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Effect of cold storage on enzyme activity and postharvest conservation of tannia (*Xanthosoma sagittifolium*) leaves

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Abstract

This study aimed to evaluate the cold tolerance of two contrasting genotypes of tannia leaves, an edible and an ornamental variety, on oxidative enzymes, accumulation of phenolic compounds and postharvest metabolism of carbohydrates. To simulate shipping storage conditions, fresh leaves were stored at 5 and 10 ºC, relative humidity of 68 ± 5%, wrapped with non-perforated low density polyethylene bag in cardboard boxes and stored for 20 days. Samples were taken at 0, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 days of storage, visually evaluated and then analyzed for the activity of peroxidase (POD), polyphenoloxidase (PPO) and catalase (CAT), concentration of soluble phenolic compounds, content of total soluble sugar, reducing sugar and starch. The storage for 20 days at temperatures of 5 or 10 ºC did not induce any degradation of starch in the leaves. The cold storage did not stimulate peroxidase activity in either genotype. The increase in catalase activity occurred after 14 days storage of leaves cv. Comum and from the 6th day in genotype BGH/UFV 5932 at 10 ºC. Cold temperature did not induce changes on polyphenoloxidase activity. The results indicate that during cold storage, the genotypes showed no symptoms of chilling, without any visual sign of browning caused by the low temperature, nevertheless shorter conservation of leaves occurred at storage of 5 ºC, indicating some chilling injury sensitivity. Further studies are underway to establish the effect of temperature on the cell membrane metabolism.

Keywords: Carbohydrates; Catalase; Cooling; Peroxidase; Polyphenoloxidase.
Abbreviations: ANOVA_analysis of variance, AU_absorbance units, BSA bovine serum albumin, CAT_catalase, H_2O_2_hydrogen peroxide, POD_peroxidase, PPO_polyphenoloxidase, PVP_polyvynil-pyrrolidone, RS_reducing sugars, TSS_total soluble sugars.

Introduction

Tannia (*Xanthosoma sagittifolium* L. Schott) is a tropical herbaceous perennial plant used as source of leaves and corms as staple food in developing countries (Mangan et al., 2008). Usually, the plant is cultivated by small farmers in tropical regions, mainly for production of leaves for consumption in regional dishes and the corms for human consumption and animal feeding. Enormous interest in this culture has been done due to the nutritional component (Pinto et al., 2001a), since the leaves are a major source of protein, fiber and vitamin C (Pinto et al., 2001b; Morais et al., 2006). Furthermore, tannia is an inexpensive vegetable, easy to propagate by plantlets, with high production of leaves, which has enormous market in the USA among the immigrant Brazilian population (Mangan et al., 2010). Nevertheless, little is known about its postharvest behavior during long term storage. As the majority of leaves, of tannia has low shelf-life after harvest, subjected to intense dehydration, senescence and as tropical species, sensitive to chilling injury. According to Seganfredo et al. (2001), after a few hours of harvest, the leaves develops visual changes, including yellowing and wilting. These attributes are determined, by the genotype, stage of development at harvest, storage temperature, and by the composition of the air and by the length of storage (Seganfredo et al., 2001). Leafy vegetables present elevated water loss, because of their large specific surface area. Loss of water through transpiration directly affects the appearance and the weight of the product, enhancing the progress of senescence (Álvares et al., 2010).

Tropical and subtropical vegetables, as tannia plants are chilling sensitive when stored at temperatures between 5 and 15 ºC. Plants susceptible to low temperature, present changes on lipid phase and alterations on proteins pattern (Lyons and Raison, 1970). However, the development rate of chilling symptoms is function of the storage temperature, time exposure and the susceptibility of the species or cultivar (Wang, 1982). A common response to chilling injury is the increase of oxidative reactions of phenolic substances, by the induction of peroxidase and polyphenoloxidase activities (Guida et al., 2011; Koksal, 2011; Tayefi-Narabadi et al., 2011). Under chilling inducing temperatures, genes unrelated to the plant defense have decreased expression, while those involved in healing the damage are increased (Kombrink and Hahlbrock, 1990). Among the genes that have increased expression are the enzymes polyphenoloxidase, peroxidase and catalase (Okey et al., 1997; Messias et al., 2006). The aims of this study were to evaluate the postharvest response of two accessions of tannia leaves to chilling inducing temperature during simulated shipping and storage conditions.

Results and Discussion

Except for the content of total soluble sugars, temperature and length of storage influenced the activity of enzymes, the concentration of soluble phenolic compounds and the carbohydrate metabolism in both genotypes. Significant triple interaction between temperatures, days of storage and cultivar was observed for the content of reducing sugars ($p \leq 0.01$), catalase ($p \leq 0.01$) and polyphenoloxidase ($p \leq 0.05$) activities.

Carbohydrate metabolism

Leafy vegetables do not store significant amount of reserve carbohydrates and the lack of organic substrates may reduce the storage potential (Finger and Vieira, 2007). Carbohydrates levels declined when the time of storage was increased in both temperatures. Variations on the total soluble sugars (Fig 1) and reducing sugars (Fig 2) contents were explained by the linear regression model, with the exception of cv. Comum stored at 5 ºC, which showed quadratic behavior, having a minimum estimated content between 12 and 14 days of storage (Fig 2). A higher percentage of total soluble sugar was detected in the leaves suitable for consumption (Fig 1). But the ornamental tannia leaves had about twice more reducing sugars in comparison to the edible cv. Comum (Fig 2). Regardless the temperature of storage, the consumption of sugars during the evaluation period was not significant (Table 1). Seganfredo et al. (2001) working with the same species determined the occurrence of sugar breakdown in leaves harvested at 5, 8 and 15 days after full expansion of the leaf blade and stored at room temperature. Glucose and fructose seem to be immediately utilized as substrates for respiration and were consumed during the storage (Fig 2). Similar trend was found in a study conducted done by Simões et al. (2010) storing cabbage at different temperatures. Percentage of starch present in the genotype BGH/UFV 5932 was equivalent to the cv. Comum, and with the storage significant decline started at 5 and 10 ºC up to the 20th day of storage (Table 1). Similar results were mentioned by Simões et al. (2010) in study with cabbage stored at different temperatures. Regardless the temperature of storage, the edible leaves had higher quantity of starch than the ornamental leaves (Fig 3). The length of storage did not affect the starch content on cv. Comum, stored at 10 ºC (Fig 3). But at 5^{o}C, the leaves of this cultivar showed significant decrease in the percentage of starch during storage, with no significant increase at the end of treatment, compared to control (Table 1). Potato tubers stored at temperatures below 10 ºC are susceptible to the process of sweetening, by increasing the degradation of starch and sucrose accumulation, resulting in increased levels of glucose and fructose (Blenkinsop et al., 2003). Ribeiro et al. (2007) had induced the accumulation of soluble sugars and intense degradation of starch in roots of Yellow Peruvian root stored at 5 and 10 ºC. In this study, despite the low temperatures influence the starch, the changes in the composition of the sugars was not significant.

Activity of enzymes

POD specific activity was fivefold greater than the CAT (Fig 4 and 5). Cultivar Comum, stored at 5 ºC showed increased POD activity up to the 12th day, followed by decrease. No effect on the length of storage on POD activity of BGH/UFV 5932 either at 5 or 10 ºC, whereas for leaves kept at 10 ºC showed twofold higher activity compared to 5 ºC (Fig 4). El-Hilali et al. (2003) analyzing fruit of mandarin 'Fortune' detected continuous increase of POD activity during the storage at 4 ºC. However, at 8 ºC the POD activity increased during the first two weeks, followed by a sharp decline until the end of storage. Chen et al. (2010) also detected a fluctuation POD activity in asparagus lettuce during storage. Menolli et al. (2011) found symptoms of internal and external injury in Yellow Peruvian roots when stored at 5 ºC, which was associated to higher activity of POD induced by the chilling temperature. POD activity did not change from day 0 up to 20 days of storage, in both genotypes, except for the leaves of cv. BGH/UFV 5932 stored at 10 ºC, which showed a peak of activity at about 2.51 fold higher at 12 days of storage (Table 2). The lower activity of CAT for the edible cultivar, stored either at 5 or 10 ºC (Fig 5) seems to be related to the higher POD activity present in the leaves (Fig 4), because both enzymes have hydrogen peroxide as common substrate. Studies with ascorbate peroxidase enzyme, in potato tubers, indicated that in response to storage at low temperatures, POD reduces H_2O_2 to water, due to its higher affinity to hydrogen peroxide compared to CAT (Kawakami et al., 2002). In a similar way this fact also explains the lower CAT activity in cv. Comum compared to the ornamental leaves (Fig 5). Changes on CAT activity at 5 ºC were similar to the activity of leaves stored at 10^{o}C. But at 10 ºC a significant increase in the activity, of 180% occurred in the last days of storage for the edible leaves, and up to 73%, from the 6th to the 20th day on the ornamental genotype compared to the day of harvest (Table 2). Similar pattern was determined by Messias et al. (2006) working with basil leaves stored for 5 days at 5 ºC, where CAT activity was higher in the last days of storage. The length of storage had no effect on PPO activity in leaves of either temperature for the ornamental leaves and for cv. Comum at 10 ºC (Fig 6). Contrarily to POD activity, the cv. Comum stored at 5 ºC increased PPO activity from the 12th day up to the end of the cold treatment (Fig 6). Nevertheless, the enzyme activity did not showed significant differences compared to the day of harvest (Table 2). Significant effect (53.2%) was observed at day two in leaves of cv. Comum at 10 ºC. The ornamental genotype BGH/UFV 5932 had higher PPO activity than treatments at lower temperatures. Significant decreases in activity were observed during storage at both temperatures, representing about 43% at 5 ºC and 41% at10 ºC at 20 days of storage (Table 2).

Phenolic compounds

Accumulation of soluble phenolic compounds in leaves the ornamental BGH/UFV 5932 accession was four fold higher than those in edible leaves (Fig 7). Regression analysis did not fit to the changes throughout storage, indicating no effect of time on accumulation of phenols. Pinto et al. (2001a) found levels of phenolics in fresh tannia accounted for 1% of the fresh. The cv. Comum fitted to a quadratic model for the phenolic content (Fig 7), with significant increase in soluble phenols at 10 ºC (Table 2). At this temperature, the genotype BGH/UFV 5932 showed a peak in the accumulation of 24% on phenolic compounds by the 10 day of storage, which may be associated with the onset of increased in POD enzyme activity at day 12, reducing the concentration in leaves, which was probably used as substrate by POD (Table 2) (Guimarães, 2006). Throughout the period in which the leaves remained chilled, no symptoms of browning associated to chilling injury was developed. This fact, might be due to the behavior of the phenolics and the activities of POD, CAT and PPO during storage, reducing the chances for

Table 1. Mean values of carbohydrate metabolism in leaves of tannia, cv. Comum and BGH/UFV 5932, stored for 20 days, at 5 and 10 ºC.

Storage time (days)	Comum		BGH/UFV 5932	
	5 ºC[1]	10 ºC	5 ºC	10 ºC
Total soluble sugars (%)				
0	2.58	2.58	1.99	1.99
2	2.83 ns	2.49 ns	2.18 ns	1.80 ns
4	2.38 ns	2.08 ns	1.83 ns	2.00 ns
6	2.68 ns	2.85 ns	1.90 ns	2.26 ns
8	2.25 ns	2.19 ns	1.56 ns	1.76 ns
10	2.18 ns	3.56 ns	1.70 ns	1.81 ns
12	2.55 ns	2.34 ns	1.42 ns	1.58 ns
14	2.60 ns	2.22 ns	1.48 ns	1.44 ns
16	2.00 ns	2.51 ns	1.23 ns	1.54 ns
18	1.87 ns	1.61 ns	1.07 ns	1.44 ns
20	1.67 ns	2.20 ns	1.33 ns	1.08 ns
Reducing sugars (%)				
0	0.38	0.38	0.67	0.67
2	0.54 ns	0.48 ns	1.01 ns	1.16 ns
4	0.43 ns	0.65 ns	0.98 ns	1.12 ns
6	0.44 ns	0.49 ns	0.55 ns	1.21 ns
8	0.31 ns	0.37 ns	0.52 ns	0.89 ns
10	0.29 ns	0.40 ns	0.89 ns	1.01 ns
12	0.31 ns	0.44 ns	0.63 ns	1.32 ns
14	0.30 ns	0.35 ns	0.61 ns	0.90 ns
16	0.25 ns	0.47 ns	0.39 ns	1.03 ns
18	0.29 ns	0.34 ns	0.53 ns	0.94 ns
20	0.39 ns	0.37 ns	0.34 ns	0.65 ns
Starch (%)				
0	3.29	3.29	3.23	3.23
2	2.91 ns	3.14 ns	1.53 *	1.65 *
4	2.55 *	2.33 *	1.32 *	0.90 *
6	2.40 *	2.46 *	1.05 *	0.97 *
8	2.12 *	2.42 *	0.77 *	0.98 *
10	2.30 *	2.93 ns	0.68 *	0.88 *
12	2.53 *	2.87 ns	0.55 *	0.87 *
14	2.78 ns	2.32 *	0.79 *	0.62 *
16	2.47 *	2.77 ns	0.42 *	0.80 *
18	2.40 *	2.68 ns	0.64 *	0.46 *
20	3.55 ns	2.70 ns	0.66 *	0.76 *

*: averages in the column differ from controls (0), at 5% probability by Dunnett's test. ns: averages in the column don't differ from controls (0), at 5% probability by Dunnett's test.

chilling injury development. Visual analysis clearly showed the yellowing of leaf during the cold storage period (Fig 8), a typical symptom of natural aging of the leaves and not a chilling injury symptom. According to Lipton (1987), the yellowing is the most common and best known senescence symptom of leafy green vegetables. According to this author, the rate of loss of chlorophyll in leaves may be influenced by temperature, by cultivating, in addition to water stress, light, hormones and modified atmosphere. Leaves of both cultivars kept at 5 ºC had higher conservation than those stored at 10 ºC, without early yellowing symptoms (Fig 8). At 20 days of storage, the genotype ornamental maintained at 10 ºC hat 51 to 75% of the leaf area yellow. However, despite the severity of symptoms, the genotype BGH/UFV 5932 was more resistant in this temperature, compared to cv. Comum, which at 18 days of stored had more than 75% of the leaf completely yellow (Fig 8). Although the temperature of 5 ºC has contributed to better maintenance of the green color during 20 days of storage, at the end of the shelf-life of tannia cv. Comum was characterized by loss of water and subsequent withering of leaves, which occurred after 10 days of storage.

Material and Methods

Preparation and storage of leaves

Fully expanded leaves of tannia (*Xanthosoma sagittifolium*) cultivar Comum (edible), obtained from a producer city Matozinhos-MG and BGH/UFV 5932 (ornamental) were harvest in the morning and selected according to the commercial standard (Seganfredo et al., 2001). The leaves were kept with 15 cm long petiole. Immediately after harvest, the leaves were hydrated for 20 minutes in water at 23ºC to reduce the fresh weight loss during storage (Shibairo and Upadhyaya, 1998).

Treatments and experimental design

The experiment was conducted in a factorial 2 × 2 × 10, comprising two varieties , two temperatures of storage (5 and 10 ºC) for 20 days storage period, arranged in a completely randomized design with five replicates of individual leaves. The leaves were accommodated inside of non-perforated low density polyethylene bags, placed inside cardboard boxes to

Table 2. Mean values of enzyme activity and concentration of phenolic compounds in leaves of tannia cv. Comum and BGH/UFV 5932, stored for 20 days at 5 and 10 ºC.

Storage time	Comum				BGH/UFV 5932			
(days)	5 ºC[1]		10 ºC		5 ºC		10 ºC	
Peroxidase (AU.min^{-1}.mg^{-1} protein)								
0	5.03		5.03		3.02		3.02	
2	2.98	ns	2.97	ns	2.30	ns	4.08	ns
4	4.61	ns	3.91	ns	2.80	ns	5.89	ns
6	4.29	ns	4.63	ns	4.23	ns	4.58	ns
8	5.35	ns	3.78	ns	4.17	ns	4.05	ns
10	4.64	ns	3.14	ns	3.52	ns	5.17	ns
12	6.16	ns	7.86	ns	2.52	ns	10.60	*
14	5.92	ns	2.41	ns	4.10	ns	4.00	ns
16	5.67	ns	6.25	ns	2.79	ns	3.67	ns
18	4.88	ns	7.71	ns	3.66	ns	4.67	ns
20	4.35	ns	6.10	ns	3.55	ns	4.47	ns
Catalase (AU.min^{-1}.mg^{-1} protein)								
0	0.33		0.33		1.26		1.26	
2	0.33	ns	0.32	ns	1.32	ns	1.54	ns
4	0.27	ns	0.46	ns	1.07	ns	1.43	ns
6	0.36	ns	0.48	ns	1.19	ns	1.90	*
8	0.16	ns	0.34	ns	1.12	ns	1.70	*
10	0.31	ns	0.41	ns	0.97	ns	2.18	*
12	0.34	ns	0.56	ns	1.03	ns	2.19	*
14	0.48	ns	0.46	ns	1.19	ns	1.67	*
16	0.25	ns	0.91	*	1.21	ns	1.96	*
18	0.32	ns	0.71	*	1.21	ns	1.72	*
20	0.24	ns	0.94	*	1.38	ns	1.82	*
Polyphenoloxidase (AU.min^{-1}.mg^{-1} protein)								
0	1.49		1.49		2.02		2.02	
2	1.62	ns	2.28	*	1.33	ns	1.33	ns
4	1.74	ns	1.77	ns	1.71	ns	1.30	*
6	1.37	ns	1.39	ns	1.56	ns	1.53	ns
8	1.51	ns	1.87	ns	1.12	*	1.24	*
10	0.91	ns	1.82	ns	1.54	ns	1.24	*
12	1.33	ns	1.21	ns	1.50	ns	1.32	ns
14	1.49	ns	1.54	ns	1.26	*	1.65	ns
16	1.46	ns	1.36	ns	1.94	ns	0.89	*
18	1.69	ns	1.50	ns	1.18	*	1.14	*
20	1.87	ns	1.25	ns	1.14	*	1.17	*
Soluble phenolic compounds (mg D-catechin.g^{-1} FW)								
0	1.36		1.36		5.04		5.04	
2	1.44	ns	1.41	ns	5.32	ns	5.24	ns
4	1.31	ns	1.41	ns	4.89	ns	5.94	ns
6	1.36	ns	1.51	ns	4.51	ns	5.04	ns
8	1.43	ns	1.34	ns	4.87	ns	5.29	ns
10	1.30	ns	1.42	ns	4.14	ns	6.23	*
12	1.31	ns	1.90	ns	5.36	ns	5.57	ns
14	1.41	ns	1.62	ns	4.37	ns	4.42	ns
16	1.42	ns	2.17	ns	4.75	ns	6.05	ns
18	1.45	ns	2.45	ns	4.61	ns	5.21	ns
20	1.72	ns	2.68	*	4.53	ns	5.96	ns

*: averages in the column differ from controls (0), at 5% probability by Dunnett's test. ns: averages in the column don't differ from controls (0), at 5% probability by Dunnett's test

simulated long distance shipping. The bocks were kept for 20 days at 5 or 10 ºC and relative humidity of 68 ± 5%. Leaf samples were taken at harvest at 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 days of storage for enzyme, chemical and visual analysis.

Chemical analysis

Samples of 0.5 g of leaf were homogenized in 80% ethanol at 65-70 ºC, centrifuged at 2,000 ×*g* for 10 min and filtered with analytical paper filter. The extraction was performed three times and the volumes were the combined. The residue on the filter paper was for the starch content quantification. Total soluble sugars quantification was according the phenol-sulfuric acid method (Dubois et al., 1956), using sucrose as standard. The values content of total soluble sugars was expressed in % TSS of fresh matter. Determination of reducing sugars was carried out by the Somogy-Nelson method (Nelson, 1944) using glucose as standard. The reducing sugar content was expressed in % RS of fresh matter. The starch extraction was performed using the residue on filter paper after the soluble sugar extraction, subsequently

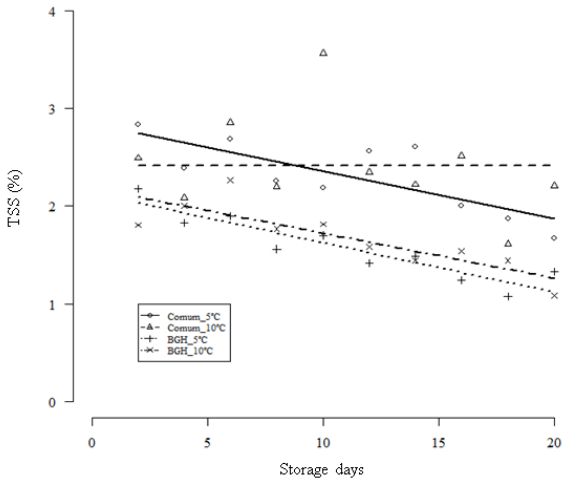


Fig 1. Total soluble sugars content in leaves of tannia, cv. Comum and BGH/UFV 5932, stored for 20 days at 5 and 10 ºC.

o _____ $\hat{Y} = 2.83973 - 0.0485504\ x$ ($r^2 = 0.6073$)
Δ _ _ _ _ _ $\hat{Y} = 2.4099$
\+ ..... $\hat{Y} = 2.13090 - 0.0507219\ x$ ($r^2 = 0.8446$)
× ..... $\hat{Y} = 2.18375 - 0.0461446\ x$ ($r^2 = 0.7157$)

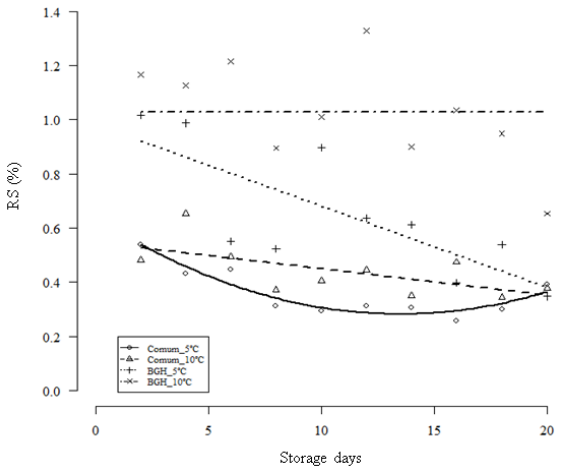


Fig 2. Reducing sugar content in leaves of tannia, cv. Comum and BGH/UFV 5932, stored for 20 days at 5 and 10 ºC.

o _____ $\hat{Y} = 0.637505 - 0.0525585\ x + 0.00194851\ x^2$ ($r^2 = 0.8823$)
Δ _ _ _ _ _ $\hat{Y} = 0.547537 - 0.00976001\ x$ ($r^2 = 0.4045$)
\+ ..... $\hat{Y} = 0.981337 - 0.0300394\ x$ ($r^2 = 0.5924$)
× ..... $\hat{Y} = 1.0280$

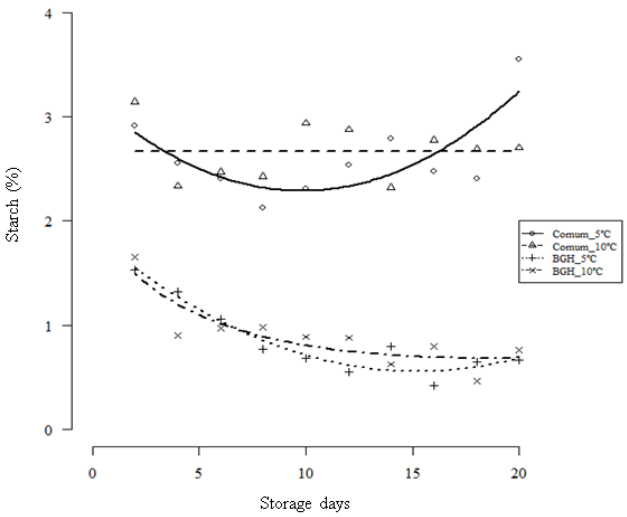


Fig 3. Starch content in leaves of tannia, cv. Comum and BGH/UFV 5932, stored for 20 days at 5 and 10 ºC.

o _____ $\hat{Y} = 3.17283 - 0.180416\ x + 0.00920317\ x^2$ ($r^2 = 0.6038$)
Δ _ _ _ _ _ $\hat{Y} = 2.6671$
\+ ..... $\hat{Y} = 1.87310 - 0.173185\ x + 0.00570106\ x^2$ ($r^2 = 0.9197$)
× ..... $\hat{Y} = 2.50434 - 0.852868\ \sqrt{x} + 0.100013\ x$ ($r^2 = 0.7509$)

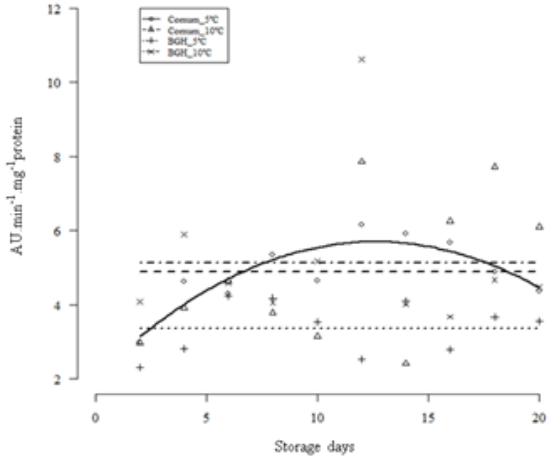


Fig 4. Peroxidase activity in leaves of tannia, cv. Comum and BGH/UFV 5932, stored for 20 days at 5 and 10 ºC.

o _____ $\hat{Y} = 2.07782 + 0.573600\ x - 0.0227114\ x^2$ ($r^2 = 0.7760$)
Δ _ _ _ _ _ $\hat{Y} = 4.8810$
\+ ..... $\hat{Y} = 3.3706$
× ..... $\hat{Y} = 5.1229$

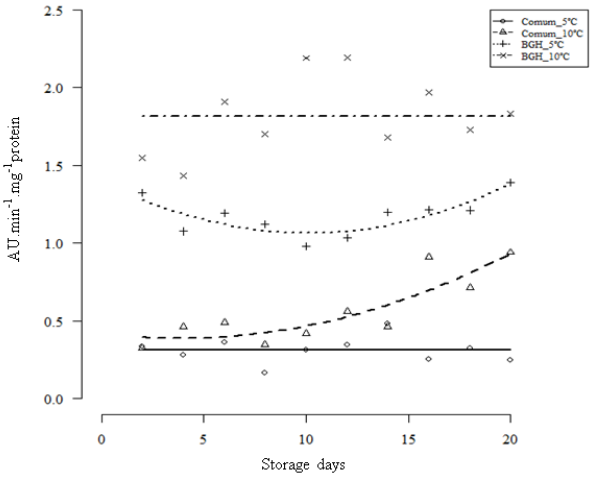


Fig 5. Catalase activity in leaves of tannia, cv. Comum and BGH/UFV 5932, stored for 20 days at 5 and 10 ºC.

o _____ $\hat{Y} = 0.3103$
Δ _ _ _ _ _ $\hat{Y} = 0.417433 - 0.0158704\ x + 0.00208297\ x^2$ (r^2 = 0.7647)
+ _ _ _ _ _ $\hat{Y} = 1.39358 - 0.0652929\ x + 0.00323464\ x^2$ (r^2 = 0.7027)
× _._._._. $\hat{Y} = 1.8173$

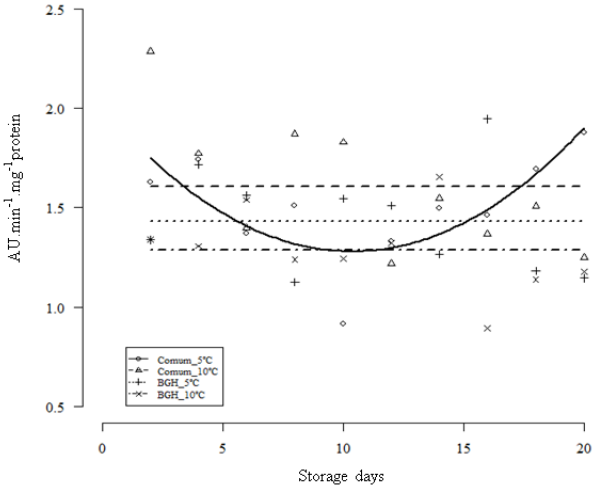


Fig 6. Polyphenoloxidase activity in leaves of tannia, cv. Comum and BGH/UFV 5932, stored for 20 days at 5 and 10 ºC.

o _____ $\hat{Y} = 1.99986 - 0.138733\ x + 0.00668442\ x^2$ (r^2 = 0.6224)
Δ _ _ _ _ _ $\hat{Y} = 1.6047$
+ _ _ _ _ _ $\hat{Y} = 1.4340$
× _._._._. $\hat{Y} = 1.2862$

dried at 65 ºC for 24 hours. The starch was hydrolyzed as described by McCready et al. (1950). Starch extracted with 52% perchloric acid, and the total carbohydrate content determined as the total soluble sugars. Sucrose was used as standard multiplied by 0.9. The starch content was expressed in % starch of fresh matter. Total leaf methanol soluble phenolic compounds was determined according to the method described by Prince and Butler (1977). The results were expressed in mg of D-catechin g^{-1} fresh weight.

Leaf visual analysis

Analysis was performed at every two days, at harvest, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 days of storage, in five leaves of each cultivar. The intensity of the senescence associated chilling symptoms, according to the rating scale, described by Ribeiro et al. (2005) with modifications, ranging from 0 to 4 where 0 = no signs of yellow at leaf surface; 1 = slightly yellowish (up to 25% of yellow surface); 2 = moderate yellowish (26 to 50% of yellow surface); 3 = extremely yellowish (51 to 75% of yellow surface); 4 = completely yellowish (more than 76% yellow surface).

Enzymes activities

Peroxidase extraction was carried out in approximately 1 g of leaf tissue was homogenized in 10 mL 100 mM Na-phosphate buffer, pH 6.5 containing 0.1% sodium bisulfite 0.1% and 150 mM sodium chloride. The homogenized tissue was filtered through four layers of gauze, followed by centrifugation at 17,000 ×*g*, for 30 min at 4 ºC. The reaction was carried out with 0.5 mL of the supernatant mixed with 1.5 mL of 100 mM Na-phosphate buffer, pH 6.5 plus 0.5 mL of 1.7% guaiacol and 0.5 mL of 1.8% H_2O_2 (Neves, 2003). The reaction was initiated by addition of the supernatant measuring the changes in absorbance in a spectrophotometer at 470 nm for 2.5 min at 25 ºC. The enzyme activity was expressed in absorbance units (AU) min^{-1} mg^{-1} of protein. Activity of catalase activity was performed in approximately 1 g of leaf tissue homogenized in 10 mL of 50 mM Na-phosphate buffer, pH 7.8 plus 1% PVP-40. The suspension was filtered through four layers of cheese cloth, followed by centrifugation at 17,000 ×*g*, at 4 ºC for 30 min. The reaction was performed in 0.85 mL of 50 mM Na-phosphate buffer, pH 7.8, 0.5 mL of 30 mM H_2O_2 and 0.15 mL of extract (Aebi, 1983). Decomposition of H_2O_2 was started by addition of extract following the changes in absorbance in a spectrophotometer at 240 nm for 2.5 minutes at 25 ºC. The enzyme activity was expressed in absorbance units (AU) min^{-1} mg^{-1} of protein. Polyphenoloxidase activity was determined using 1 g of leaf tissue homogenized in 10 mL of 100 mM Na-phosphate buffer, pH 6.5, 1% PVP-40 and 0.1% Triton X-100 (Concellon et al., 2004). The filtrate was passed through four layers of cheese cloth and centrifuged at 17,000 ×*g* at 4 ºC for 30 min. Activity was determined using 0.5 mL of supernatant mixed to 0.5 mL of 100 mM Na-phosphate buffer, pH 7.0 plus 0.5 mL of 15 mM catechol (Neves, 2003). The reaction was initiated by adding the supernatant and following the changes in absorbance in a spectrophotometer at 420 nm for 2.5 minutes at 25 ºC. The enzyme activity was expressed in absorbance units (AU) min^{-1} mg^{-1} of protein. Total protein concentration in all enzyme preparations was determined according to Bradford (1976), and BSA as standard.

Statistical analysis

The data of enzymatic activity, content of phenolic compounds and carbohydrates were subjected to analysis of variance (ANOVA) and regression in SAEG statistical program version 9.1 (2007). To compare the means, it was used the Dunnett test, at 5% probability. The models were obtained based on the significance of the regression coefficients, using the test "t", adopting the level of 5% probability, in the determination coefficient and the behavior of the phenomenon under study.

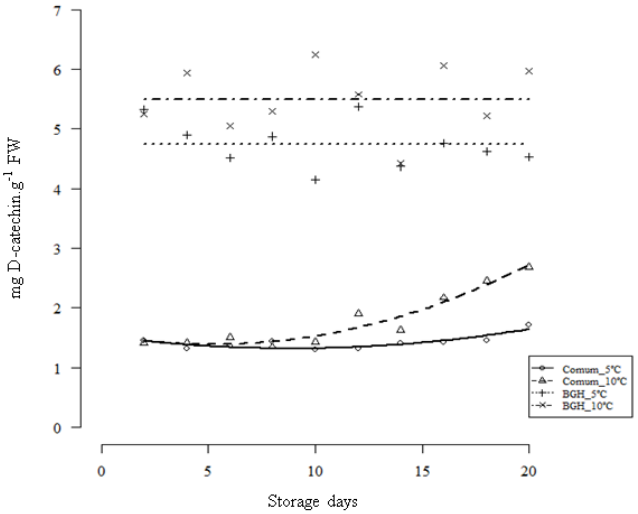
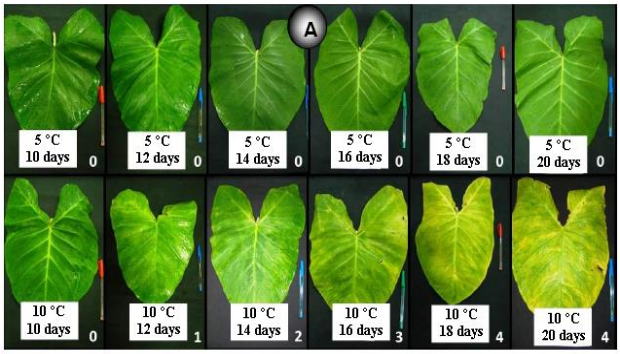


Fig 7. Concentration of soluble phenolic compounds in leaves of tannia, cv. Comum and BGH/UFV 5932, stored for 20 days at 5 and 10 ºC.

o _____ $\hat{Y} = 1.53170 - 0.0471623\ x + 0.00264790\ x^2$ (r^2 = 0.7467)
Δ _ _ _ _ _ $\hat{Y} = 1.55409 - 0.0631958\ x + 0.00608895\ x^2$ (r^2 = 0.9275)
+ ---------- $\hat{Y} = 4.7404$
× ._._._._. $\hat{Y} = 5.5015$



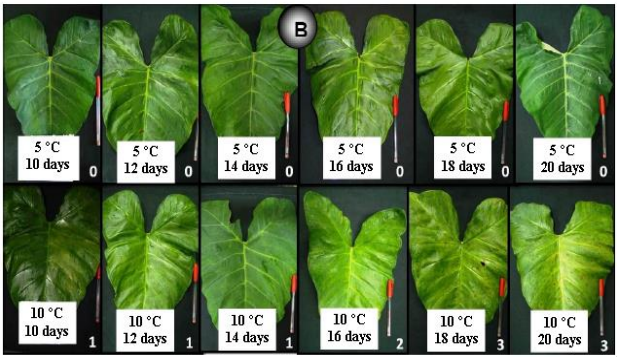


Fig 8. Visual evaluation in tannia leaves cv. Comum (A) and cv. BGH/UFV 5932 (B), stored at 5 ºC and 10 ºC for 20 days. 0 = no sign of yellowing; 1 = slightly yellowish (25%); 2 = moderate yellowish (26 to 50%); 3 = extremely yellowish (51 to 75%); 4 = completely yellowish (> 76%).

Conclusions

Storage for 20 days at 5 and 10 ºC, do not induce changes in the contents of total soluble and reducing sugars, but induce the degradation of starch in leaves of tannia cv. Comum and BGH/UFV 5932. Edible leaves have a higher starch content compared to the ornamental. Cold storage does not stimulate peroxidase activity in both genotypes. The increase in catalase activity occurs after 14 days of storage of the leaves cv. Comum and from the 6th day in the genotype BGH/UFV 5932 chilled to 10 ºC. Low temperature does not induce changes in PPO activity of edible tannia. Ornamental leaves have phenolics accumulation 4 times larger than the leaves of cv. Comum, which are not altered by refrigeration. The two genotypes are insensitive to low temperatures, with no signs of browning caused by cold. Longer shelf-life of the leaves occurs at 5 ºC.

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