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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

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Abstract

Côte d'Ivoire is one of several developing nations where plantain banana (Musa paradisiaca) farming is increasing. The use of chemical fertilizers is necessary, however they raise soil salinity and reduce crop yields. The purpose of this study is to learn how banana plants respond to salt stress by measuring the amounts of phenols produced by young plants at 2 weeks and mature plants at 12 weeks, as well as by analyzing the activities of peroxidases and polyphenoloxidases. The plants' necks were watered with salt solutions containing 50 and 100 g/l. The results showed that both young and elderly plants' leaves showed an increase in enzymatic activity when treated with 50g/l of NaCl. Specifically, 547 (24%) polyphenoloxidases and 560 (4.5%) peroxidases. Plants treated with 50 g/l of NaCl had greater phenol content in their roots (100 10-3 mg AG/g mf for young plants) and leaves (50 and 55 10-3 mg AG/g mf for elderly plants, respectively). Finally, NaCl can be used to make plantain bananas act like they're under salt stress, and phenols, polyphenoloxidases, and peroxidases may be utilized to identify when plants have adapted.

Key words: Phenols, salt stress, Musa paradisiaca, peroxidases, polyphenol oxidases

Introduction

The plantain banana, scientifically known as Musa paradisiaca, provides a steady source of income for many farmers and is an essential food source for thousands of people around the globe. Plantain bananas are the fourth most significant crop in the world, behind rice, wheat, and maize, with an annual output of about 20 million tons [1]. Over 120 tropical nations cultivate banana plants [2] [3]. Plantain is native to Africa, most specifically Côte d'Ivoire, is seeing a lot of local interest [4] [5]. Viruses, bacteria, nematodes, and fungus are only some of the plant diseases that may harm plantain bananas and lower their productivity [6]. Plantain bananas face extreme biotic variables as well as climatic and edaphic challenges. In order to boost production, massive plantations are being established, necessitating thorough upkeep such the use of chemical fertilizers and heavy irrigation. But chemical inputs raise soil salinity over time, which exerts a lot more osmotic pressure on soil roots compared to water [7]. There may be a long-term threat to food security posed by this continuously occurring phenomena in banana farms. An imbalance in nutrition may occur as a consequence of osmotic stress, which causes cells to accumulate harmful ions [8]. Plants use adaption mechanisms when confronted with these challenging environments. In order to provide a response that is more or less targeted to received signals, they rely on intricate perception and signaling systems

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[9] [10]. Several signaling pathways are activated, cell wall enhancement occurs, enzymes like peroxidases and polyphenol oxidases are stimulated, antibiotic compounds such phenols are produced, and so on [11]. One way that peroxidases are thought to promote cell proliferation is by facilitating the production of hydroxyl radicals, which in turn break down the polymers that make up the cell wall. This makes room for the cell to enlarge [12]. Plant defense systems against biotic and abiotic stresses rely heavily on this production. Research by Martinez and Whitaker [13] suggests that phenolic chemicals help plants withstand harmful environmental factors and infections caused by viruses and microbes. They act as a barrier during microbial infection, preventing the infection from spreading and limiting tissue damage to the plant[14]. Phenolic substances are used as substrates by peroxidases and polyphenoloxidases. Adaptation to salt stress in plants varies with age, species, genotype, and the physiological condition of organs, according to some research [15]. Researching how sodium chloride affects plantain's peroxidase and polyphenol oxidase activities, as well as phenolic compound production and accumulation, was the primary goal of this research.

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 subdividedinto 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 adjustedto 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 5g of fresh leaves or roots was milled in presence of 5 ml of ethanol 80%

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(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.

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/mgprot). 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 ofNaCl, 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, polyphenoloxidaseswere highest at same time (72 h) in both young and old plants. However, this enzymatic activity remainedimportant 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)

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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^{-} DO/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.

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 synthesisof 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

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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.

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.

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