2D) and from 40 days for the BRS Cuia (Figure 2E). On the other hand, storage temperatures did not influence POX activity in cultivar Beauregard (Figure 2F).

Similar to CAT, APX activity in cultivars BRS Rubissol and BRS Cuia exhibited a late response to the cold induced stress. In these cold-sensitive genotypes, only at 60 days, storage at 6 °C induced higher APX activity than 13 °C (Figure 2G and 2H). In contrast, cv. Beauregard maintained higher APX activity throughout storage at 6 °C compared to 13 °C (Figure 2I).

3.3 Proline, total phenolics and carotenoids content

The non-enzymatic antioxidant system was evaluated for proline, total soluble phenolics and carotenoid content. Overall, compared to storage at 13 °C, roots stored at 6 °C showed increased proline and phenolic content in the roots of the all three cultivars (Figure 3). The proline content in cvs. BRS Rubissol and BRS Cuia at 6 °C exceeded by 4.5 (Figure 3A) and 2.7 times (Figure 3A), respectively, the content determined in roots stored at 13 °C. During storage at 6 °C, proline content in cv. Beauregard increased up to 40 days, reaching value 4.4 times higher than storage at 13 °C (Figure 3C). In addition, proline content reduced at 60 days at 6 °C to a statistically similar value to 13 °C (Figure 3C).

Compared to storage at 13 °C, the total phenolic content in the three cultivars studied was significantly higher at 6 °C (Figure 3). However, the magnitude of phenolic accumulation in storage at 6 °C was different between genotypes. In roots of cvs. BRS Rubissol and BRS Cuia stored at 6 °C, the phenolic content exceeded up to 3.5 (Figure 3D) and 1.6 (Figure 3E) times that observed at 13 °C. In contrast, phenolic accumulation in BRS Beauregard at 6 °C was modest (Figure 3F).

Among the cultivars studied, carotenoids were detected only in the pulp of the cv.Beauregard. Carotenoid content increased from 94.8 to 110.4 mg 100 g^{-1} FM during the first 20 days of storage at 6 °C, remaining virtually stable until 40 days (Figure 3I). In this period, the carotenoid content at 6 °C significantly exceeded up to 1.3 times that obtained at 13 °C. However, at 60 days, this content declined, reaching a value similar to that measured at 13 °C

(Figure 3I).

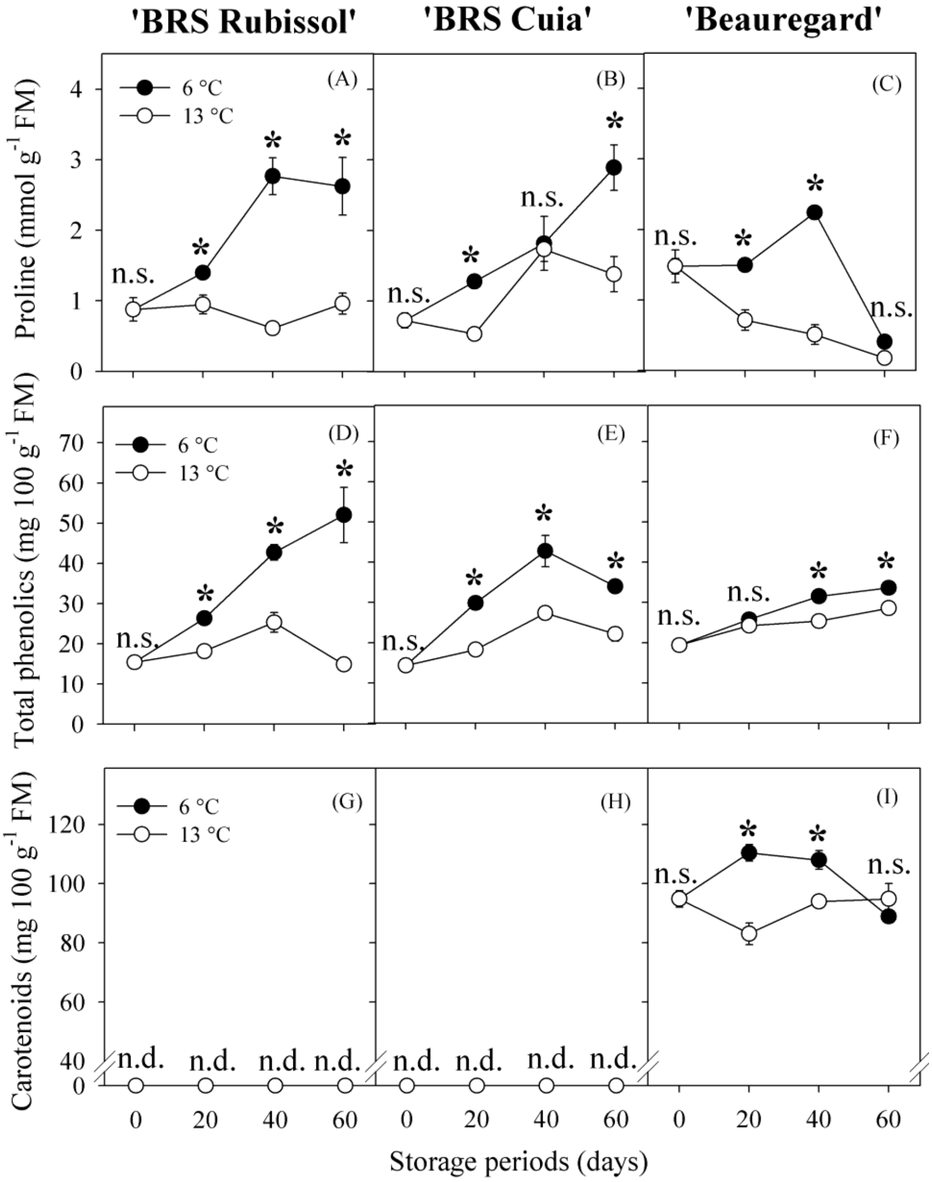

Figure 3. Content of proline (A, B, C), total phenolics (D, E, F) and carotenoids (G, H,I) in roots of sweet potato cultivars BRS Rubissol, BRS Cuia and Beauregard stored for up to 60 days at 6 or 13 °C. Data represent the mean ± standard error (n = 5). n.s. and * indicate non-significant ($P > 0.05$) and significant ($P \leq 0.05$) difference by F test. n.d: not detected.

3.4 Phenylalanine ammonia-lyase (PAL) and polyphenoloxidase (PPO) activity

PAL activity remained constant during 60 days of storage at 13 °C, regardless of the cultivar evaluated (Figure 4). For the cvs. BRS Rubissol and BRS Cuia, PAL activity was strongly induced by storage at 6 °C between 20 and 60 days of storage (Figure 4A and 4B). PAL activity in the cold-sensitive cultivars stored at 6 °C exceeded up to 3.6- (Figure 4A) and 2.5-fold (Figure 4A) respectively, the activity measured at storage at 13 °C. In contrast, the activity of PAL in tolerant cv. Beauregard did not change during storage at 6 °C (Figure 4C).

Storage at 6 °C accentuated PPO activity in cold-sensitive and cold-tolerant sweet potato cultivars, compared to 13 °C. PPO activity in cv. BRS Rubissol stored for 60 days at 6 °C was 5.1 times higher than at 13 °C (Figure 4D). The highest PPO activity was observed in the roots of cv. BRS Cuia stored at 6 °C (Figure 4E), corroborating the widespread darkening of the root bark of this genotype (data not shown). In contrast to cold-sensitive cultivars, the difference between temperatures on PPO activity of the cultivar Beauregard was detected only at 60 days of storage (Figure 4F).

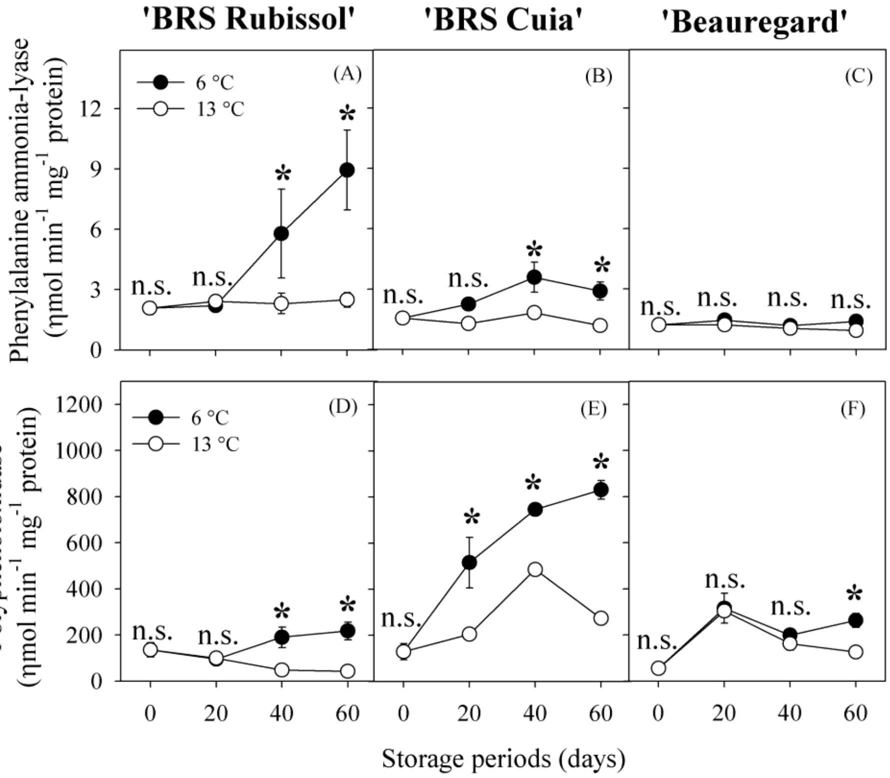

Figure 4. Phenylalanine ammonia-lyase (A, B, C) and polyphenoloxidase (D, E, F) activity in roots of sweet potato cultivars BRS Rubissol (RB), BRS Cuia (CA) and Beauregard (BG) stored for up to 60 days at 6 or 13 °C. Data represent mean ± standard error (n = 4). $^{\text{n.s.}}$ and * indicate non-significant ($P > 0.05$) and significant ($P \leq 0.05$) difference by the F test.

3.5 3.5 Multivariate analysis

HCA allowed the identification of three groups, ordering them according to the level of metabolic dysfunctions promoted by cold storage (Figure 5). Group I included the cvs. BRS Rubissol (RB) and BRS Cuia (CA) stored for 40 and 60 days at 6 °C, whose roots showed intense symptoms of CI. The group II grouped the cold-sensitive cultivars BRS Rubissol and BRS Cuia stored at 13 °C, without symptoms of CI, and at 6 °C with mild symptoms due to the short time of exposure to low temperature. Group III, in turn, was formed only by the cold-tolerant cv. Beauregard, whose roots did not exhibit symptoms of CI, regardless of temperature and storage period. The high value of the cophenetic coefficient of correlation (CCC= 0.81) observed here for the hierarchical cluster constructed denotes the reliability of this approach to summarize the information of dissimilarity matrices.

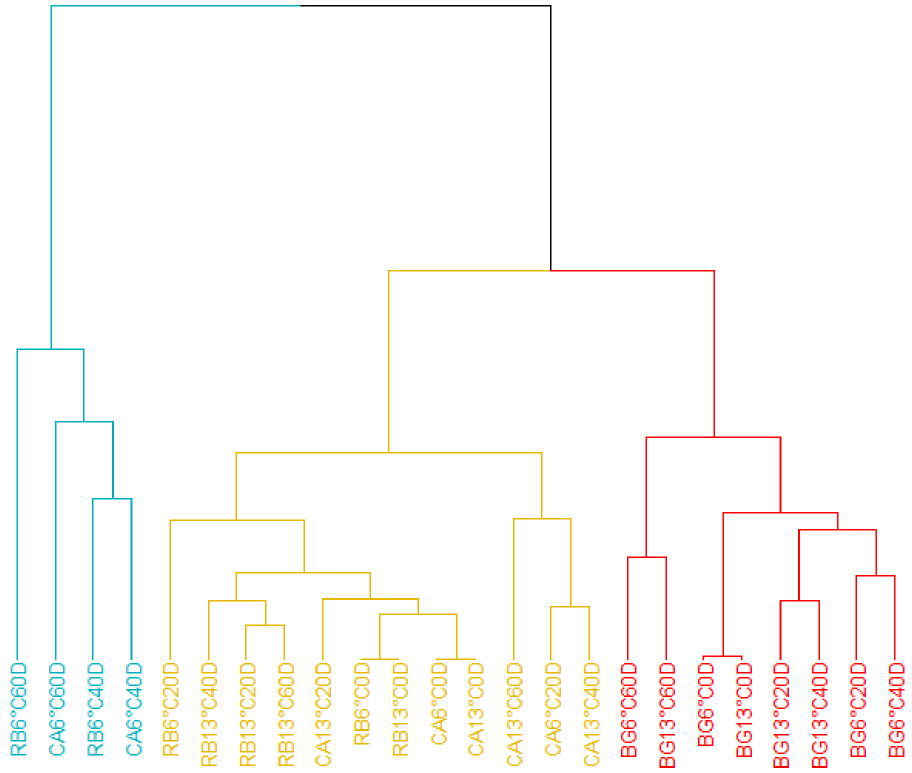

Figure 5. Hierarchical cluster analysis in roots of sweet potato cultivars BRS Rubissol (RB), BRS Cuia (CA) and Beauregard (BG) stored at 6 or 13 °C for 0, 20, 40 and 60 days (D). Cophenetic correlation coefficient equal to 0.81.

Similar to HCA, PCA also revealed a clear separation between cold-tolerant and cold-sensitive cultivar (Figure 6). The variation of the first principal component (PC_1) and the second principal component (PC_2) was 52.9 and 19.4 %, respectively. In total, the variation of 72.3%

explained by both PCs is sufficient to explain much of the variation in the original data without the need for continue the analysis of the other PCs (Wijewardana et al., 2015). PC_1 separates roots that exhibit severe symptoms of chilling injury (Group 1 in the HCA) from roots without symptoms and mild symptoms of CI (Group 2 in the HCA), while PC_2 separates cold-sensitive cultivars (Group 2 in the HCA) from cold-tolerant cultivar (Group 3 in the HCA) (Figure 6).

To better understand the association between variables and the level of CI tolerance, we also examined the loadings of biochemical and physiological parameters on the first two components. Highly weighted variables under PC_1 included CII, EL, H_2O_2, MDA, PAL and PN, whose sharp angle between their vectors suggests a positive correlation between these parameters. Under PC_2, CAT and CT were highly weighted variables. Since the angle between the CAT and CII vector is obtuse, these variables have a negative correlation between them.

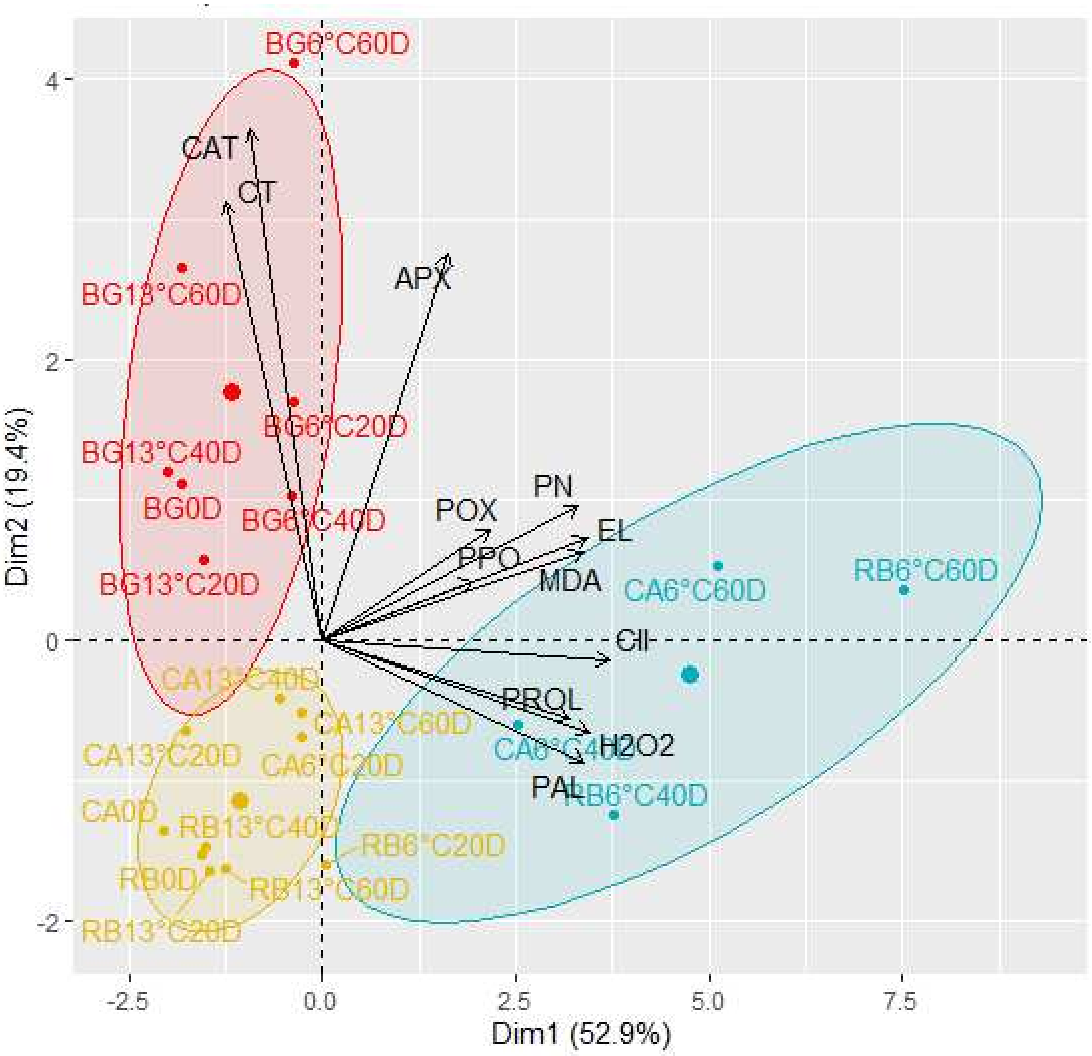

Figure 6. Biplot projection of chilling injury index (CII), hydrogen peroxide (H_2O_2),

malondialdehyde (MDA), electrolyte leakage (EL), catalase (CAT), ascorbate peroxidase (APX), peroxidases (POX), phenylalanine ammonia-lyase (PAL), polyphenoloxidase (PPO), total soluble phenolics (PN), carotenoids (CT), proline (PROL) in roots of sweet potato cultivars BRS Rubissol (RB), BRS Cuia (CA) and Beauregard (BG) stored at 6 or 13 °C for 0, 20, 40 and 60 days (D). Ellipses indicate a 95% confidence interval.

4 ***Discussion***

CI is one of the most important indicators of abiotic stress in fruits and vegetables stored at low temperatures. Our previous study showed that CI symptoms were intensely present in the roots of sweet potato cultivars BRS Rubissol and BRS Cuia stored up to 60 days at 6 °C, but absent in the roots of cultivar Beauregard (unpublished data). In the present study, univariate and multivariate analyzes (HCA and PCA) reinforce the difference in responses of these cultivars to CI inducing temperature, allowing to discriminate the tolerance level of these genotypes to cold and the main biochemical parameters related to the absence or presence of this physiological disorder on sweet potato roots.

Biochemical studies suggest that the irreversible symptoms of CI are secondary events associated with overproduction of superoxide anion, hydroxyl radical, hydrogen peroxide, among others. These ROS are responsible for lipid peroxidation of cell membranes, contributing to the loss of their integrity (Ji et al., 2017; Vighi et al., 2017). In the present study, there was a strong accumulation of H_2O_2 in cultivar BRS Rubissol (Figure 1D) and moderate in cultivar BRS Cuia stored at 6 °C (Figure 1E). In these cold-sensitive cultivars, H_2O_2 production, MDA accumulation and electrolyte leakage followed the same trend, indicating a close relationship between ROS, lipid peroxidation and loss of membrane integrity. In contrast, the contents of H_2O_2 and MDA in cv. Beauregard were virtually unaffected by storage temperatures. Lipid peroxidation measured by MDA content and electrolyte leakage are effective indicators of cellular oxidative stress (Li et al., 2018). Therefore, Beauregard's ability to maintain H_2O_2 content at baseline levels may be indicative of the tolerance of this genotype at low temperatures, delaying lipid peroxidation and electrolyte leakage. (Li et al., 2016).

H_2O_2 is one of the most important non-radical ROS produced under stress conditions (Ślesak et al., 2007) and its physiological role in vegetables includes a paradox: high concentration H_2O_2 causes oxidative damage to lipids, DNA and proteins, while at low concentrations it triggers intracellular signaling mechanisms (Černý et al., 2018). The high content of H_2O_2 found in the roots of the cultivars BRS Rubissol and BRS Cuia explain, at least in part, the intense symptoms of CI and the rapid deterioration of the product stored at 6 °C. This phenomenon was also evidenced by Li et al., (2018) in roots of cold-sensitive sweet potato cultivars stored at 4 °C for 28 days.

Control of cellular levels of ROS is maintained by enzymatic and non-enzymatic antioxidant mechanisms (Singh et al., 2016). There are reports in several species that variability in cold sensitivity depends on the efficient balance between ROS production and elimination (Vighi et al., 2017; Mutlu et al., 2016; Shen et al., 1999). In the present study, the inability of cv. BRS Rubissol and BRS Cuia to efficiently activate the enzymatic antioxidant system may explain the higher sensitivity of these genotypes to cold. In these cold-sensitive cultivars, CAT activity remained unchanged at both temperatures and the increase of APX and POX activity in storage at 6 °C was late, not being efficient to reduce H_2O_2 content to non harmful levels under these storage conditions. In contrast, the enzymatic antioxidant system in cv. Beauregard was cold responsive, evidenced by the higher activity of CAT and APX in storage at 6 °C than at 13 °C. This view obtained from univariate techniques corroborates the interpretation of multivariate techniques.

Our results show PCA and HCA techniques clearly separate the cold-tolerant cultivar from the cold-sensitive cultivars (Figure 5 and 6). In addition, PCA was able discriminate which variables that most contributed to the difference in cold sensitivity of the sweet potato cultivars, which was supported well by the observations made in corn hybrids by Wijewardana et al. (2015). Cluster I included the roots of cold-sensitive cultivars stored for 40 and 60 days at 6 °C. They had a higher score for variables related to the physical symptoms of IC (CII) as well as for variables related to oxidative stress, such as H_2O_2, MDA and EL, justifying their cold sensitivity. In contrast, cluster III, including the roots of cv. Beauregard only, regardless of temperature and storage period, had a low score for variables associated with oxidative stress but a high score for variables associated with the enzymatic (CAT and APX) and non-enzymatic antioxidant system (CT). This multivariate approach point to an ability of the cv. Beauregard to balance components of oxidative stress by inducing some components of the enzymatic and non-enzymatic antioxidant system.

The enzymes CAT and APX are able to eliminate H_2O_2, but through different mechanisms. APX, unlike CAT, requires an ascorbate and glutathione regeneration system to convert H_2O_2 to H_2O (Kaur et al., 2016). While the APX catalyzed reaction is more involved in regulating H_2O_2 as a signaling molecule, the CAT reaction is directly related to H_2O_2 detoxification (Sofo et al., 2015). In this study, the increased activity of CAT and APX was important to keep H_2O_2 at low concentrations and to ensure redox homeostasis in cv.

Beauregard. This phenomenon decreased oxidative stress and seems to have allowed storage of the cv. Beauregard at 6 °C without CI symptoms.

Few researches has focused on the relationship between CI manifestation and non-enzymatic antioxidant compounds content such as proline, phenolics and carotenoids. In addition to its role as osmolite, proline also plays an important role in tolerating different stress conditions by acting as an antioxidant molecule (Signorelli et al., 2015). In the present study, both cold-sensitive and cold-tolerant cultivars increased proline content in response to storage at 6 °C. However, at the end of storage, while proline content remained high in the cold-sensitive cultivars, proline levels decreased dramatically in cv. Beauregard. These results suggest that Beauregard has the greatest ability to adjust intracellular proline content, possibly preventing levels of this amino acid from reaching toxic concentrations (Hellmann et al., 2000; Nanjo et al., 2003). These results corroborates the results obtained in barley, where proline was not a promising parameter for differentiating cold-sensitive from cold-tolerant cultivars (Murelli et al., 1995). In contrast, proline content correlated with cold tolerance in Chinese sweet potato roots (Wang et al., 2019). Therefore, in view of these controversial results, further studies are needed to conclude on the role of proline at low temperatures.

Carotenoids, especially β-carotene, are lipophilic pigments responsible for the orange colour of the pulp of cv. Beauregard (Baafi et al., 2016), but are not present in BRS Rubissol (Figure 3G) and BRS Cuia (Figure 3H). Increasing the content of these pigments at 6 °C may have increased the antioxidant capacity of cv. Beauregard to extinguish ROS, preventing the incidence of CI symptoms in this genotype (Figure 4I). Previous studies on tomato (Whitaker, 1994) and grapefruit (Lado et al., 2015) fruits corroborate this view by reporting the protective role of carotenoids under suboptimal temperature conditions. In maize plants, the largest accumulation of carotenoid was associated with cold tolerance (Haldimann, 1995).

Phenolic compounds are secondary metabolites that under low temperatures may act as antioxidants, relieving symptoms of CI, and / or as substrate for enzymatic browning reactions. In our study, the higher accumulation of phenolics in cold-sensitive cultivars contradicts the function of these compounds as non-enzymatic antioxidants in sweet potato roots. Therefore, it is reasonable to consider that the high phenolic content acted as a biological marker of cold sensitivity in sweet potato roots, contrary to the results obtained by Wang et al. (2019) in sweet potato roots stored at 4 or 16 °C for up to 16 days. According to these authors, phenolic accumulation was associated with cold tolerance in sweet potato roots.

The phenolic content in plant tissues depends on the rate of its synthesis and degradation. The high phenolic levels in the cultivars BRS Rubissol and BRS Cuia at 6 °C resulted from increase in phenylalanine ammonia-lyase (PAL) activity, the key enzyme that diverts the central flux of carbon from primary metabolism to the synthesis of myriad phenolic compounds (Wang et al., 2019). In the present work, the high activity of PAL in sensitive genotypes was crucial for maintaining the phenolic group, which are used in enzymatic darkening reactions catalyzed, for example, by polyphenoloxidase (PPO).

PPO, like POX, is an important oxidizing enzyme that converts phenolics into *o*-quinone. Usually under optimal temperature conditions, phenolic compounds and PPO are spatially separated (Li et al., 2016). However, under sub-optimal temperatures, the loss of membrane integrity leads to the breakdown of cell membranes, favoring the oxidation of phenolic compounds by PPO (Wang et al, 2016). Therefore, the high PPO activity induced by the temperature of 6 °C in cvs. BRS Rubissol and, mainly, in BRS Cuia explains the darkening of the peel under these storage conditions. In contrast, PPO activity in cv. Beauregard was practically unaffected by storage temperature, which corroborates the absence of darkening on the root surface maintained at 6 °C.

In summary, this study shows that, unlike cultivars BRS Rubissol and BRS Cuia, the cultivar Beauregard activates enzymatic antioxidant system components and increases carotenoid content in low temperature conditions. Increased catalase and ascorbate peroxidase activity and the accumulation of carotenoids correlated with H_2O_2 reduction in cultivar Beauregard. These events decreased lipid peroxidation and conferred cold tolerance to Beauregard roots stored at 6 °C, without the manifestation of symptoms of chilling injury. The incidence of chilling injury in the cultivars BRS Rubissol and BRS Cuia at 6 °C was due to the inability of these genotypes to balance the mechanisms of production and elimination of H_2O_2. In these cold-sensitive cultivars, the high proline and total phenolic contents as well as the high activity of phenylalanine ammonia-lyase in storage at 6 °C acted as biochemical markers of cold sensitivity.

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GENERAL CONCLUSIONS

Differences in chilling sensitivity among roots of sweet potato cultivars stored at low temperatures were shown in this study. The American cultivar Beauregard was considered chilling-tolerant, while the Brazilian cultivars BRS Rubissol and BRS Cuia were considered chilling-sensitive.

The cold-induced sweetening (CIS) was observed in the roots of the three studied cultivars, with a predominance of non-reducing sugars and low invertase activity in the cultivars BRS Rubissol and BRS Cuia. In cultivar Beauregard, CIS was observed by the high activity of invertase and accumulation in a relatively equivalent proportion of reducing and non-reducing sugars. Sucrose synthase activity was induced by storage at 6 °C only in cold-sensitive cultivars, acting as a possible energy conservation mechanism under low-temperature stress conditions.

The symptoms of chilling injury were observed only in the roots of the cultivars BRS Rubissol and BRS Cuia resulted from the imbalance between oxidative stress and antioxidant metabolism. Beauregard roots stored at 6 °C showed no symptoms of chilling injury by reducing oxidative stress through the induction of enzymatic and non-enzymatic antioxidant components.