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(Research Article)

Determinism of some phytohormones and potassium nitrate on the vegetative growth of Tangelo (*Citrus x tangelo*) in the Sudano-Sahelian area of Mali

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Abstract

In Mali, citrus fruits contribute significantly to the nutritional balance of the population due to their high content of vitamin C, carotenoids and polyphenols. The citrus sector in Mali is rich in several species, but the current trend in citrus production is towards the production of Tangelo (or *Citrus×tangelo*). The objective of this study was to determine the effects of phytohormones 2,4,5-T, AG_3 and potassium nitrate (KNO_3) on vegetative growth of Tangelo. The trial was conducted in Koulikoro district, especially on the experimental plot of the IPR/IFRA of Katibougou. The plant material used is *Tangelo orlando* with white pulp. The experimental design was a Fisher block with three replications. The three products and water (check) were applied by spraying the leaves of the young plants at 110 days after planting. Observations were made on the following growth parameters: graft diameter, graft length and number of vegetative branches at 90th, 180th and 270th days after the application. The results of the analysis of variance of the different treatments revealed significant ($p<1\%$ and $p<5\%$) and non-significant differences. The phytohormone 2,4,5-T followed by AG3, showed the best growth in height, diameter and number of vegetative branches at all measurement periods. Weak to very weak effects of KNO3, and water were found on all studied parameters. The application of 2,4,5-T on Tangelo at the plant's seedling stage improved vegetative growth which may result in early flowering and could improve yield and fruit quality in Sudano-Sahelian area of Mali.

Keywords: Phytohormone; Growth; Tangelo; Citrus; Sudano-Sahelian area

1. Introduction

Citrus trees are an important economic resource in the production of their fruits that are appreciated worldwide. They are also called under the name of agrumes and belong to the family Rutaceae, grouping trees and shrubs count 3725 known varieties [1]. The origin of the genus Citrus is precisely from Southeast Asia in Indian region [2]. Citrus growing occupies an important place in Africa in general and in tropical Africa in particular [3]. Citrus fruits contribute significantly to the nutritional balance of the populations. The orange, mandarin, lemon and pamplemousse trees are the four dominant citrus species in Mali. However, the current trend in citrus production is towards the production of Tangelo, which is derived from hybridization between the mandarin and pamplemousse trees [4]. For the past five years, new citrus plantations have been made up mainly of Tangelo plants. The citrus processing sector is essentially domestic and artisanal for the moment [4]. Malian citrus production is not yet sufficient to satisfy the growing demand

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due to population growth and changing eating habits. Part of the citrus supply, particularly clementines in the country's urban centers, comes from Morocco [5]. According to growers, the fight against the massive importation of clementines on the Malian market will only succeed with the Malian Tangelo, which is also highly appreciated by consumers [4].

One of the main challenges for citrus growers in Mali is the low productivity of the trees. This study was initiated to assess strategies for improving citrus and especially Tangelo production in Mali. In other countries such as Morocco, growers apply hormones on different phases of plant development, especially citrus [6]. Hormones and salts play a crucial role in plant growth and development.

The objectives of this study are to determine the effects of two phytohormones (2,4,5-T and AG3) and a mineral salt (KNO3) on vegetative growth of Tangelo.

2. Material and methods

2.1. Experimental site

The trial was conducted in Koulikoro district, precisely in the orchard of the “Institut Polytechnique Rural de Formation et de Recherche Appliquée” (IPR/IFRA) of Katibougou. The urban commune of Koulikoro is located about 60 kilometers east of Bamako. It extends over 14 kilometers along the Niger River, wedged for the most part between the river and the hills of Mount Mandingue. Katibougou is a village in the urban commune of Koulikoro (Figure 1). The geographical coordinates of the site where the trial was conducted are: 12°54.866' North latitude; 07°31.852' West longitude and 288 m altitude. With an average annual rainfall of 850 mm. The average temperature is 39°C in the hot season and 21°C in the cool season (data from the local weather [7]. The main soil types found at the site are tropical ferruginous soils with a silty to sandy-silty texture at the surface and a silty texture at depth [8].

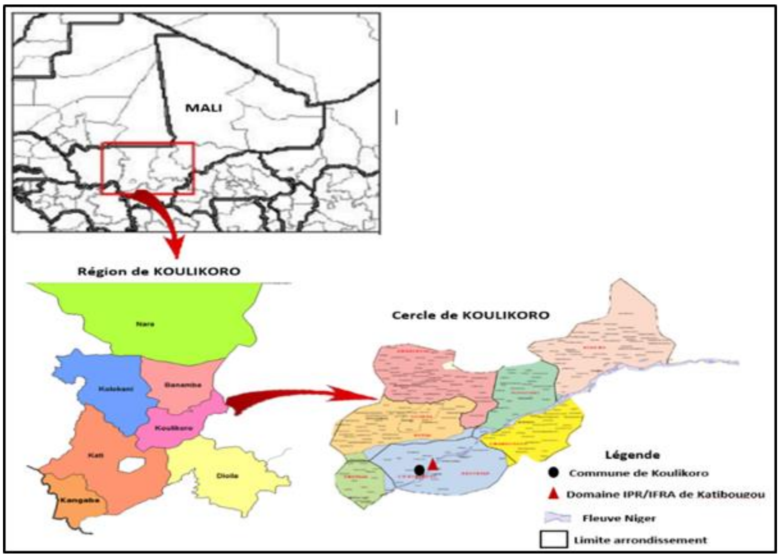

Figure 1 Location map of Koulikoro District [9]

2.2. Material

2.2.1. Plant material

The plant material used is the white-fleshed Tangelo orlando, a citrus variety whose seedlings were obtained from the Genetic Resources Unit (URG) of the Rural Economy Institute (IER) of Bamako. The plants were grafted five months before being transplanted. The grafting stock was young bigaradier plants of eight months old, bearing Tangelo branches of an average size of 30 cm from grafted mother plants that had fruited well.

2.2.2. Phytohormones and salt used

2, 4, 5 - trichlorophenoxyacetic acid (or 2, 4, 5 - T).

2, 4, 5-T, is a synthetic auxin with the gross formula $C_8H_5Cl_3O_3$. It is an herbicidal active substance that was, among other things, a component of Agent Orange, an herbicide used on a large scale during the Vietnam War [10].

Gibberellins (GA)

Gibberellins (GA) are phytohormones that regulate various developmental processes including stem elongation, germination, dormancy, flower development, leaf and fruit senescence. Gibberellins are referred to as AG_1 through AGn in order of discovery. Gibberellic acid, which was the first gibberellin to be structurally characterized, is AG_3 [11].

Potassium nitrate (KNO3)

It is a mineral salt, which contains potassium, the third nutrient of the plant after nitrogen and phosphorus. In contrast to hormones, the plants produce sap from water and mineral salts from the soil. They extract these from their substrates (soil). Potassium can be associated with phosphorus and nitrogen (NPK) [12].

2.3. Methods

In order to improve the vegetative growth of Tangelo, a trial was conducted with three growth products 2,4,5-T; AG_3; KNO_3 and plus simple water (H_2O) as a check on young plants (Seedlings). That is to say a total of four treatments:

T0 = Young plants sprayed with water
T1 = Young plants sprayed with AG_3
T2 = Young plants sprayed with 2,4,5-T
T3 = Young plants sprayed with KNO_3.

2.3.1. Method of application of the products

All three products plus water were applied by spray (Technoma 15 sprayer) to the leaves of young plants at 110 days after planting in July 2016. The 2,4,5-T phytohormone was applied at a concentration of 5 mg/liter of water. As this hormone is insoluble in water, we dissolved it in 10ml of 70° alcohol. The AG_3 hormone is applied at a concentration of 2 mg/liter of water. The salt KNO_3 is applied at a concentration of 80 g/liter of water. And finally, the check plot was sprayed (treated) with simple water.

2.3.2. Experimental design

The trial was conducted in a simple Fischer block design consisting of three blocks or replicates. A repetition is made up of four elementary plots of 20x5 m, i.e. an area of 100 m^2.

Planting rows were drawn before the holes were marked out. This was done by staking the plot using the 3-4-5 method to identify right angles. Along each transect, the locations of the holes were marked. Holes of 50 cm in diameter and depth were dug before receiving 30 kg of organic fertilizer or 12 tons per ha. In addition to this organic fertilization, 30 g of carbofuran per hole was applied to control nematodes and insects. Grafted seedlings were planted on July 25, 2016, at the exact location of the holes so that the tree collar was 10 cm from the soil surface. The young plants were assigned to the holes in the elementary plots randomly. After planting, a 50 cm radius basin was created around each plant. The young plants were then watered at a rate of 20 liters of water per plant.

2.3.3. Trial management

Irrigation

To avoid water stress to the plants, a supplementary irrigation was necessary. For this purpose, the principle of the double basin was adopted; a small basin of 50 cm in diameter which prevents direct contact of water with the stem of the tree (risk of diseases such as citrus gum disease or phytophtora). And a large basin of 100 cm diameter to retain the irrigation water.

Fertilization

An initial uniform dose of 100 g of urea, cereal complex and K_2SO_4 was applied per Tangelo plant to the whole of the foliage plumb line on the ground. The other doses of fertilizer 44 g of ammonia phosphate, 40 g of potassium sulfate and 92 g of urea were buried in the soil when irrigation was restarted.

Maintenance works on the experimental plot

In the first year some works of periodic weeding of the basins, of pulverizing with discs or a superficial plowing in order not to destroy the superficial roots were carried out. These works are followed by scarification for the emollition and the cleaning of the soil. In the second and third year; the same maintenance works were carried out.

2.3.4. Observations and measurements

The reference point for the measurements was marked with red paint at 1 cm above the grafting point. A preliminary follow-up of the evolution of the length (height) and the diameter of the graft of the seedlings before the treatment (110th day) with the four products was made.

The growth parameters observed were as follows:

Graft length

The length (height) of the graft was measured immediately on the day of application of the products and on the 90th, 180th, 270th day after application products. The difference between the initial length and the length obtained during the observation periods is made to obtain the height increase (or growth gain) of the graft (cm).

Graft diameter

Graft diameter (cm) at day 90, 180, 270 after application products was measured.

Number of vegetative branches

Number of vegetative branches at day 180, 270 after application products.

2.3.5. Data analysis

The statistical analysis was based on the comparison of the different treatments using an analysis of variance with the STATITCF 5.0 statistical software, followed by a comparison of the means using the Newman and Keuls test at the 5% threshold. This analysis concerned all the measured parameters (length, diameter and number of vegetative branches). The results obtained are then represented in the form of graphs according to the treatments, using EXCEL software.

3. Results

3.1. Experimental conditions

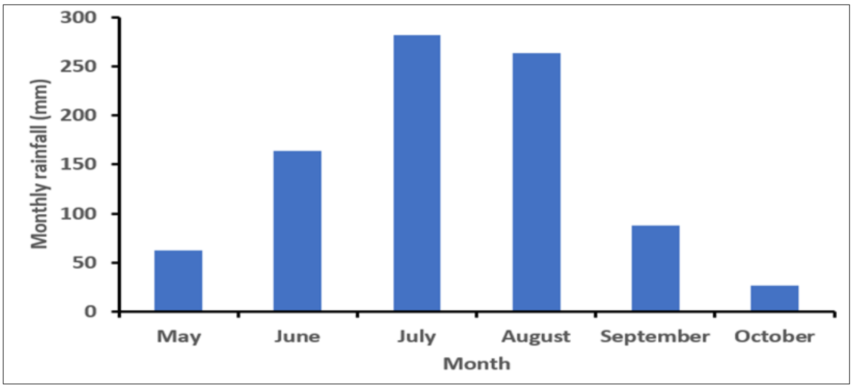

Figure 2 Data rainfall during the first year of planting in IPR/IFRA of Katibougou 2016 [7]

Rainfall amounts by month in the first year of planting in 2016 are shown in Figure 2. July (planting period) was the month in which the highest rainfall was recorded (281.8 mm sufficient for planting needs). August was the second most rainy month with a total of 263 mm, followed by June (163.9 mm). October can be considered the driest month of the season. The water needs of the plants were covered by supplemental irrigation.

3.2. Experimental results

Before the treatment on day 110, the monitoring of the evolution of the length (height) and diameter of the graft showed no significant difference between the plants. All grafts had statistically the same lengths and diameters.

3.2.1. Length (Height) of the graft (cm)

This parameter in our example concerned mainly the differences in size between the initial length and the length at the measurement period. The gains in graft height growth are shown in Figure 3. The analysis of variance of the results revealed a highly significant difference ($p<1\%$) between the effects of the four products sprayed on the plants.

The application of the Newman-Keuls test at the 5% significance level, at 90 days after application (90 DAA) products, showed four distinct homogeneous groups (A, B, C and D). According to this classification, we find the greatest increase in length of the seedlings sprayed with the 2,4,5-T hormone (T2) and significantly higher than the control sprayed with water (T0). It is followed by the seedlings sprayed with AG3 hormone (T1) and KNO3 salt (T3) (Figure 3).

At 180 days after application (180 DAA) products, the use of Newman-Keuls test at 5% significance level showed three distinct homogeneous groups (A, B and C). We record an average increase of 0.15 cm and 0.16 cm in graft length of seedlings sprayed with AG3 and 2,4,5-T hormone respectively (Figure 3). The smallest increase in graft height, is observed in seedlings sprayed with KNO3 salt and water with a gain of 0.09 and 0.05 cm.

At 270 days after application (270 DAA) products, the results show us, three distinct homogeneous groups (A, B and C) following the application of the Newman-Keuls test at the 5% significance level (Figure 3). The highest growth in height of the graft was observed in treatments T2 and T1, both statistically equal, and the lowest in treatments T3 and T0.

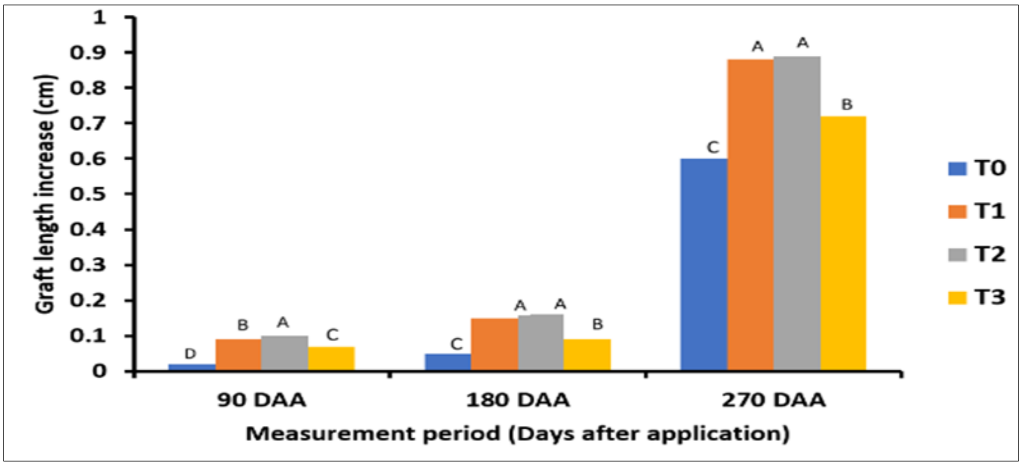

Figure 3 Increase in graft length at day 90, 180, 270 after application products

3.2.2. Graft diameter

Analysis of variance on graft diameter at day 90 after application products revealed a Highly Significant difference between treatments ($p< 1\%$). The use of the Newman-Keuls test at the 5% significance level showed four distinct homogeneous groups (A, AB, B and C). According to this classification, we observe that in treatments T2 and T1, the increase in diameter the most rapid with respective diameters of 0.52 and 0.50 cm (Figure 4). These growths are statistically higher than those observed for treatments T3 and T0 with 0.45 and 0.20 cm in diameter respectively.

Analysis of variance of graft diameter at day 180 after application revealed a highly significant difference ($p< 1\%$) between treatments. Four homogeneous groups were obtained with the use of the Newman-Keuls test at the 5% significance level (A, B, BC and C). The graft of T2 increased more in diameter at day 180 (0.73 cm), followed by T1 and T3 with 0.55cm and 0.47 cm respectively (Figure 4). The graft of the T0 also increased slightly at day 180 after treatment but statistically different from the T1 and T2 treatments.

Highly significant difference (p< 1%) between treatments was found in the analysis of variance of the results on graft diameter at day 270 after application the four products. And three homogeneous groups (A, B and C) were obtained with the use of the Newman-Keuls test at 5% significance level (Figure 4). The treatments T2 and T1 still have the highest average values of growth in diameter and statistically superior to T3 and T0 (Figure 4).

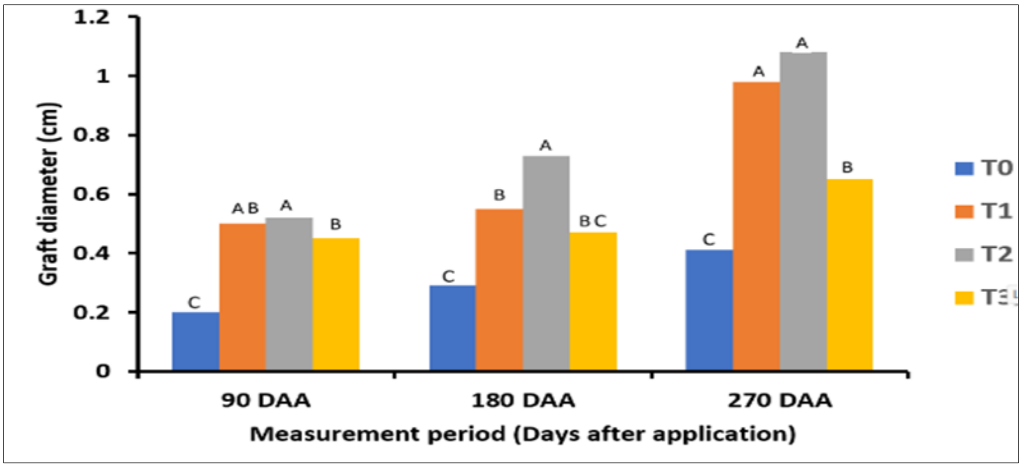

Figure 4 Graft diameter at day 90, 180, 270 after application products

3.2.3. Number of vegetative branches

We observed the emergence of vegetative branches on the grafts from the 90th day after application products. The analysis of variance on the number of branches at day 180 after application did not show a significant difference between treatments. The Newman-Keuls test at the 5% significance level showed a single homogeneous group (Figure 5). This indicates that the number of observed vegetative branches was statistically identical for all four treatments.

At day 270 after application, analysis of variance on the number of vegetative branches revealed a highly significant difference (p< 1%) between treatments. Application of the Newman-Keuls test at the 5% significance level showed three distinct homogeneous groups (A, B, and C). According to the classification, we note that grafts from treatments T2 and T1 produced on average the highest number of vegetative branches with 8.42 and 8.18 branches, respectively, and that these values are statistically equal but different from the values in treatments T3 and T0.

After day 270, we observed a cessation of vegetative growth in all grafts and the beginning of reproductive growth.

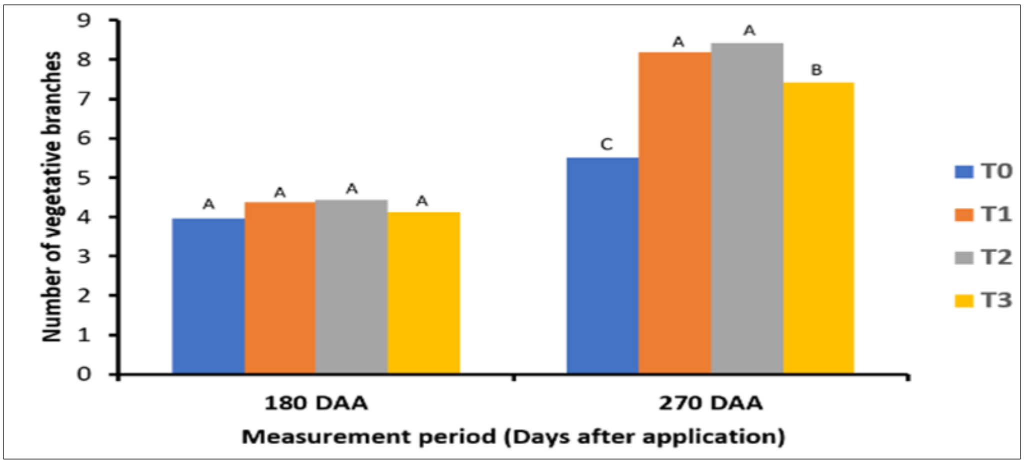

Figure 5 Number of vegetative branches on days 180, 270 after application products

4. Discussion

The difference in vegetative growth between Tangelo seedlings reveals the influence of phytohormones on their development.

The evolution of the graft length and diameter showed no significant difference between plants before the application of the products at day 110 after planting. These results are in agreement with the works of Gener [13] who reported that the growth and homogeneity of a grafted plantation, depend on the rootstock family and the scion, the rootstock/graft association. In citrus, the rootstock plays a determining role in the growth rate and vigor of the trees [14]. The phytohormones 2,4,5-T (T2) and AG3 (T1) showed a difference in growth (size, diameter and number of vegetative branches), which was observed from the third month of product use. This result is confirmed by the hypotheses of [15] and [16]. Duval et al. [15] stated that early spraying with GA3 at a concentration of 5x10-4 g.ml-1 induces an increase in height and diameter in the plant. Conde [16] also said that foliar sprays of auxins at 25 and 40 days after sowing showed an increase in the rate of stem elongation and leaf formation. According to Hannah and Jan [17], the growth of a crop represents the quantitative and qualitative transformations that accompany the course of the different stages of its life from implantation to maturity. The use of hormones has led to an earlier development of the plants (the plants enter reproduction very early because they reach vigor and size early). This is in agreement with the work of Champagnat et al. [18[8]], who showed that growth (increase in height, diameter and appearance of branches) in fruiting plants, especially citrus, stops at the time of floral induction.

5. Conclusion

Citrus is economically important, representing the most important group of fruits in international trade. The goal of this study was to evaluate the influence of some phytohormones and potassium nitrate on Tangelo or *Citrus×tangelo* vegetative growth.

The vegetative growth parameters (height, diameter and number of vegetative branches) depend globally on the effect of phytohormones (2,4,5-T and AG_3) and salt (KNO_3). The grafts of plants sprayed with phytohormones have a better vegetative growth. The effect of the mineral salt (KNO_3) was much less, but better than that of water. This shows that the treatment of seedlings with phytohormones improves their growth compared to mineral salt. We find that 2,4,5-T is the best phytohormone for the growth of Tangelo seedlings and it also allows the seedlings to go quickly from vegetative to reproductive growth under the growing conditions in the Sudano-Sahelian area of Mali.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declared that there is no conflict of interest.

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