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Effects of Potassium Fertilization for Pineapple on Internal Browning of Fruit in Post-Harvest Conservation

Coulibaly Souleymane*
Université Nangui Abrogoua, UFR Sciences de la Nature, 02 BP 801 Abidjan 02, Côte d'Ivoire

Yapo Sopie Edwige-Salomé
Université Jean Lorougnon Guédé, UFR Agroforesterie, BP 150 Daloa, Côte d'Ivoire

N'cho Achi Laurent
Université Nangui Abrogoua, UFR Sciences de la Nature, 02 BP 801 Abidjan 02, Côte d'Ivoire

Kouadio Oi Kouadio Samuel
Université Peleforo Gon Coulibaly, UFR Sciences Biologiques, BP 1328 Korhogo, Côte d'Ivoire

Kouakou Tanoh Hilaire
Université Nangui Abrogoua, UFR des Sciences de la Nature, 02 BP 801 Abidjan 02, Côte d'Ivoire

Abstract

Resistance to internal browning of pineapple fruits depends on several factors such as the cultural techniques through mineral fertilization. The objective of this work is to study the effects of the potassium fertilization for pineapple on internal browning of fruit in post-harvest conservation. The experiments have been carried out on the site of the University Nangui Abrogoua (Abidjan, Côte d'Ivoire) of July 2015 to the end of October 2016. Potassium has been applied according to four modalities of treatments (T0; T1; T2 and T3) made in 2nd, 4, 6 and 7th months (respectively) after plantation. The incidence of internal browning (IB), the phenolic content, sugars and ascorbic acid have been determined after post-harvest conservation of fruit. The activity of phenolic biosynthesis enzymes (PAL and TAL) and oxidation enzymes (PPO and POD) were evaluated. The results showed that BI intensity in pineapple fruit decreases with the potassium amount applied in field. This IB drop was correlated with the content of reducing sugars, total phenols, activity of PAL and the PPO. No symptom of IB was observed on pineapple fruits under treatment T2 (34 g of K_2O/plant). Potassium has a depressive effect on phenolic biosynthesis. In effect, it inhibits the IB in the both varieties of pineapple studied that are Smooth Cayenne and MD2.

Keywords: Internal browning; Pineapple; Post-harvest conservation; Potassium; Smooth cayenne; MD2.

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1. Introduction

The pineapple is one of the main export crops of Côte d'Ivoire. In the recent past, pineapple export generated more than 45 billion CFA per year to the Ivorian State and contributed 0.6% to the GDP [1]. Pineapple is an important source of foreign exchange for Côte d'Ivoire, it also supports a large number of producers. However, the 1980s have marked, in the sector of the pineapple, the beginning of a long crisis which is accentuated after the political crisis of 2002 [2]. As consequences, there has been a drastic decline of the Ivorian production (213 620 tones in 1999 to 20 900 tones in 2014), followed by the loss of its status as a premier supplier of fresh pineapple on the European market The causes of this crisis are multiple, among which there was the aging of planting material, the depletion of soils due to the monoculture, the acidity of the fruit, the high rate of chemical residues, the internal browning etc. However, the internal browning is by far one of the most important causes of this crisis in the sector pineapple [2]. The internal browning of the fruits of pineapple depends on several factors such as the planting, the cultivation techniques and especially the conservation procedures post-harvest (storage at low temperature, 10 °C, during the export). Given the economic losses enormous related to internal browning during the export of the fresh pineapple, several works have been undertaken on the potassium to control this phenomenon. In fact, the plants absorb nutrients necessary for their different physiological functions (growth, development, reproduction) [3]. Thus, an appropriate concentration of potassium in the soil is necessary for the production of pineapples of good quality [4]. Potassium is absorbed by the plants under its ionic form K^+ to regulate the osmotic pressure as well as the opening and closing of the stomata [5, 6]. This mineral is also used as a cofactor in the enzymatic reactions and biochemical [5]. According to Antonio, *et al.* [7], the application of a high amount of potassium in the pineapple inhibits the internal browning of the fruit at maturity.

The objective of this study was to evaluate the effects of potassium fertilization on pineapple plants and impact on fruit internal browning in post-harvest conservation.

*Corresponding Author

2. Material and Methods

2.1. Plant Material

The plant material is constituted of rejects of pineapples of varieties MD2 and Smooth Cayenne (*Ananas Comosus* L. Merr.), grown on a large scale in Côte d'Ivoire. Pineapple fruits from four different treatments of potassium plants were harvested. After nine months of culture, the floral induction treatment (FIT) was performed to homogenize the flowering of the plants. The pineapples studied were labeled once flowering started.

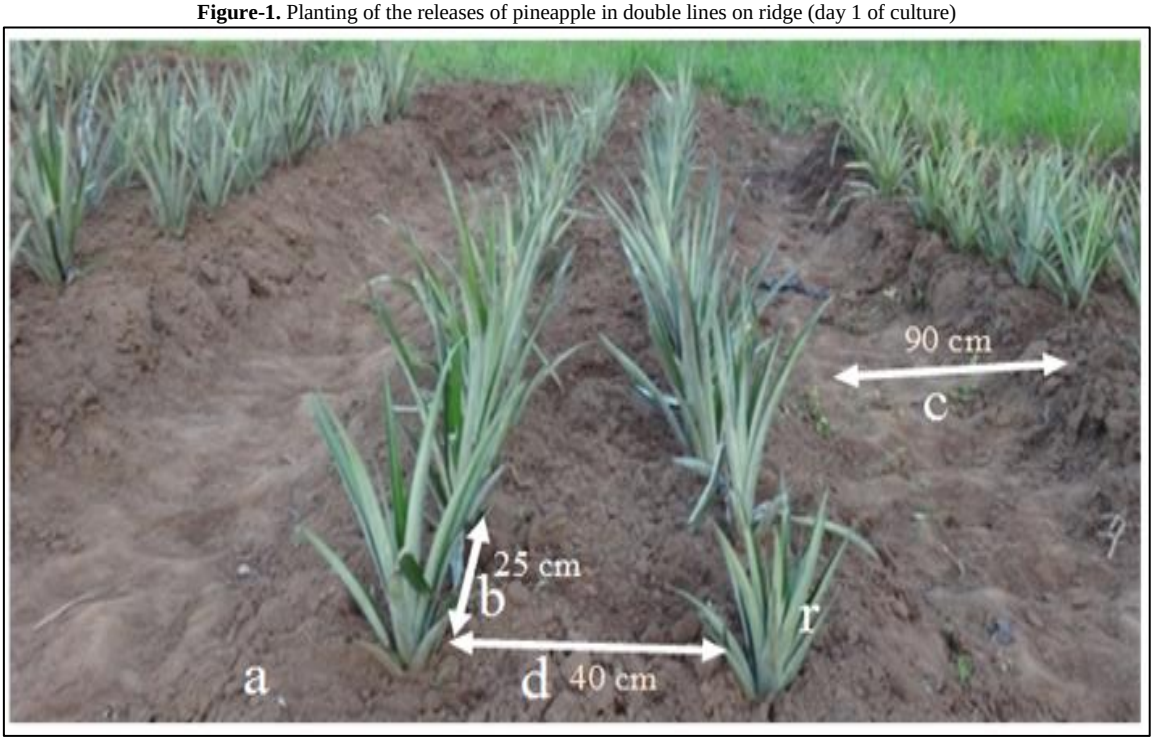
The field was planted in July 2015, on a sandy and low fertility in experimental farm of University Nangui Abrogoua (Abidjan, Côte d'Ivoire) farm at the city of Abidjan (5º23'N; 4º11'W), Côte d'Ivoire.

2.2. Methods

2.2.1 Establishment of the Culture

After the chemical analysis and then treatment of soil by fumigation, ridges of dimensions 6.5 m × 1 m have been made on an area of 150 m^2. Each ridge has been amended with 1 kg of dolomite and 500 g of calcium triphosphate. For each variety of pineapple, the plants of approximately 400 g are subcultured on the ridges in double rows in a device in block. Each treatment consisting of 12 seedlings is repeated three times. The spacing between plants is 25 cm on the line and 40 cm between the lines. The ridges are spaced 90 cm (Fig 1).

Figure-1. Planting of the releases of pineapple in double lines on ridge (day 1 of culture)



a (ridge); b (distance between two plants on the same line of a ridge); c (distance between two ridges); d (distance between two plants on a ridge); r (reject of pineapple);

2.2.2. Phytosanitary Treatment

Phytosanitary treatments have been made with an insecticide (chlorpyrifos-étyl 480 g/L) at a dose of 2 L/ha and a fungicide (fosetyl aluminum) at a dose of 7 kg/ha. A nematicide (Némacur 40 EC) at the dose of 25 mL/L has also been applied at the foot of plants due to 6 mL of the mixture by plant. All these treatments were carried out as early as in the second month of culture and/or the fifth month according to the need.

2.2.3. Application of Different Doses of Potassium

The fertilization has been done according to the four modalities of manure or treatments (T0; T1; T2 and T3). All manure have received the same quantities of urea to 46 % of nitrogen (16 g/plant), Fertilizer complete in a proportion of (10 g/plant) consisting of 11 per cent of nitrogen (N); 5% phosphorus (P_2O_3); 27 % of potassium oxide (K_2O); 15 % of sulfur (S); 5% of magnesium oxide (MgO) and a fertilizer enriched in trace elements boron (0.51 g/L; copper chelated EDTA 0.25 g/L; chelated iron EDTA 0.16 g/L; Molybdenum 0.05 g/L; zinc 0.47 g/L ; chelated manganese EDTA 0.51 g/L). For the potash, the quantity has varied according to the different manure: T1 (28 g of K_2O /plant), T2 (34 g of K_2O /plant) and T3 (40 g of K_2O /plant). The quantity of potash applied by the peasants was taken as control treatment T0 (20 g of K_2O /plant) [8]. All these manure have been made, to plants of pineapple MD2 and Smooth Cayenne, during their vegetative phases, respective to the frequency of four applications (at 2, 4, 6 and 7^{th} months of culture). From the second month, the initial input of fertilizers has been done in solid form (granular) in the axils of basal leaves. The other three applications have been carried out in liquid form (fog) on all leaves.

2.2.4. Harvest and Fruit Packaging

After nine months of culture. The treatment of floral induction (TIF) has been carried out to homogenize the flowering of plants [3]. The TIF was realized when the sheet has reached 70 g. The product used is the calcium carbide prepared at the dose of 2 kg in 200 L of water. A volume of 50 mL of the liquid obtained was immediately paid in the heart of each plant, using a backpack sprayer to Adjustable flow control. This operation was resumed 48 hours later in order to ensure the success of the floral induction.

For each variety, a batch of 3 harvested fruit has been constituted by treatment. The lots are marked T0, T1, T2, T3. A solution fungicide (benomyl) has been applied to the crown of the fruit before its conservation to 10 °C for 14 days (time needed for the export). The fruits have then been removed and retained again to 22 °C for 5 days (time of marketing) before being used for the different analyzes.

2.2.5. Impact of the Browning

The fruits of each treatment have been split lengthwise in two (2). The incidence (intensity) of the browning was appreciated to the naked eye at the level of the Flesh and the heart of the pineapple according to the following scale: absence of the symptom (-); the presence of the Symptom (+); intense symptom (++); symptom very intense (+++).

2.2.6. Rate of Soluble Dry Extract of Fruit

The rate of soluble dry extract measured in degree Brix (°Brix) [9]. The pulp of the half-portion of the fruit was sectioned longitudinally. A slice of pulp has been pressed to extract the juice. The soluble dry extract expressed in °Brix of the juice has been measured with a portable refractometer equipped with a temperature corrector (model FG106/113, Milan, Italy). The values (°Brix) displayed on the screen of the device have been noted in order to establish an average.

2.2.7. Content of Vitamin C

Ten (10) grams of pulp of pineapple is pressed to extract the juice. A volume of 1 mL of the juice is titrated by 2.6-dichlorophenol indophenol. The appearance of a pink color champagne persistent during 15 s, marks the end of the dosing. A volume of 1 mL of a standard solution of ascorbic acid pure (1 mg/mL) was also titrated by 2.6-dichlorophenol. The ascorbic acid content was obtained by the equation:

$$\text{Quantity of Vitamin C (mg/100g)} = \frac{(Ve - V0) \times 20}{(Vc - V0) \times 10} \times 100$$

Where, Vc is the volume of the 2,6-dichlorophenol indophenol used to titrate 1 mL of the standard solution of vitamin C pure (1 mg/mL); V0 is the volume of the 2,6-dichlorophenol indophenol used to titrate 1 mL methaphosphoric acid/ acetic acid and Ve is the volume of the 2,6-dichlorophenol indophenol used to titrate 1 mL of juice obtained from the sample of pineapple.

2.2.8. Sample Extraction

The sample extraction was carried out according to the method of Kouakou, *et al.* [10]. As well, 50 mg of pineapple pulps freeze-dried have been placed in a tube to hemolysis and 10 ml of methanol (96%) have been added. The whole is placed in the dark for 10 h at 4 °C. After a centrifugation at 5000 rev/min for 10 min, the supernatant obtained was filtered on a Millipore membrane (0.45 µm) and constituted the sample extract for sugars and phenols analysis.

2.2.9. Determination of Total Sugars

The assay was done according to the method of Dubois Dubois, *et al.* [11]. In this method, sulfuric acid makes it possible to break the osidic bonds between D-glucose and D-fructose putting in solution all the sugars present, which will be revealed by phenol. The reaction mixture was contained 0.2 mL phenol 5%, 1 mL distilled water, 1 mL sulfuric acid (97%) and 0.2 mL sample extract. After incubation for 5 min in a boiling water bath, the mixture was cooled in the dark for 30 min at room temperature. The intensity of the coloration produced by the reaction is measured with a spectrophotometer at 490 nm against a control. The amount of total sugars was determined using a calibration curve with glucose at 1 mg/mL and expressed in g/100 g dw.

2.3. Determination of Reducing Sugars

The reducing sugars were determined according to the method of Bernfeld [12] using 3, 5-dinitrosalicylic acid (DNS). The reaction mixture was constituted of 0.5 mL DNS, 0.9 mL distilled water and 0.1 mL sample extract. The mixture was incubated in a boiling water bath for 5 min and cooled for 5 min at room temperature. Then, 3.5 mL distilled water was added again to the mixture reaction. The intensity of the coloration produced by the reaction is measured with a spectrophotometer at 540 nm against a control. The amount of reducing sugars was determined using a calibration curve with glucose at 1 mg/mL and expressed in g/100 g dw.

2.4. Determination of Total Phenols Content

Total phenols content was determined using Folin-Ciocalteu's reagent according to the method of Singh [13]. The reaction mixture was contained 0.5 mL reagent Folin-Ciocalteu, 0.9 mL distilled water, 1.5 mL sodium carbonate 17% and 0.1 mL sample extract. The mixture was incubated for 35 min in darkness and absorbance was measured at 765 nm. The total phenols content was determined with a calibration curve made with gallic acid at 200 µg/mL ($y = 0,586x$; $R^2 = 0.999$, where y is the absorbance and x is the concentration of gallic acid. Total phenols content was expressed in mg gallic acid/ g dw.

2.5. Enzyme Extraction and Assay

The extraction of enzymes is performed to cold (4°C) by the crushing of 2 g of lyophilized pulp in 10 ml of extraction buffer in the presence of the PVP (0.5 %) and sodium phosphate buffer 0.1 Mr. the extraction buffer, is composed of 0.5 mL polyethylene glycol 6000 (PEG 6000), 0.25% sodium thiosulphate, 15% Glycerol, 1 mM EDTA and 15 mM mercaptoethanol. The filtrate is centrifuged at 5000 rpm for 20 min. The supernatant obtained constituting the crude extract of enzymes is maintained at 4°C. A crude extract contains a large number of inhibitors. Most of the inhibitors are of ionic nature. For the fix, a the anion exchange resin basic, the Dowex 2 is used. It is dissolved in the crude extract and then incubated for 30 min with agitation. Centrifugation is performed in order to eliminate the Dowex 2 which has fixed the inhibitor ions. The supernatant obtained was the enzyme fraction purified ready to be analyzed.

2.6. Polyphenoloxydase Activity

Polyphenoloxydase (PPO) activity was determined according the method of Zhou, *et al.* [14]. Briefly, the final reaction mixture was composed of 1 mL pyrocatechol 130 mM, 1.8 mL citrate phosphate buffer 0.1 M (pH 6.5) and 0.2 mL of purified enzyme extract. The oxidation of pyrocatechol is followed in the spectrophotometer at 500 nm. PPO activity was expressed in µkat/min/g dw (µmol substrate converted/s/g dw), considering that the molar extinction coefficient of the formed product as 1400 M^{-1} cm^{-1}.

2.7. Peroxidase Activity

Peroxidase (POD) activity was estimated by the method described by Blancas, *et al.* [15]. The reaction mixture was contained 1 mL gaiacol 25 mM, 0.1 mL hydrogen peroxide 10 mM, 1.8 mL sodium phosphate buffer 0.1 M (pH 6.0) and 0.1 mL of purified enzyme extract. The mixture was incubated for 3 min in the dark. POD activity was determined by following the oxidation of gaiacol with the spectrophotometer at 470 nm and expressed in µkat/min/g dw (µmol substrate converted/s/g dw) taking the molar extinction coefficient of the tetraguaiacol formed as 26.6 $x10^{-3}$ M^{-1} cm^{-1}.

2.8. PAl and TAL Activities

Ammonia-lyase enzymes were constituted of phenylalanine ammonia-lyase (PAL) tyrosine ammonia-lyase (TAL) and essayed with the modified method of Beaudoin-Eagan and Thorpe [16]. Both enzymes were assayed spectrophotometrically by measuring the amount of trans-cinnamic acid formed for PAL and p-coumaric acid formed for TAL at 290 nm. The reaction mixture consisted of 1 mL phenylalanine 100 mM for PAL or 1 mL tyrosine 100 mM for TAL, 1.9 mL sodium borate buffer 0.2 M (pH 8.8) and 0.1 mL enzyme extract. The mixture was incubated for 10 min at the ambient temperature. PAL or TAL activity was expressed in µkat/min/g dw (µmol substrate converted/s/g dw), considering the molar extinction coefficient of the cinnamic acid formed as 19600 M^{-1} cm^{-1} and that of the acid p-coumaric formed as 17600 M^{-1} cm^{-1} for PAL and TAL, respectively.

2.9. Catalase

Catalase (CAT) activity was estimated by the method of Patterson, *et al.* [17] modified by Zhou, *et al.* [14]. CAT activity was determined by measuring the decomposition of H_2O_2 in absorbance at 240 nm. The reaction mixture consisted of 1 mL H_2O_2 10 mM, 0.1 mL EDTA 1 mM, 1.8 mL Tris-HCl buffer 0.1 M (pH 7.0) and 0.1 mL enzyme extract. The mixture was incubated for 3 min in the dark. CAT activity was expressed in µkat/min/g dw (µmol substrate converted/s/g dw), taking the molar extinction coefficient of the formed product as 43.6 $x10^{-3}$ M^{-1} cm^{-1}.

2.10. Ascorbate Peroxidase

Ascorbate Peroxidase (APX) activity was determined according to the method of Nakano [18] modified by Zhou, *et al.* [14]. The reaction mixture contained 1 mL ascorbic acid 1.0 mM, 0.2 mL H_2O_2 10 mM, 0.2 mL EDTA 1 mM, 1.5 mL Tris-HCl buffer 0.1 M (pH 7.0) and 0.1 mL enzyme extract. The mixture was incubated for 3 min in the dark. The oxidation of ascorbic acid is followed in the spectrophotometer at 290 nm. APX activity was expressed in µkat/min/g dw (µmol substrate converted/s/g dw), considering the molar extinction coefficient of the formed product as 2.8 $x10^{-3}$ M^{-1} cm^{-1}.

2.11. Statistical Analysis

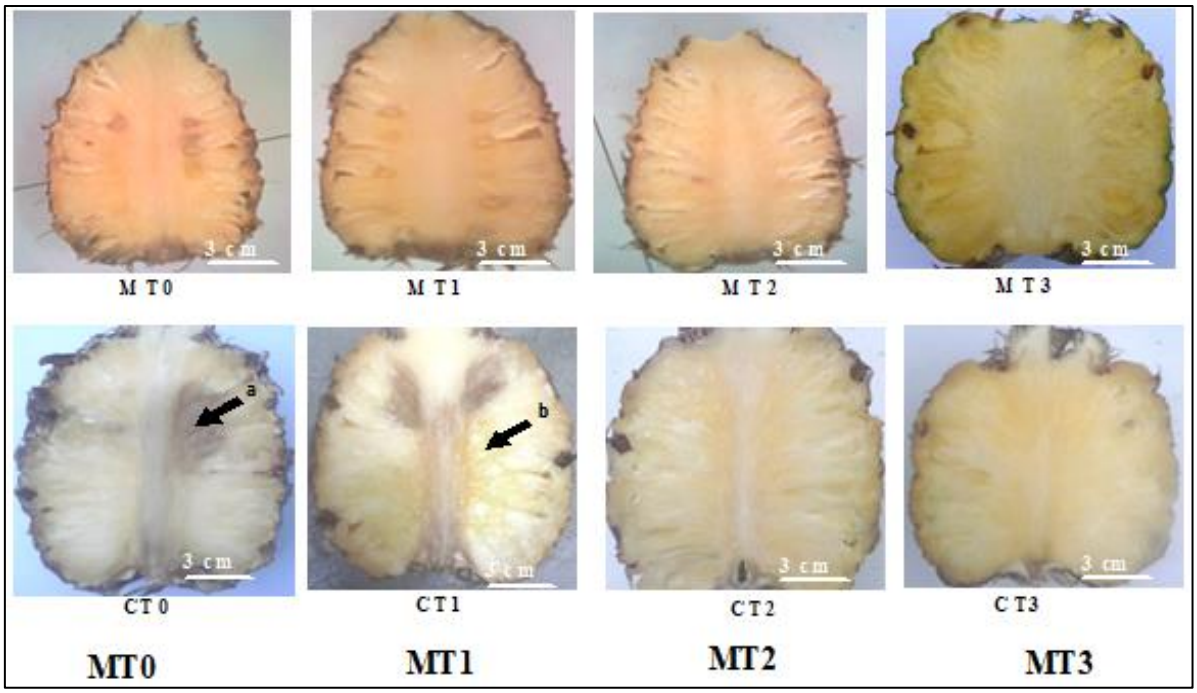
The data were subjected to statistical analysis using Statistica 7.5 software. Data were represented as mean values. The significant differences between mean values were determined by ANOVA using Newman Keuls test at 5% of significance level. Data are the mean of three replicates

3. Results

3.1. Aspect of the Pulp

The internal browning of the pulp is shows more intense in the pineapple Smooth Cayenne that among the variety MD2. No symptoms of translucency is observed at the level of the pineapple MD2. In contrast, in the pineapple Smooth Cayenne, this symptom is observable at the level of salaries T1 and T2. The analysis of the results suggests that the translucency of the pulp precedes its Browning (Fig 2).

Figure-2. Evolution of the symptoms of internal browning of pineapple MD2 and Smooth Cayenne in function of the concentration of potassium applied to the soil



a (symptom of browning); b (symptom of translucency); MT0 (fruit witnesses of the variety MD2 resulting from the treatment 20 g of K_2O/plant); MT1 (fruit of the variety MD2 resulting from the treatment 28 g of K_2O/plant); MT2 (fruit of the variety MD2 resulting from the treatment 34 g of K_2O/plant); MT3 (fruit of the variety MD2 from the treatment 40 g of K_2O/plant); CT0 (fruit witnesses of the variety Smooth Cayenne from the treatment 20 g of K_2O/plant); CT1 (fruit of the variety Smooth Cayenne from the treatment 28 g of K_2O/plant); CT2 (fruit of the variety Smooth Cayenne from the treatment 34 g of K_2O/plant); CT3 (fruits Of the variety Smooth Cayenne from the treatment 40 g of K_2O/plant).

Table 1 shows that the intensity of browning of pineapple studied decreases with the concentration of potassium applied in culture.

Table-1. Effect of potassium on the symptoms of internal browning and the translucency of the pulp of the pineapple Smooth Cayenne and MD2

Ananas variety	Symptoms		
	Treatments	Browning	Translucency
MD2	T0	+	-
	T1	-	-
	T2	-	-
	T3	-	-
Smooth Cayenne	T0	+++	-
	T1	++	++
	T2	-	+
	T3	-	-

T0 (fruit witnesses from the treatment 20 g of K_2O/plant); T1 (fruit from the treatment 28 g of K_2O/plant); T2 (fruit from the treatment 34 g of K_2O/plant); T3 (fruit from the treatment 40 g K_2O/plant). (-): Absence of the symptom; (+): presence of the symptom; (++): Symptom intense; (+++): Symptom very intense.

3.2. Fruit Soluble Dry Extract

The content of fruit soluble dry extract varies with the potassium treatment in both pineapple varieties (Table 2). The values are between 12.76 and 14.86 °Brix in MD2 and 12.06 and 13.9 °Brix in smooth Cayenne. The increase in fruit soluble dry extract from the potassium-treated plants starts from the T2 treatment (34 g K_2O/plant), regardless of the variety. The fruits of MD2 variety were significantly richer in soluble dry extract than those of Smooth Cayenne variety.

Table-2. Evolution of fruit soluble dry extract content as a function of plant-treated with potassium in two pineapple varieties

Potassium Treatment	Fruit soluble dry extract (°Brix)	
	MD2	Smooth Cayenne
T0	12.76 ± 0.54 c	12.06 ± 0.37 d
T1	12.86 ± 0.81 c	12.18 ± 0.23 d
T2	13.66 ± 1.31 b	12.84 ± 0.2 c
T3	14.86 ± 0.16 a	13.9 ± 0.25 b

T0 (fruit witnesses from the treatment 20 g of K_2O/plant); T1 (fruit from the treatment 28 g of K_2O/plant); T2 (fruit from the treatment 34 g of K_2O/plant); T3 (fruit from the treatment 40 g K_2O/plant). In column and line, the values followed by the same letter are not significantly different (Newman-Keuls test at 5%).

3.3. Total and Reducing Sugars

The results shown in Table 3 reveal that the total sugars content increases proportionally with the amount of potassium supplied to the pineapple plant. So, at treatment T3, total sugars content is 6.35 g/100 g of fruit dry weight and 6.02 g/100 g of fruit dry weight respectively for MD2 and Smooth Cayenne. On the other hand, the content of reducing sugars decreases as the amount of potassium added to the plants increases. The lowest levels of 1.34 g/100 g dw for MD2 pineapple and 0.85 g/100 g dw for Smooth Cayenne were obtained with treatment T3. However, it is worth noting that the MD2 variety is richer in sugar than the Smooth Cayenne.

Table 3. Evolution of soluble sugars content in fruit as a function of plant-treated with potassium in two pineapple varieties

		Sugars content (g/100 g dw)	
Sugars	Potassium treatment	MD2	Smooth Cayenne
Total sugars	T0	3.78 ± 0.07 g	2.64 ± 0.07 f
	T1	3.95 ± 0.17 g	3.95 ± 0.15 e
	T2	5.40 ± 0.20 c	4.30 ± 0.20 d
	T3	6.35 ± 0.12 a	6.02 ± 0.10 b
Reducing sugars	T0	2.58 ± 0.03 a	2.46 ± 0.01 a
	T1	2.12 ± 0.02 b	1.58 ± 0.03 c
	T2	1.68 ± 0.02 c	1.20 ± 0.03 d
	T3	1.34 ± 0.04 d	0.85 ± 0.03 e

dw (dry weight); T0 (fruit witnesses from the treatment 20 g of K_2O/plant); T1 (fruit from the treatment 28 g of K_2O/plant); T2 (fruit from the treatment 34 g of K_2O/plant); T3 (fruit from the treatment 40 g K_2O/plant). In the same column and on the same line, the values followed by the same letter are not significantly different (Test of Newman-Keuls at 5%).

3.4. Ascorbic Acid

Table 4 shows that ascorbic acid content does not vary significantly with the amount of potassium applied to the plant for a pineapple variety. At the level of Smooth Cayenne, a significant increase is observed compared to the value of the control fruit which is 11.16 mg/100 mL. However, the ascorbic acid content in the MD2 pineapple variety is 4-times higher than in the Smooth Cayenne pineapple variety.

Table-4. Evolution of ascorbic acid content in fruit as a function of plant-treated with potassium in two pineapple varieties

Potassium treatment	Ascorbic acid content (mg/100 mL)	
	MD2	Smooth Cayenne
T0	45.06 ± 0.54 a	11.16 ± 0.37 c
T1	44.26 ± 0.81 a	11.80 ± 0.23 b
T2	45.20 ± 1.31 a	12.20 ± 0.20 b
T3	45.86 ± 0.16 a	12.90 ± 0.25 b

T0 (fruit witnesses from the treatment 20 g of K_2O/plant); T1 (fruit from the treatment 28 g of K_2O/plant); T2 (fruit from the treatment 34 g of K_2O/plant); T3 (fruit from the treatment 40 g K_2O/plant). In the same column and on the same line, the values followed by the same letter are not significantly different (Test of Newman-Keuls at 5%).

3.5. Total Phenols

Table 5 indicates that the total phenols content of both pineapple varieties decreases significantly with the increase in the amount of potassium applied to the pineapple plant compared to the control. Total phenols content of Smooth Cayenne fruit at about 1.29 mg/g dw decreases to the lowest value to reach 1.02 mg/g dw at the potassium treatment T3. Likewise, with MD2, the total phenol content of the fruits from 1.09 mg/g dw in control drops to 0.91

mg/g dw at the potassium treatment T3. Smooth Cayenne variety is richer in total phenols than the MD2 pineapple variety.

Table-5. Evolution of total phenols content in fruit as a function of plant-treated with potassium in two pineapple varieties

Potassium treatment	Total phenol content (mg/g dw)	
	MD2	Smooth Cayenne
T0	1.09 ± 0.02 c	1.29 ± 0.01 a
T1	1.09 ± 0.04 c	1.18 ± 0.06 b
T2	1.01 ± 0.03 d	1.09 ± 0.16 c
T3	0.91 ± 0.10 e	1.02 ± 0.04 d

T0 (fruit witnesses from the treatment 20 g of K_2O/plant); T1 (fruit from the treatment 28 g of K_2O/plant); T2 (fruit from the treatment 34 g of K_2O/plant); T3 (fruit from the treatment 40 g K_2O/plant). In the same column and on the same line, the values followed by the same letter are not significantly different (Test of Newman-Keuls at 5%).

3.6. PAL and TAL Activities

The results shown in Table 6 reveal that phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase (TAL) activities in fruits of both pineapple were inversely proportional to the amount of potassium brought to the pineapple plant with pineapple varieties Smooth Cayenne and MD2. Thus, the lowest activities of PAL (1.33 µkat/min/g dw for MD2 and 2.05 µkat/min/g dw for smooth Cayenne) were observed at potassium treatment T3, as was TAL (0. µkat/min/g dw for MD2 and 0.83 µkat/min/g dw for Smooth Cayenne). It should be noted whatever the enzyme, activity is more important with Smooth Cayenne than with MD2. In addition, the PAL/TAL ratio is greater than 1 regardless of the potassium treatment. However, it decreases from treatment T0 (20 g K_2O/plant) to treatment T3 (40 g K_2O/plant) with MD2 (2 to 1.86). On the other hand, PAL/TAL ratio increases progressively when the amount of potassium increases (1.75 to 2.51).

Table-6. Activities of PAL and TAL in fruit as a function of plant-treated with potassium in two pineapple varieties

		Enzyme activities (µkat/min/g dw)	
The enzymes	Treatments	MD2	Smooth Cayenne
PAL	T0	2.38 ± 0.10 b	2.69 ± 0.87 a
	T1	2.16 ± 0.13c	2.68 ± 0.13 a
	T2	1.38 ± 0.10 d	2.09 ± 0.17 c
	T3	1.33 ± 0.42 d	2.05 ± 0.18 c
TAL	T0	1.19 ± 0.01 bc	1.53 ± 0.01 a
	T1	1.08 ± 0.02 c	1.39 ± 0.02 b
	T2	1.01 ± 0.03 c	1.07 ± 0.03 c
	T3	0.74 ± 0.05 d	0.83 ± 0.01 d
PAL/TAL	T0	2.00 ± 0.03 a	1.75 ± 0.03 c
	T1	2.00 ± 0.01 a	1.92 ± 0.01 b
	T2	1.31 ± 0.01 d	1.91 ± 0.03 b
	T3	1.86 ± 0.02 b	2.51 ± 0.03 a

PAL (phenylalanine ammonia-lyase); TAL (tyrosine ammonia-lyase); T0 (fruit witnesses from the treatment 20 g of K_2O/plant); T1 (fruit from the treatment 28 g of K2O/plant); T2 (fruit from the treatment 34 g of K_2O/plant); T3 (fruit from the treatment 40 g K_2O/plant). For a parameter, the values followed by the same letter are not significantly different (Test of Newman-Keuls at 5%).

3.7. POD and PPO Activities

Table 7 shows that the phenolic compounds oxidation enzymes (PPO and POD) activities in pineapple fruits depends on the amount of potassium applied to the plants. In addition, the results reveal a significant varietal effect with PPO and POD. In addition, a strong enzyme activity was observed in Smooth Cayenne compared to MD2. However, PPO and POD activities are suppressed by the increase content of potassium in both pineapple varieties. Thus, for PPO, enzyme activity goes from 7.11 to 4.61 µkat/min/g dw with Smooth Cayenne and 5.93 to 2.36 µkat/min/g dw with MD2. Similarly, POD activity increased from 1.91 to 1.58 µkat/min/g dw with Smooth Cayenne and 1.83 to 1.45 µkat/min/dw g with MD2. Otherwise, PPO activity is twice as high in Smooth Cayenne fruits as in MD2 fruits. On the other hand, POD activity is almost identical in both pineapple varieties and varies little with the type of potassium treatment. However, this POD activity is significantly lower compared to those of PPO. In addition, PPO/POD ratio is greater than 1 regardless of the variety and type of potassium treatment. But, this ratio decreases significantly with the increase in the amount of potassium applied to the plants. For example, potassium treatment T3 showed the lowest PPO/POD ratios in Smooth Cayenne (2.91 µkat/min/dw g) and MD2 (1.62 µkat/min/dw).

Table-7. Activities of PPO and POD in fruit as a function of plant-treated with potassium in two pineapple varieties

		Enzyme activities (µkat/min/g dw)	
The enzymes	**Treatments**	**MD2**	**Smooth Cayenne**
PPO	T0	5.93 ± 0.04 b	7.11 ± 0.33 a
	T1	3.26 ± 0.01 d	7.09 ± 0.25 a
	T2	2.37 ± 0.07 e	6.00 ± 0.09 b
	T3	2.36 ± 0.02 e	4.61 ± 0.06 c
POD	T0	1.83 ± 0.40 a	1.90 ± 0.60 a
	T1	1.64 ± 0.88 b	1.90 ± 0.36 a
	T2	1.42 ± 0.25 c	1.64 ± 0.62 b
	T3	1.45 ± 0.70 c	1.58 ± 0.36 b
PPO/POD	T0	3.24 ± 0.13 a	3.74 ± 0.11 a
	T1	1.98 ± 0.11 cd	3.73 ± 0.10 b
	T2	1.67 ± 0.08 d	3.65 ± 0.11 b
	T3	1.62 ± 0.12 d	2.91± 0.12 c

PPO (polyphenoloxidase); POD (peroxidase); T0 (fruit witnesses from the treatment 20 g of K_2O/plant); T1 (fruit from the treatment 28 g of K2O/plant); T2 (fruit from the treatment 34 g of K_2O/plant); T3 (fruit from the treatment 40 g K_2O/plant). For a parameter, the values followed by the same letter are not significantly different (Test of Newman-Keuls at 5%).

4. Discussion

Fruits of the pineapple variety Smooth Cayenne show intense symptoms of translucency and browning of the flesh, unlike the non-symptomatic variety MD2. These results seem to reveal a differential sensitivity of pineapple varieties with internal browning, as reported by Raimbault [18]. MD2 is therefore resistant to the translucency and internal browning of fruit flesh. However, in Smooth Cayenne, these both phenomena decrease regularly with increasing amounts of potassium applied to the plants until they disappear with potassium treatments of 34 to 40 g of K_2O per plant. These observations clearly indicate that potassium supply to the plants has a positive impact on pineapple fruit quality. The internal browning of the fruit is believed to be due to abiotic constraints that cause vacuolar rearrangements and invade intercellular spaces resulting in translucency flesh [19, 20]. Thus, the contact of vacuolar phenols with chloroplastic PPOs would be responsible for the browning of translucent zones as reported by Matos, *et al.* [21]. Potassium seems to increase membrane resistance and even the firmness of the fruit flesh, resulting in a decrease in browning symptoms with the increase in the amount of potassium applied in pineapple culture.

A significant decrease in total phenols content of the fruit pulp is observed in both pineapple varieties with the increase in the amount of potassium supplied to the plants. These results are correlated with those obtained on the aspect of enzymatic browning of pulp and could show that the total phenols content of pineapple pulp increases with enzymatic browning. In addition, the decrease in total phenols is also positively correlated with the activity of biosynthetic (PAL and TAL) and oxidation (POD and PPO) enzymes of phenols. The significant increase in total sugars content according to the amount of potassium supplied to the plant would mean that the potassium intake has a positive effect on the sugars content of pineapple fruits. This is also reflected in the good soluble dry extract of fruit content between 12.06 and 14.86 °Brix observed at harvest fruit. However, despite the increase in total sugars content as a function of potassium treatments, a negative correlation of reducing sugars values is observed in the same fruits. This decrease is believed to be due to a decrease in the activity of cell wall and starch degradation enzymes. Indeed, according to Emaga, *et al.* [22], the enzymes that break down cell walls and starch (complex sugar such as polyholoside) hydrolyze pectin, hemicelluloses and starch into fructose, glucose and sucrose (simple sugars such as oligoholosides) thus contributing to the softening of the fruit. Moreover, Sancho, *et al.* [23] showed that the change in fruit texture is related to the increase in cell wall degradation enzymes activity. In view of all the above, we can say that a decrease in enzyme activity that break down cell walls will also result in the preservation and maintenance of the firmness of pineapple fruits. The soluble dry extract of fruit content at harvest reached the reference value of 12 °Brix for all treatments [24]. According to the standard, pineapple intended to be delivered fresh to consumers after packaging and wrapping must have at least 12 °Brix [25]. This shows the efficiency and importance of the potassium treatment provided to pineapple plants to obtain fruits for export.

5. Conclusion

The application of a high amount of potassium in pineapple cultivation leads to a decrease in phenol biosynthetic enzymes which are PAL and TAL, hence a reduction of total phenol content in the pineapple fruits. Phenol biosynthesis is more oriented towards PAL pathway than that of TAL. Increasing the amount of potassium would increase the membrane resistance making the flesh of the pineapple fruit firmer. This resistance is reflected in the decrease in the incidence of internal browning of fruits.

PPO activity which is the key enzyme for fruit browning, is also inhibited by this membrane resistance. The decrease in PPO and PAL activities is correlated with the total phenols content as well as the internal browning intensity of the pineapple fruit studied. The results clearly show that increasing the amount of potassium in pineapple plants is a simple method that reduces the internal browning process in pineapple fruit. This method also improves the commercial and nutritional quality of pineapple fruits.

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