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Study of salt stress in plantain banana (*Musa paradisiaca* L.): peroxydases and polyphenol oxidases activities, synthesis and accumulation of phenolic compounds in leaves and roots

SeuJonathan GOGBEU[1*], Kouassi Joseph KOUAKOU[2], Koutoua AYOLIE[1], Denezon Odette DOGBO[2]

1. Laboratory of Improvement of Agricultural Production, University Jean Lorougnon Guédé, Côte d'Ivoire

2. Laboratory of Biology and Improvement of Plant Productions, University Nangui Abrogoua, Côte d'Ivoire

**Laboratory of Improvement of Agricultural Production, University Jean Lorougnon Guédé, Côte d'Ivoire, Box 150 Daloa, jgogbeu@yahoo.fr*

CorrespondenceAuthor

Seu Jonathan GOGBEU

jgogbeu@yahoo.fr

Abstract

Plantain banana (Musa paradisiaca)cultivation is booming in many underdeveloped countries such as Côte d'Ivoire. It requires application of chemical fertilizers that make soil salinity and lead to lower plant yields. Aim of this work is to understand behavior of banana against salt stress by evaluation of peroxidases and polyphenoloxidases activities as well as amount of phenols synthesized in plants of 2 (young) and 12 (old) weeks. Salt solutions of 50 and 100 g/l were applied to neck of plants by watering. Results indicated an amplification of enzymatic activities in leaves of young [polyphenoloxidases, 547 (24%) 10^{-3}ΔDO/min/mg prot] and old plants [peroxidases, 560 (4.5%) 10^{-3}ΔDO/min/mg prot] with NaCl 50g/l treatment. Phenol content was higher in leaves [50 (old) and 55 (young) 10^{-3} mg AG/g mf] and roots [100 10^{-3} mg AG/g mf (young)] of plants with NaCl 50 g/l. In conclusion, NaCl can be used to mimic salt stress in plantain banana and, peroxidases, polyphenoloxidases and phenols as markers of plant adaptation.

Key words:*Musa paradisiaca*, phenols, peroxidases, polyphenol oxidases, salt stress

I. Introduction

Plantain banana (*Musa paradisiaca*) is a staple food for thousands of people around world and its crop generates permanent income for a large number of farmers. With an estimated production of more than 20 million tonnes per year [1], plantain bananais fourth most important agricultural product after rice, wheat and maize. Banana trees are grown in more than 120 tropical countries [2] [3]. In Africa, and particularly in Côte d'Ivoire, plantain

is experiencing significant local consumption [4] [5]. Like all plants, plantain banana is subject to attack by several pathogens such as viruses, bacteria, nematodes and fungi that reduce yield [6]. In addition to these biotic factors, plantain banana is exposed to often drastic climatic and edaphic conditions. To increase yield, large-scale plantations are being set up, requiring adequate maintenance such as use of chemical fertilizers and extensive irrigation. However, chemical inputs create a long-term increase in soil salinity, causing a very high osmotic pressure of soil relative to plant roots [7]. This ever-increasing phenomenon in banana plantations may be a long-term danger to food security. Osmotic stress essentially results in toxic accumulation of ions in cells creating a nutritional imbalance [8]. Faced with these stressful conditions, plants implement adaptation strategies. They have complex perception and signaling mechanisms that allow them to produce a more or less specific response to perceived signals [9] [10]. These defenses include cell wall enhancement, stimulation of enzymes such as peroxidases and polyphenol oxidases, production of antibiotic substances such as phenols and activation of several signaling pathways [11]. Peroxidases have been described as inducing growth by catalyzing formation of hydroxyl radicals which cause cleavage of polymers in wall; this eases latter, allowing extension of cell [12]. This production is of great importance in mechanisms of plant defenses against biotic and abiotic constraints. According to Martinez and Whitaker [13] work, phenolic compounds play a role of resistance against microbial and viral infections and also against bad climatic conditions. During microbial infection, they form a barrier that limits proliferation of infection and alteration of plant tissues[14]. Peroxidases and polyphenoloxidases use phenolic compounds as substrates. For adaptation of plants to salt stress, some studies have mentioned that it differs according to species, genotype, physiological state of organs and their ages [15]. Aim of this work was to study influence of sodium chloride on peroxidase and polyphenol oxidase activities as well as synthesis and accumulation of phenolic compounds in plantain leaves and roots.

II. Material and methods

2.1. Biological material

Biological material consists of plantain banana seedlings aged 2 (young plant) and 12 (old plant) weeks. Explants for obtaining seedlings were provided free of charge by a food production group of Daloa, Côte d'Ivoire.

2.2. Methods

2.2.1. Collection and treatment of plants

A sprouter 2.25 m long, 0.5 m wide and 0.40 m deep was used to obtain seedlings. This sprouter was subdivided into 6 bins of same size. Inside of bins was lined with black plastic tarpaulin perforated to let excess water. Each bin was filled with substrate, sawdust. In nursery, substrate used was sawdust mixed with decomposed coffee parsley (½-½). For obtaining plants, PIF technique was used. Plants obtained aged 2 and 12 weeks were arranged in two blocks according to age. Each block was subdivided into two sub-blocks according to concentrations (50 and 100 g/l) in salt solution. For a given concentration, 18 plants were used due to 6 sampling periods. Each solution was provided at 50 ml per plant during root watering. After watering, second row leaves bloomed from apex and roots were harvested at 0, 24, 72, 96, 120 and 168 hours and stored in freezer at 0 °C for previous use. Control plant was 0 h.

2.2.2. Extraction and determination of peroxidases and polyphenoloxidases

Extraction of enzymes was carried out from 5 g of fresh leaves or roots milled in 5 ml of 0.2 M sodium phosphate buffer pH 6 and 10 µl of triton X-100. Ground material was transferred to tubes and centrifuged at 3000 rpm for 12 min at 4 °C. Collected supernatant constituted enzymatic extract. Peroxidase assay was performed according to Criquet *et al*. [16] modified method. Reaction medium contains 1 ml of 2.0 M H_2O_2, 1 ml of 0.01 M pyrocatechol and 0.5 ml of enzymatic extract. Volume of reaction medium was supplemented to 3 ml with sodium phosphate buffer. Incubation was performed at 30 °C for 5 min in dark. Peroxidase activity was determined using a spectrophotometer at 470 nm against a control containing no enzyme extract. As for determination of polyphenoloxidases, it was carried out according to Constabel *et al*. [17] method. Reaction medium is composed of 1 ml of 0.01 M pyrocatechol and 0.5 ml of enzymatic extract. This volume was adjusted to 3 ml with 0.2M sodium phosphate buffer, pH 6. Incubation was carried out at 30 °C for 5 min in dark and

absorbance measured at 595 nm against a control not containing enzymatic extract.Enzymatic activities were expressed as absorbance per minute and per milligram of protein (ΔDO/min/mg prot).

2.2.3. Quantification of phenolic compounds

Extraction of phenolic compounds was carried out according to Gogbeu*et al*. [18] modified method. A mass of 5 g of fresh leaves or roots was milled in presence of 5 ml of ethanol 80% (v/v). Ground material obtained was centrifuged at 5000 rpm for 10 min. This extract was used for determination of phenolic compounds. Assay was done according to Singleton method using Folin-Ciocalteu reagent [19]. For this purpose, 0.8 ml of sodium bicarbonate (Na_2CO_3) 7.5% (w/v) was added to 0.2 ml of phenol extract. After 5 min of incubation, 1 ml of 0.5 N Folin-Ciocalteu reagent was added. Reaction mixture was homogenized and incubated for 45 min at 30 °C. Absorbance was read spectrophotometer at 765 nm against a control containing no phenolic extract. Amount of phenols contained in extract was estimated using a calibration curve made with different concentrations of gallic acid and expressed in milligram equivalent of gallic acid per gram of fresh material (mg AG/g mf).

2.2.4. Quantification of proteins

Extracts used for enzyme assay were also used for determining amount of protein. Protein assay was performed according to colorimetric method of Bradford [20]. Reaction medium is composed of 0.5 ml of enzymatic extract and 3 ml of Bradford reagent. It was adjusted to 4 ml with distilled water and incubated for 30 min at 30 °C. Absorbance was measured at 595 nm. Amount of protein was determined using a standard curve consisting of different concentrations of bovine serum albumin solution. It is expressed in milligrams per gram of protein (mg/gprot).

2.3. Statistical analysis of the data

SPSS software version 11.5 was used to analyze data. Variance analysis (ANOVA) with one and two classification criteria was made at 5% threshold. When $p \leq 0.05$, difference is said to be significant. Homogeneous group'sindividuals are then determined by Duncan's method.

III. Results

3.1. Activities of peroxidases extracted from leaves and roots of plantain banana treated with NaCl

Analysis of Table 1 shows that in control plants peroxidase activity was higher in leaves of young plants than in roots. As for aged plants, enzymatic activity was greater in roots. After treatment of plants with NaCl 50 g/l, in general, enzymatic activity increased in both leaves and roots. In leaves of young plants, it was 73 10^{-3}ΔDO/min/mg prot after 96 hours of treatment. On other hand, in leaves of old plants, it reached value of 560 10^{-3} ΔDO/min/mg prot after 72 hours of treatment with NaCl 50 g/l after a drop observed at 24 h. In roots of same plants, activity of enzyme fluctuated with NaCl contact time. In young plants, a small peak was recorded at 72 h (36 10^{-3}ΔDO/min/mg prot). Peroxidase activity was significant at 168 h (77 10^{-3}ΔDO/min/mg prot). When banana plants were treated with 100 g/l NaCl, in leaves of young and old plants, peroxidase activity was early. It reached value of 100 10^{-3} and 158 10^{-3}Δ DO/min/mg prot, respectively in young and old plants after 24 hours of treatment. After this period, enzyme activity decreased considerably with NaCl contact time. In roots, however, response of plants differed according to age. In young plants, activity of enzyme increased with contact time to reach its maximum at 120 h of treatment. Value was 118 10^{-3}ΔDO/min/mg prot. As for roots of old plants, treatment with NaCl 100 g/l strongly inhibited peroxidase activity.

3.2. Activities of polyphenoloxidases extracted from leaves and roots of plantain banana treated with NaCl

Table 2 shows that polyphenoloxidases activities varied according to organs and age of plants. In young control plants, enzymatic activity was higher in roots (77 10^{-3}ΔDO/min/mg prot) than leaves (22 10^{-3}ΔDO/min/mg prot). In old plants, however, it is the leaves that accumulate more activity of polyphenoloxidases. It was 300 10^{-3}ΔDO/min/mg prot in leaves against 214 10^{-3}ΔDO/min/mg prot in roots. When plants were treated with 50 g/l of NaCl, enzymatic activity increased rapidly in both leaves and roots. In leaves, it was maximum at 24 (old plants) and 72 h (young plants). Values were 485 10^{-3} and 547 10^{-3}ΔDO/min/mg prot. In roots, polyphenoloxidases were highest at same time (72 h) in both young and old plants. However, this enzymatic activity remained important until end of experiment. After treatment of plants with NaCl 100 g/l, two peaks of enzymatic activity

were recorded at 24 (70 10^{-3} ΔDO/min/mg prot) and 96 h (198 10^{-3} ΔDO/min/mg prot.) for young plants and a peak at 72 h (327 10^{-3}ΔDO/min/mg prot) for old plants. In roots, however, in young plants, polyphenoloxidase activity doubled at 72 h (167 10^{-3}ΔDO/min/mg prot) and remained elevated for up to 120 h (130 10^{-3}ΔDO/min/mg prot) before decreasing. As for old plants, a small amplitude was recorded at 72 h (327 10^{-3}ΔDO/min/mg prot). After this time, enzyme activity decreased with NaCl contact time.

3.3. Leaf and root content of phenolic compounds in NaCl treated plantain banana plants

Amount of phenolic compounds varied according to age of plants, organs and concentration of NaCl in medium (Fig. 1). In control plants, leaves accumulated more phenols than roots. In leaves of young plants, value was 2010^{-3} against 25 10^{-3} mg AG/g mf for old plants leaves. At root level, it's also old plants that have more synthesized phenols compared to young plants. When plants were treated with NaCl 50 g/l, 24 hours after treatment, amount of phenols increased very rapidly in roots of young plants (100 10^{-3} mg AG/g mf), before decreasing and increasing at 96 h (64 10^{-3} mg AG/g mf). In leaves of young plants, maximum values were recorded at 24 (42 10^{-3} mg AG/g mf), 120 (55 10^{-3} mg AG/g mf) and 168 h (54 10^{-3} mg AG/g mf) after treatment. On other hand, in leaves and roots of old plants, values were respectively 50 10^{-3} (72 h) and 34 10^{-3} (168 h) mg AG/g mf. After treatment of banana plants with NaCl 100 g/l, no significant increase was recorded. A small amplitude was however observed at 120 h (26 10^{-3} mg AG/g mf) in leaves of old plants.

IV. Discussion

These works in plantain have shown existence of peroxidase and polyphenoloxidase activities as well as presence of phenolic compounds in leaves and roots. In control plants, presence of these enzymes and compounds in these different organs shows that they play a role in growth and development of plant. Moreover, work done by Delamny *et al*. [21] mentioned that peroxidases were involved in several plant functions, including regulation ofhormones, defense against microorganisms and growth. Phenolic compounds contribute to stiffening of cell walls [22]. In same control plants, peroxidase and polyphenoloxidase activities were important in leaves and roots of old plants. On other hand, leaves have accumulated more phenols in both young and old plants. This enzymatic predisposition observed in old plants has been mentioned by some authors. Indeed, work of Kra*et al*. [23] in cassava showed that peroxidase activity was important in old leaves. In general, in rice, Gogbeu*et al*. [24] showed that phenolic compounds were more important in leaves than roots. In plantain banana, same observations were made. Presence of phenolic compounds was greater in leaves (young and old plants) than roots. When plants were subjected to salt stress, enzymatic activities were induced as well as synthesis and accumulation of phenolic compounds. These observations indicate that plantain banana was reactive to saline treatment. Low concentration of 50 g/l stimulated these different metabolisms. In young plants, peroxidases and polyphenoloxidases were stimulated 1.5 and 24-fold, respectivelyin leaves. For old plants, values were 4.5 (leaves) and 2.25 times (roots). Induction of these enzymes suggests synthesis of new forms or latent form activation. For this purpose, works of Gogbeu*et al*. [25] on cassava have shown synthesis of several isoenzymes after treatment of plant with salicylic and phosphorous acids. With the 100 g/l treatment, enzymatic activities were much lower. This inactivation of enzymatic activities would probably be due to an effect of toxicity of NaCl in culture medium. Indeed, in plant acclimation mechanisms, vacuolar compartmentalization or exclusion of toxic ions have been widely evoked by Blumwald*et al.* [26] and Munns and Tester [27]. For these authors, excess of sodium in cytoplasm is rejected to apoplasm to avoid their high concentrations in cytoplasm. This mechanism would thus contribute to maintenance of cell growth under salt stress conditions [28]. In plantain banana, sensitivity to salinity varied by organ, NaCl concentration, and treatment time. However, maximum time ofenzymatic activities was between 24 and 72 hours after treatment. As for synthesis and accumulation of phenolic compounds, high values were recorded in young plants 24 hours after treatment with NaCl 50 g/l. After this period, amount of phenols decreased with NaCl contact time. This strong increase in roots would probably be related to a low level of enzymatic activities at this time or other biosynthetic pathways. According to Hiraga *et al.* [29] and Dogbo*et al.* [30], peroxidases and polyphenoloxidases would probably be cause of decrease or increase of this quantity. In plantain banana, these enzymes would also play the same role found in other plants. Grant and Lamb [31] discuss ability of a plant species to resist microorganisms by content of these organs in phenolic compounds. Salt stress has probably

induced similar effects as biotic stress in plantain banana; which activated enzymes and accumulation of phenols.

V. Conclusion

Peroxidases and polyphenoloxidases as well as phenolic compounds wereactivated in plantain banana aftertreatmentwithlow concentration of NaCl. These enzymes and compoundsthatcontribute to plant resistance to pathogensmaycontribute to adaptation of plantain banana to salt stress. Theycanconstitute markers in study of this plant withsalinity.

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Table 1: Peroxidases activities (10^{-3}ΔDO/min/ mg prot) in organs of plantain banana (*Musa paradisiaca*) plants treated with NaCl

Concentration of NaCl (g/l)	Ages of plants	Organs	Contact time of NaCl (h)					
			Control plant	24	72	96	120	168
50	young	Leaves	50±0.01^{c2}	52±1.04^{c}	54±1.08^{c}	73±1.46^{d}	41±0.82^{b}	2±0.04^{a}
		Roots	21±0.00^{a1}	17±0.80^{a}	36±1.17^{b}	25±1.19^{a}	51±0.42^{c}	77±0.60^{c}
	old	Leaves	124±0.02^{c3}	38±0.30^{a}	560±0.50^{d}	108±0.50^{b}	35±0.28^{a}	24±0.19^{a}
		Roots	169±0.20^{c4}	38±0.22^{a}	310±0.80^{d}	108±0.63^{b}	35±0.20^{a}	95±0.50^{b}
100	young	Leaves	50±0.01^{c2}	100±0.20^{d}	65±1.30^{c}	12±0.20^{b}	45±1.00^{c}	2±0.04^{a}
		Roots	21±0.00^{b1}	38±1.80^{b}	98±0.60^{c}	86±0.09^{c}	118±0.61^{c}	5±0.23^{a}
	old	Leaves	124±0.02^{c3}	158±1.30^{c}	105±0.80^{bc}	124±1.00^{c}	54±0.40^{a}	55±0.40^{a}
		Roots	169±0.20^{c4}	6±0.03^{a}	3±0.01^{a}	58±0.30^{b}	95±0.50^{b}	3±0.01^{a}

Each value isaverage of 3 replicates ± standard deviation. For eachline, meansfollowed a single alphabeticalletter are not statisticallydifferent for a threshold of 5% according to Dancun test. For control plantcolumn, meansfollowed by same figure not statisticallydifferent for a threshold of 5% according to Dancun test.

Concentration of NaCl (g/l)	Age of plants	Organs	Contact time of NaCl (h)					
			Control plant	24	72	96	120	168
50	young	Leaves	22 ± 0.04^{a1}	282 ± 0.80^{c}	547 ± 2.00^{d}	284 ± 1.00^{c}	293 ± 0.31^{c}	148 ± 0.70^{b}
		Roots	77 ± 0.01^{a2}	262 ± 0.40^{b}	300 ± 3.00^{c}	242 ± 0.14^{b}	234 ± 3.00^{b}	84 ± 1.09^{a}
	old	Leaves	300 ± 1.00^{a4}	485 ± 0.60^{c}	473 ± 1.00^{c}	428 ± 0.41^{b}	426 ± 0.40^{b}	413 ± 0.37^{b}
		Roots	214 ± 0.30^{a3}	330 ± 1.00^{b}	482 ± 0.25^{c}	453 ± 0.11^{c}	316 ± 0.47^{b}	300 ± 0.41^{b}
100	young	Leaves	22 ± 0.04^{a1}	70 ± 0.10^{c}	36 ± 0.60^{ab}	198 ± 0.90^{d}	95 ± 0.30^{c}	25 ± 0.13^{a}
		Roots	77 ± 0.01^{b2}	75 ± 0.90^{b}	167 ± 0.16^{d}	150 ± 1.90^{cd}	130 ± 0.60^{c}	44 ± 0.50^{a}
	old	Leaves	300 ± 1.00^{c4}	313 ± 1.01^{c}	327 ± 0.09^{c}	186 ± 0.62^{b}	118 ± 0.39^{a}	119 ± 0.39^{a}
		Roots	214 ± 0.30^{b3}	210 ± 1.00^{b}	327 ± 0.52^{c}	168 ± 0.70^{ab}	125 ± 0.50^{a}	85 ± 0.30^{a}

Table 2: Polyphenoloxidasesactivities ($10^{-3}\Delta$DO/min/mg prot) in organs of plantain banana (*Musa paradisiaca*) plants treatedwith NaCl

Each value isaverage of 3 replicates ± standard deviation. For eachline, meansfollowed a single alphabeticalletter are not statisticallydifferent for a threshold of 5% according to Dancun test. For control plantcolumn, meansfollowed by same figure not statisticallydifferent for a threshold of 5% according to Dancun test

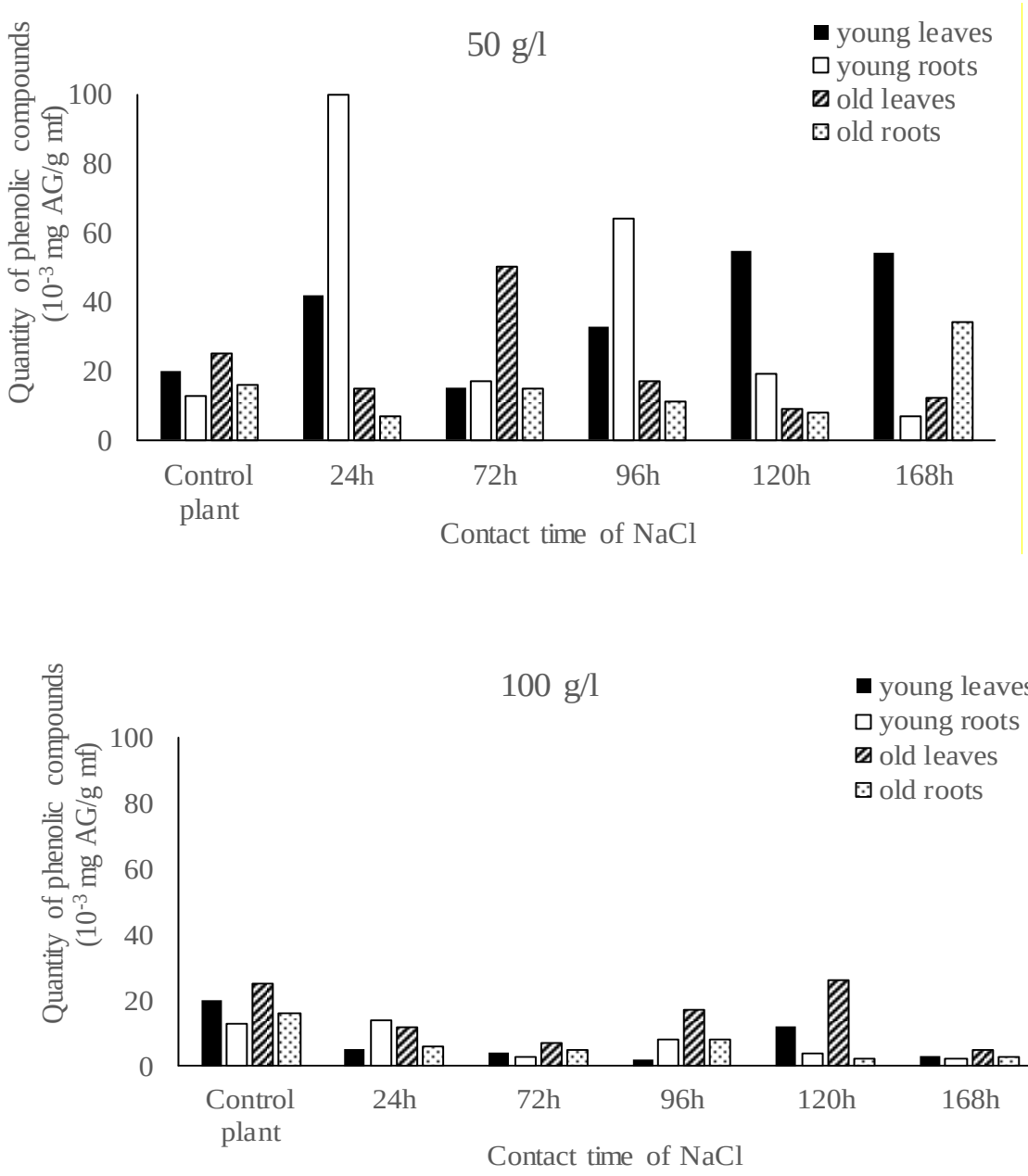

Figure 2 : Quantity of phenoliccompounds in leaves and roots of plantain banana plants treatedwith NaCl