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Effect of Potassium Concentration on Some Agrophysiological Parameters of Pineapple Plants (*Ananas comosus* **L. cv. Smooth Cayenne and MD2) Grown in Côte d'Ivoire**

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Authors' contributions

This work was carried out in collaboration between all authors. Author CS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors YSES and OG managed the analyses of the study. Author KTH managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Introduction: The Ivorian production of pineapple was on the decrease from 213620 t in 1999 to 30000 t in 2013. This decline in the production of pineapple would in part be linked to the quality of the fruit that is acid and especially the sensitivity of the fruit to browning enzymatic. The deterioration of the fruit quality would be due to the absence of good adequate cultural practice. **Objective:** The aim of this study was to determine the effect of the level of concentration of potassium applied on the physiological state of the pineapple plant.

___ **Place and Duration of the Study:** The experimental tests were conducted on the site of Nangui Abrogoua University (Abidjan, Côte d'Ivoire) from July 2015 to the end of October 2016.

Methodology: Four applications of potassium were applied in the 2^{nd} , 4^{th} , 6^{th} and 7^{th} month after sowing, with 20 g of K₂O/plant (T0), 28 g of K₂O/plant (T1), 34 g of K₂O/plant (T2), 40 g of $K₂O/plant$ (T3). The chlorophyll content, potassium and proline accumulation as the biomass of the sheet of pineapples to the floral induction have been determined as a function of the amount of potassium applied to the plant.

Results: The results showed that the biomass and leaf area of pineapple increase with the amount of potassium applied up to 34 g of $K₂O$ /plant. The excess of this nutrient entails a reduction of the chlorophyll synthesis and accumulation of proline in the leaves.

Conclusion: In addition to the report K/N (which must not exceed 2.5), the additional inputs of potassium in the culture of the pineapples must also consider the report K/Mg=1 for a good productivity.

Keywords: Pineapple; biomass; chlorophyll; proline; Côte d'Ivoire; Ananas comosus.

1. INTRODUCTION

Pineapple [*Ananas comosus* (L.) Merrill] is a tropical plant and resistant to drought. It can be grown under schemes in rainfall ranging from 600 to 4000 mm per year. A good rainfall is essential to good yields [1]. Pineapple occupies the third rank of world production of tropical fruits [2] and plays an important role in the economy of many countries. Ivory Coast came first among the African exporters countries with 59,000 tons of fruit exported in 2007. The resources generated by this sector contributed to 1.6% in the agricultural gross domestic product and 0.6% to the national gross domestic product [3]. The Ivorian production of fresh pineapple was declined from 213,620 to 30,000 tons between 1999 to 2013 [4]. This decline in the production of pineapple in Côte d'Ivoire would in part be linked to the fruit quality that becomes too acid and especially the sensitivity of the fruit to browning enzymatic. The deterioration of the quality of the fruit would be due to the absence of an adequate cultural practice. In effect, according to Gomes et al*.* [5], the quality of the fruit depends on several factors such as the planting and procedures postharvest. An appropriate concentration of potassium in the soil is necessary for the production of pineapples of good quality. Therefore, an adequate mineral nutrition is crucial for both a good quality of fruit and a high productivity. In fact, the plants absorb nutrients necessary for their different physiological functions: growth, development, reproduction. A complete mineral nutrition (N, P, K, Mg, Fe, Zn, Bo, Ca) would be indispensable to the growth of the plants as well as to the maintenance of genetic characteristics and the taste of the fruit [6]. Potassium is absorbed by the plants under its ionic form K^+ . It intervenes in the osmotic regulation as well as in the process of opening and closing of the stomata [7]. Potassium is also necessary for several enzymatic functions.

The objective of this work is to study the effect of potassium level application on the physiological state of the pineapple plant. Also, it aims to determine the content of chlorophyll, potassium, proline accumulation as well as the biomass of the sheet of pineapples to the floral induction.

2. MATERIALS AND METHODS

2.1 Site Study

The experimental tests were conducted at Nangui Abrogoua University (Abidjan, Côte d'Ivoire) from July 2015 to October 2016. This site is located between 523' north latitude, 411'west longitude and 100 m above the sea level. The soil is ferruginous and furniture type [8]. The subequatorial climate is characterized by two rainy seasons and two dry seasons. The first rainy season usually takes place from March to July followed by a short dry season between August and September. Then, a short rainy season occurs from October to November. followed by the great dry season that begins in December and ends at the end of February. The precipitation was of 2161.80 and 1433.34 mm with mean annual temperatures of 26.7 and 27°C in 2015 and 2016, respectively according the SODEXAM (Fig. 1).

2.2 Plant Materiel

For this study, the pineapple (*Ananas Comosus* L. Merrill) plant material is obtained from the two varieties, Smooth Cayenne and MD2, cultivated on a large scale in Côte d'Ivoire and which has been rejected from export. These rejections of which the mass is between 350 and 500 g, come from plantations of the area of Bonoua (south of Côte d'Ivoire), a traditional zone production of pineapple. These both pineapple varieties have a production cycle between 13 and 14 months.

2.3 Methods

The preparation of the ground began with place setting of plot. The ridges of 6.5 m length and 1 m width became ready to receive pineapple seedling (3 months old). For every variety, the plantation of the shoots (400 g) was made in staggered rows on four billions according to a device in double line and altogether. The maintenance of the plot of land consisted of a manual and chemical weeding. The manual weeding is regularly made once every month. The chemical weed control was done by means of a Weed-killer (Ampa 30 WP) in the dose of 3 kg for 3000 l/ha. The phytosanitary treatments were applied with an insecticide (chlorpyrifos-etyl 480 g/l) in the dose of 2 l/ha and the fungicide (aluminum phosethyl) in the dose of 7 kg/ha every two months after the burial of the shoots. A nematicide (Nemacur 40 EC) at the dose of 25 ml/l has also been spread at roots zone of plants at dose of 6 ml of the mixture per plant as early as in the second month of culture and/or the fifth month according to the need.

2.3.1 Experimental device

The experimental device adopted for the implementation is in blocks with the repetition of four treatments: T0 (20 g of K_2O /plant), T1 (28 g of K₂O/plant), T2 (34 g of K₂O/plant) and T3 (40 g of K_2O /plant). Blocks measured 6.5 m length \times 1 m width and elementary blocks 1.6 m ×1 m. Every treatment established with 12 plants and triplicate.

2.3.2 Fertilization

The manure used for the planting fertilization had a basic formulation consisting of simple fertilizer represented mainly by the urea to 46% nitrogen (N) and potash in the form of potassium sulphate to 50% potassium oxide (K_2O) and 17% sulfur (S). Fertilizer was completed with 11% nitrogen (N), 5% phosphorus (P_2O_3) , 27% potassium oxide (K_2O) , 15% sulfur (S) , 5% magnesium oxide (MgO) and enriched with oligoelements such as 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) whose Zn chelated EDTA (0.0065 g/l) and manganese chelated EDTA (0.51 g/l). At vegetative growth all treatments (T0, T1, T2, T3) received same amount of fertilization as shown Table 1.

Potassium oxide (K_2O) was applied as a supplement to the classic fertilization of plants. Four applications were applied in 2^{nd} , 4^{th} , 6^{th} and 7^{tn} month after sowing. The quantity varied according to various manures or treatments (T0, T1, T2 and T3). The quantity of potassium at present practiced in a culture of pineapple [9] was taken as a treatment witness (T0). The quantities used according to the applications are recorded in the Table 2.

Fig. 1. Ombrothermic diagram of the experimental site during the essays

CFTE: Complete Fertilizer + trace elements (0.06 ml); Urea (46% N)

Table 2. Total quantities of potassium according to treatments successful candidates by plant of pineapple during the vegetative phase of the cycle

Treatments	Potassium content (g/plant)				Total potassium content
	$2nd$ month	$4th$ month	6 th month	⊸th month	during treatment (g/plant)
			14		28
			16		34
		10			40

2.3.3 Effect of potassium concentration on morphological parameters of pineapple plants

2.3.3.1 Fresh and dry mass, and dimensions of the leaf D

The mass of the fruit at the harvest depends on that of the plant in the TIF, which is closely linked to the mass of fresh material of the leaf D. The latter has to be on average, at least equal to 70 g to activate the TIF to obtain in the harvest, fruits of acceptable caliber in the export [10]. The mass of fresh material of the leaf D taken monthly, is determined by means of an electronic balance of type Sartorius's precision, until the obtaining of an average value at least equal to 70 g [11]. Besides, the length and the width of the leaf D are measured.

2.3.3.2 Surface of the leaf

The surface of the leaf has been determined according to the method described by Ouattara et al. [9]. The leaf D of average weight equal to at least 70 g, has been attached to the assistance of bedbugs on paper of type Canson for weight determination. The paper has been divided according to the form of the leaf and then weighed using a precision balance of type Sartorius. After checking the weight indicated by the manufacturer on the paper, the surface of the

leaf has been determined according to the following formula:

$$
Sf (cm2) = M1 (g)/ M2 (g.cm2)
$$

Where Sf is surface of the leaf D, M1 is weight of paper cut according to the shape of the leaf D and M2 is weight of the paper.

2.3.4 Potassium and physiological parameters of pineapple plants

2.3.4.1 Extraction and content of chlorophyll pigments and carotenoids

The content of chlorophylls (a, b, total) and carotenoids of leaves give an approximation of the photosynthetic activity and the physiological state of the plants. The extraction and proportion of chlorophyll pigments and carotenoids are made according to the method described by Lichtenthaler [12]. Also, for each treatment, 0.1 g of fragment taken from the center of the distal half of the leaf D was incubated in a test tube containing 10 ml of acetone 20% and kept in the dark at 4°C for 48 hours. The whole is then crushed in the presence of a pinch of calcium carbonate and sand of Fontainebleau. The residue of extraction is washed twice with acetone 20%. The ground material was centrifuged at 5000 rpm for 15 min at 4°C. The mass of crushed leaves and the volume of the crude extract are determined. The absorbance is

measured using a spectrophotometer at 663 nm and 647 nm for chlorophyll a and b respectively, and 470 nm for carotenoids. The calculation of the different concentrations is carried out according to the following formulas:

Chl a (μg/g FM) = [(12.25 x A663 – 2.79 x A647) x V]/W

Chl b (μ g/g FM) = [(21.5 x A647 – 5.10 x A663) x V]/W

Chl total (μ g/g FM) = [(7.15 x A663 + 18.71x A647) x V]/W

Car (μ g/g FM) = [(1000 x A470 – 1.82 x Chl a – 85.02 x Chl b) / 198) x V]/W

Where, V is final volume of crude extract, W is weight of leaves, FM is fresh material, Chl is chlorophyll, Car is carotenoids and A is absorbance.

2.3.4.2 Proline content of the leaf D

The proportion of proline is carried out according to the method of Dreier and Göring [13]. The extraction is done on 100 mg of fresh material (FM) of the leaf D, which have been kneaded in the presence of 3 ml of methanol to 40%. The whole is heated in a bain-marie (85°C) for 30 min. After cooling in the melting ice and centrifugation (4000 rpm, 10 min), take 1 ml of extract to which 1 ml of glacial acetic acid is added, 25 mg of ninhydrin and 1 ml of the mixture containing (120 ml of distilled water, 300 ml of acetic acid and 80 ml of phosphoric acid. The solution, well mixed with the help of a vortex, is brought to the boil (100°C, 45 min) until it turns red. After cooling, 5 ml of toluene is added to the solution followed by agitation and then left to rest during 30 min. The upper phase is retrieved and its absorbance is read using spectrophotometer at 528 nm. Proline concentration (C) of leaf D (longest leaf by grouping all the leaves towards the top of the plant) was determined using the following formula:

$$
C = A / (\varepsilon.L)
$$

Where C is proline concentration (mg/g MF), ϵ is the extinction coefficient (0.9986 mM/L/cm), L (cm) is length of the tube spectrophotometer, and A is absorbance.

2.3.4.3 Potassium content of the leaf D

The leaves D were collected at the $11th$ month after growing, just after the beginning of the flowering period. A sample of three leaves D has been taken for potassium determination.

The sample is washed with distilled water and dried in the drying oven 65°C [14]. For every sample, 2 g of ash is transferred in an Erlenmeyer flask and 15 ml nitric acid, 10 ml perchloric acid and 5 ml sulfuric acid were added. The mixture is heated in a boiling bath for 5 min and then left to cool down to the ambient temperature. The whole is filtered on Whatman paper. The filtrate was used for the determination of potassium with the assistance of an atomic absorption spectrometer to flame of Varian type AA 20.

2.4 Statistical Analysis

The data obtained are subject to an analysis of variance (ANOVA). This statistical analysis has been carried out from the test of Newman-Keuls at 5%). The significance of the difference of the medium is determined by comparing the probability *P* associated with the statistics of the test of the Fischer-Snedecor theoretical threshold of $\alpha = 0.05$.

3. RESULTS

3.1 Effect of Potassium Concentration on Proline Content of the Leaf D

The results revealed that there is no significant difference between proline content of the leaves D of Smooth Cayenne and MD2 in the treatment T0 (20 g of K₂O/plant) to T2 (34 g K₂O/plant). On the other hand, a varietal effect was observed with the treatment T3 $(40 \text{ q K}_2O/\text{plant})$. So, the quantity of proline accumulated by the leaves D of the variety MD2 (2.63 μg/g MF) is significantly more important than that of the witness T0. The treatment T3 showed an increase of proline content at MD2 and Smooth Cayenne (Table 3).

3.2 Effect of Potassium Concentration on Foliar Pigments Content in the Leaf D

The results in Table 4 indicated that the content of foliar pigments increases significantly with the quantity of potassium applied for both varieties. The content in chlorophyll (a) is widely higher than that of the chlorophyll (b). That means, there is more chlorophyll (a) than chlorophyll (b) in the old leaves of the studied varieties of pineapple. The analyzed pigments reached their optimum content in the treatment T2 (34 g $K₂O$) plant): the chlorophyll a reached 760 μg/g FM in MD2 and 420 μg/g FM in Smooth cayenne while the chlorophyll b reached 170 μg/g FM in MD2

and 140 μg/g FM in Smooth cayenne. Otherwise, total chlorophyll reached 1470 μg/g FM in MD2 and 1010 μg /g FM in Smooth cayenne. For the treatment T3 (40 g $K_2O\prime$ plant), the results showed that pigments content were appreciably equal to those obtained in the treatment T2 (34 g $K₂O$ / plant). It is necessary to note that the content in chlorophyll pigments is higher in the pineapple MD2 than in the variety Smooth cayenne.

3.3 Effect of Potassium Concentration on Weight and Surface of the Leaf D

The Table 5 showed that the weight of fresh and dry material as well as the surface of the leaf D increases significantly with the applied quantity of potassium. This increase is observed up to a dose of 34 g K_2O /plant (T2). For this treatment, the mass of fresh material gives optimal values of 46.95 and 34.45 g respectively for pineapple MD2 and Smooth Cayenne. The weight of the dried materials gave us optimal values of 9.78 and 6.48 g as well as foliar surfaces respective of 25.08 and 21.09 cm^2 .

For the dose of 40 g K_2O /plant of the treatment T3, the mass of fresh and material as well as the surface of the leaf D give us values statically below those of the treatment T2. The results also show that the biomass of the pineapple MD2 is higher than that of the variety Smooth cayenne.

In column and on a line, the values followed by the same letter are not significantly different (Test of Newman-Keuls to 5%). T0 (treatment with 20 g of K2O/plant); T1 (treatment with 28 g of K2O/plant); T2 (treatment with 34 g of K2O/plant); T3 (treatment with 40 g of K2O/plant)

In column and on a line, the values followed by the same letter are not significantly different (Test of Newman-Keuls to 5%). T0 (treatment with 20 g of K2O/plant); T1 (treatment with 28 g of K2O/plant); T2 (treatment with 34 g of K2O/plant); T3 (treatment with 40 g of K2O/plant)

Treatments	Pineapple varieties		
	MD ₂	Smooth Cayenne	
T0	37.49 ± 0.39 d	25.16 ± 0.39 a	
Τ1	39.87 ± 1.14 c	25.32 ± 0.68 a	
T2	46.95 ± 0.53 a	34.45 ± 0.37 e	
T3	44.75 ± 0.64 b	31.93 ± 0.65 f	
T0	4.26 ± 0.27 d	3.93 ± 0.11 d	
T1	6.49 ± 0.31 b	4.11 \pm 0.34 d	
T2	9.78 ± 0.44 a	6.48 ± 0.39 b	
T3	7.54 ± 0.33 b	5.57 ± 0.39 b	
T0	17.32 ± 0.51 c	16.19 ± 0.23 c	
Τ1	19.26 ± 1.04 b	17.31 ± 0.91 c	
T2	25.08 ± 0.67 a	21.09 ± 1.06 b	
T3	21.47 ± 0.36 b	19.00 ± 0.57 b	

Table 5. Evolution of fresh and dry material mass as well as the surface of the leaf D according to the various treatments

*For a parameter, in a column and on a line, the values followed by the same letter are not significantly different (Test of Newman-Keuls at 5%). T0 (treatment with 20 g K2O/plant); T1 (treatment with 28 g K*₂O/plant); *T2* (treatment with 34 g *K*₂O/plant); *T3* (treatment with 40 g *K*₂O/plant)

3.4 Effect of Pineapple Plant Treatment by Potassium on Its Accumulation in the Leaf D

The potassium content in leaves is significantly more important in MD2 than in Smooth cayenne whatever the applied potassium treatment (Fig. 2). However, a progressive increase of the content in potassium is observed when the quantity of potassium brought to plants is high. So, treatment T3 allows an accumulation of potassium in the leaves more significantly important than T2 followed by T1 and finally T0. For example, in T2, the accumulation of potassium in leaves is 25 mg/kg in MD2 while it is 15 mg/kg in Smooth Cayenne. Also, with T3, the content in potassium of leaves increased in MD2 (29.96 mg/kg) with regard to Smooth cayenne (20 mg/kg).

Fig. 2. Evolution of potassium content in the leaf D of both pineapple varieties in function of the fertilizing treatments

The histograms topped by different letters have significantly different contents (Test of Newman-Keuls at 5%). T0 (treatment with 20 g of K2O/plant); T1 (treatment with 28 g of K2O/plant); T2 (treatment with 34 g of $K_2O\prime$ *plant);* T3 (treatment with 40 g of $K_2O\prime$ *plant)*

4. DISCUSSION

The good vegetative growth of a plant is translated by its photosynthetic activity and its biomass. This growth requires a good mineral nutrition. In our study, an increase of the biomass and the value surface of pineapple are observed with the quantities of potassium which go from 20 to 34 g from K_2O /plant. This result is likely to show that potassium is an essential element in the nutrition of the pineapple as testified to by several authors [5,10]. These results are observed even though all the plants received the same quantity of nitrogen (16 g/plant) which is the essential element for the vegetative growth of plants [15]. Indeed, potassium seems to act in an indirect way on the chlorophylls synthesis through the magnesium *via* the report $K₂O/MgO$ = 1 [7]. The decrease of the biomass in the treatment T3 (40 g $K_2O\prime$ plant) shows that this quantity of potassium is excessive for the good growth of the pineapple plants. The excess of potassium seems to cause a stress at the nutritional level of the plant. Consequently, the accumulation of proline in plants as an answer to the stress would be more important [16,17]. In the plants under treatment T3 (40 g $K_2O\prime$ plant), the strong accumulation of proline compared with the other treatments, would be a sign of metabolic disturbance [18]. This stressful situation would be bound to the increase of the applied quantity of potash. Indeed, this amino acid is a part of osmoticums which the plants synthesize once exposed to the abiotic stress [19-21]. Furthermore, proline is responsible for the osmotic adjustment and its accumulation allows the plant to resist the stress [22].

This stress could be saline because the zones of culture are close to the sea. Besides, the role of potassium in the resistance to this stress is already reported by several studies [23,24]. The resistance of the pineapple plants to the degree of soil salinity is going to be noticed in their growth and their development. Indeed, the analysis of the physiological state of plants through the quantification of the foliar pigments would reflect the vigor and the good growth of plants [25]. Thus, the pigments analyzed in this study showed the existence of a correlation between the content and the brought quantity of potassium. The content in chlorophylls a, b and total as well as in carotenoids increases with the quantity of potassium applied per plant (from 20 to 34 g per plant). These results revealed a good photosynthetic activity and then a good

physiological state of pineapple plants. As a result, the secondary metabolism responsible for the formation of the molecules of defenses is activated [26].

However, the content of pigments remains static beyond the concentration of 34 g $K₂O$ /plant. The quantity of potassium of the treatment T3 (40 g of $K₂O/plant$ would be too plentiful and then stressful for the plant. Therefore, this stress would cause a reduction in the synthesis of foliar pigments. The excessive contribution of potassium can cause a magnesium deficiency [7]. Indeed, magnesium is a mineral not a chelating agent which intervenes in the constitution of the chlorophyll. It permits to maintain the integrity of the chlorophyll. Thus, its absence causes a destruction of the chlorophyll and thus a loss of vigor of the plants [25]. This mineral also plays the role of cofactor in several enzymatic reactions. So, the accumulation of potassium beyond 34 g/plant is fatal in the good physiological growth of the pineapple. It will be thus essential in the collection (quest) of reduction of the internal tanning of the pineapple to consider this effect denial of potassium not to reduce the potential of production of the cultivated plants. The excess of potassium would thus justify the reduction in the chlorophylls synthesis observed with the treatment T3 (40 g/plant). The high quantity of potassium might cause a magnesium deficiency which would engender a stress to the pineapple plants, hence the accumulation of proline in the treatment T3 [27-29]. Furthermore, the potassium stress also causes an inhibition of the acid 5 aminolévulinique which is a precursor for chlorophylls synthesis [30,31].

Thus, it could also justify the content in lower chlorophyll a less important photosynthetic activity of plants handled by important quantities of potassium of the treatment T3 (40 g/plant in our case) compared with the plants of the treatment T2 (34 g/plant). Moreover, several works also confirm the existence of a competition between proline and the chlorophyll for the same precursor that is the glutamate [32,33]. Concerning the potassium concentration in the leaf D, the results revealed that it increases as the applied quantity of potassium increases. The additional contribution of potassium in the soil would be a good technique to increase the potassium concentration in the plant [5]. These results indicated that potassium absorbed by the roots is forwarded to the shoot part of the plant. The increase of plant potassium content could be

an indicator for salt tolerance of the plant [34, 35].

The results showed a reduction of the chlorophyll synthesis in spite of the increase of potassium content of leaves in the treatment T3. It indicates that a high concentration of the ion K^+ in leaves would be a limiting factor for the photosynthetic activity. The excess of potassium would cause a magnesium deficiency [7]. This magnesium deficiency could be assuming as a nutritional stress. Indeed, chlorophyll is a chlorine chelating cation of magnesium (Mg^{2+}) in the center of the macrocycle and esterifying a terpenoïd alcohol in $C₂O$, the phytol, which is hydrophobic and using as anchoring of proteins of thylacoïds membranes [36]. In addition, the decrease of chlorophyll content could be due to a progressive loss of magnesium ion [27].

5. CONCLUSION

At the end of this study, it should be noted that potassium is an essential element in the pineapple plant nutrition. According the results, the maximum amount of potassium to be applied per pineapple plant is about 34 g. Beyond this dose, potassium becomes toxic and disrupts the development and growth of pineapple plants. It also has negative impacts on the physiological parameters of the plants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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