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Full Length Research Paper

Effect of two cytokinins in combination with acetic acid α-naphthalene on yams (*Dioscorea* spp.) genotypes' response to *in vitro* morphogenesis

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The effect of two growth regulator combinations was studied on the morphogenesis *in vitro* of 3 genotypes of yams (*Kounondakou, Gnon-boya* and *RB 89579*). Benzyl aminopurin (BAP) and zeatin (ZEAT) were tested, respectively at a concentration of 0.5 mg/l with the galzy glutamine basic medium containing naphtalene acetic acid (NAA) (0.5 mg/l). Stem fragments were used as explants. The number of stripped buds and explants having stem and roots are sampled after 2 weeks in culture. The dry matter content, the number of roots and leaves and the height of each young sprout were determined after 5 weeks in culture. The results obtained indicated that no break in leaf growth was observed on the control medium (without cytokinin) but media with BAP and zeatin presented a good plants aerial part development. A significant interaction ($p < 0.05$) was observed between the genotypes and the type of cytokinin. However, the highest bud sprouting and shoot development were obtained with BAP. Thus, BAP can be considered as cytokinin having a good morphogenic aptitude when compared to zeatin for yam micropropagation.

Key words: Cytokinin, auxin, morphogenesis, yam, Benin.

INTRODUCTION

Yam (*Dioscorea* spp.) is a tuber plant of the family Dioscoreaceae with great importance in feeding and is widely cultivated in West Africa. Benin Republic is in the fourth world rank with a production of about 6% of the worldwide production, compared to Nigeria (67%), Ghana (10%) and Côte d'Ivoire (8%) (FAO, 2005a). About half of the population in Benin Republic uses yam as basic food (Dansi et al., 1999). The quantity of fresh tubers of yam produced in 2005 is evaluated to 2.7 million tons compared to 1.2 million tons in 1995 (FAO, 2005b). Despite this importance, yam remains one of the scarce plants with less farming techniques improvement. An important part of the harvest is used as seeds for the subsequent season, and this reduces the part of the production available for food consumption. The average proportion of the harvested yam converted in seed beets was estimated to be between 25 and 50% (Foua-Bi, 1993; Zoundjihékpon, 1993; Hinvi and Nonfon, 2000). Thus, it is important to find out an alternative way for seed beets production which could increase yam availability. Plant tissue and organ *in vitro* culture seems to be the best way to solve this problem. However, the success of *in vitro* plant cell culture depends on several factors which are mainly the genotype of donor plant (Arzani and Mirodjagh, 1999; Schween and Schwenkel, 2003; Gandonou et al., 2005), the age of the explant (Caswell et al., 2000; Delporte et al, 2001) and the culture medium composition (Murashige and Skoog, 1962; Saharan et al., 2004; Ahanhanzo et al., 2003, Ahanhanzo et al., 2008). Among these factors, the culture medium composition,

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Abbreviations: BAP, Benzyl aminopurin; **ZEAT,** zeatin; **NAA,** naphtalene acetic acid; **2GG,** glutamined galzy medium; **MC,** control medium; **ANA,** acetic acid α-naphthalene.

Table 1. Effect of BAP (0.5 mg/l) and zeatin (0.5 mg/l) on the number of stripped buds and the number of explants with stem of 3 genotypes of *Dioscorea* after 2 weeks in culture.

Genotypes	Number of stripped buds			Number of explants formed stem		
	MC	MC + BAP	MC + Zeat	MC	MC+ BAP	MC + Zeat
Kounondakou	10.33	12.33	8.67	0	4.67	0.33
Gnon-boya	5.33	6.33	1.67	0	2.33	0
RB 89579	7.33	4.67	4.2	0	0	0

MC: Control medium containing ANA and without cytokinin.

especially the nature and the concentration of the growth regulators used in its composition need to be investigated. The purpose of this study was to improve yam *in vitro* propagation with the objectives to study the influence of two cytokinins associated with an auxin on *in vitro* morphogenesis of three yams varieties from Benin.

MATERIAL AND METHODS

Plant material

The plant material used consists of three (3) genotypes of yams (*Kounondakou, Gnon-boya* and *RB89579*) collected at Ina in the experimental station of the Institut National des Recherches Agricoles du Bénin (INRAB). *Kounondakou* (KD) and *Gnon-boya* (GB) are respectively, early and late varieties belonging to the complex *Dioscorea cayenensis-rotundata*, while variety RB89579 (RB) belongs to the species *Dioscorea alata*. The choice of these 3 varieties is guided by the fact that they are well adapted to all the agroecologic zones of Benin. In addition, they are less required, in regards to the climatic conditions and their tubers are snuffed and appreciated by the consumers.

Methods

The tubers of these various yam genotypes were put in a greenhouse for germination to obtain the mothers plants. Fragments of stem were taken on these mothers plants as explants. The explants were disinfected with alcohol 70% and mercuric chloride 0.1%. Explants were laid out vertically in test tubes containing 20 ml of culture medium with one explant per tube. The three culture media used have as base, the glutamined galzy medium (2 GG), according to Doukouré (2000) and Ahanhanzo et al. (2003). The basic medium was supplemented with various types of growth regulators. The control medium (MC) contains only 0.5 mg/l of acetic acid α-naphthalene (ANA); the two other media consisted of the control medium (MC) to which either 0.5 mg/l of 6-benzylaminopurine was added (MC + BAP), or 0.5 mg/l of zeatin was added (MC + ZEAT). The pH of media was adjusted to 5.7 ± 0.1 with NaOH solution (0.1 N) before the addition of sucrose (30 g/l). Media were solidified by bacteriological agar (8 g/l) before autoclaving for 15 min at 121°C. Cultures were placed in a culture room at 27 ± 1°C equipped with lamps ensuring a light intensity of 5.000 lux. The photoperiod was 12 h of light per day. Tests were followed during five (5) weeks of culture. At the end of the second week, parameters such as the number of explants having strip, the number of explants having stems and the number of explants with roots have been sampled to evaluate explants development. At the end of the fifth (5th) week of culture, the dry matter, the leaf and root numbers as well as the plant height were determined. All the experiments were repeated separately at two different occasions. For each experiment, 30 explants of each variety of yams were used for each culture medium.

Statistical analysis

The analysis of the main effects of BAP or zeatin was based on a 1-way analysis of variance (ANOVA). All statistical analyses were performed by SAS 92 software. Means were calculated and Student, Newman and Keuls' test was used to classify these means.

RESULTS

Effect of the cytokinins used on buds strip, stem and roots formation

The results of the effect of BAP (0.5 mg/l) and zeatin (0.5 mg/l) on buds growth and development of the three (3) yam genotypes used after 2 weeks of culture are presented on Table 1. The average number of buds stripped on control medium (MC) is 10.33 for the variety *Kounondakou* against, 5.33 and 7.33, respectively, for varieties *Gnon-boya* and RB89579 (Table 1). On medium MC + BAP, the average number of stripped buds is 12.33 for *Kounondakou*, 6.33 for *Gnon-boya* and 4.67 for *RB89579*; while on medium MC + ZEAT, variety *Kounondakou* presents the highest means value (8.67). In addition, variety *Gnon-boya* presents the smallest mean value (1.67). Moreover, at the end of the second week, *Kounondakou* presents a strong aptitude to be stripped in comparison to the two other varieties and BAP improved buds stripping. On the control medium, no variety had stem. The mean number of stems formed on medium MC+BAP is 4.67 for variety *Kounondakou* against 2.33 for variety *Gnon-boya* and none for variety *RB89579*. On medium MC+ZEAT, only *Kounondakou* formed stems with an average of 0.33 (Table 1). At the end of the second week of culture, BAP induced the building of stems for varieties *Kounondakou* and *Gnon-boya.* No root formation was observed in the presence of cytokinin *(*neither with BAP nor with zeatin*)* but the control medium induced roots formation with an average of 5.67 explants forming roots for *Kounondakou* against 2.33 for *RB89579* and 0.67 for *Gnon-boya* (Table 2).

Table 2. Effect of BAP (0.5 mg/l) and zeatin (0.5 mg/l) on the number of explants formed roots of 3 genotypes of *Dioscorea* after 2 weeks in culture.

Genotypes	Medium		
	MC	MC + BAP	MC + Zeat
Kounondakou	5.67	0	0
Gnon-boya	0.67	0	0
RB 89579	2.33	0	0

MC: Control medium containing ANA and without cytokinin.

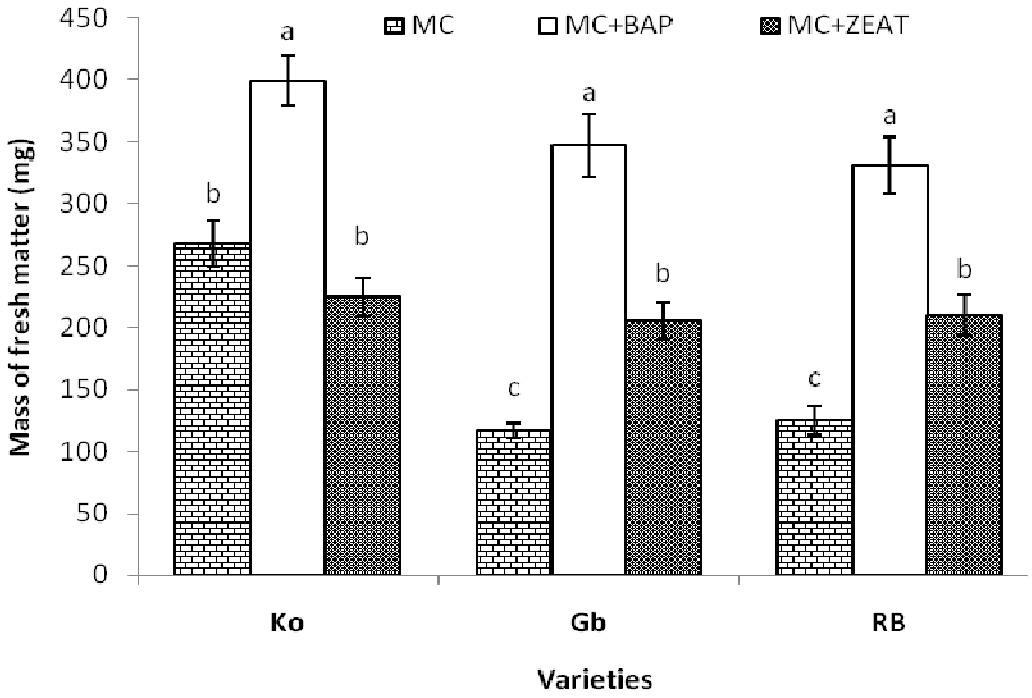

Figure 1. Effect of BAP (0.5 mg/l) and zeatin (0.5 mg/l) on mass of fresh matter of 3 genotypes of *Dioscorea* after 5 weeks culture on three different media. Values with different letters are significantly different ($p < 0.05$). MC: Control medium containing ANA and without cytokinin.

Effects of the two studied cytokinins on the growth of the plants obtained *in vitro*

Effect on the mass of fresh matter

The results of the effect of BAP (0.5 mg/l) and zeatin (0.5 mg/l) on the mass of fresh matter are presented on Figure 1. From this figure, the mass of fresh matter obtained on control medium, varies according to the genotype. The strongest mean value of the mass of fresh matter is obtained with variety *Kounondakou* (260 mg) against 102 and 115 mg for varieties *Gnon-boya* and *RB89579,* respectively. The application of BAP at the concentration of 0.5 mg/l shows a significant increase ($p < 0.001$) of the mass of fresh matter for the three varieties. The highest mass of the fresh matter (399.64 mg) was obtained with variety *Kounondakou* against 267.84 mg on the control medium. However, the action of the BAP showed more with the varieties *Gnon-boya* and *RB89579,* when the mass of fresh matter obtained on medium MC + BAP was compared with the one obtained on the control medium. Zeatin applied at the same concentration did not show any effect on the mass of fresh matter of *Kounondakou*. On the other hand, it caused a significant increase ($p < 0.05$) of the mass of fresh matter of varieties *Gnon-boya* and *RB89579*, comparatively to the control medium.

Effect on the mass of dry matter

The mass of dry matter produced by the three varieties varies according to the genotype and culture medium (Table 3). The mass averages of dry matter obtained on

Table 3. Effect of BAP (0.5 mg/l) and zeatin (0.5 mg/l) on the mass of dry matter of 3 genotypes of *Dioscorea* after 5 weeks culture on three different media.

Varieties	Culture medium	Mass of dry matter
Kounondakou	MC	43.74± 3.27b
	MC + BAP	67.17 ±3.78 a
	MC + ZEA	38.05 ± 2.79 b
	P>F	<0.0001
	CV (%)	36.46
	Means	49.65 ±2.31
Gnon-boya	MC	14.62 ±0.6 c
	MC + BAP	65.49 ±5.3 a
	MC + ZEA	38.97 ±3.09 b
	P > F	<0.0001
	CV (%)	50.19
	Means	39.64 ± 3.02
RB89579	MC	17.02 ±1.93 c
	MC + BAP	52.93 ±3.73 a
	MC + ZEA	38.76 ±3.18 b
	P > F	<0.0001
	CV (%)	46.02
	Means	36.24 ±2.34

Values with different letters are significantly different ($p < 0.05$).
MC: Control medium containing ANA and without cytokinin.

the control medium are 43.74 mg for variety *Kounondakou*, 14.62 mg for *Gnon-boya* and 17.02 mg for *RB89579*. These averages showed a clear increase in the medium containing BAP where the weakest average (52.93 mg) was obtained for *RB89579* and the highest (67.17 mg) was recorded for *Kounondakou*. However, it is important to note that the strongest increases were observed for varieties *Gnon-boya* and *RB89579* when compared to *Kounondakou*. Except for the variety *Kounondakou* for which zeatin did not have any signi-ficant effect, the dry mass of *Gnon-boya* and *RB89579* increased to a significant level ($p < 0.05$) with zeatin.

Effect on leaf number

BAP induced a significant increase ($p < 0.05$) in leaf number of varieties *Kounondakou* and *Gnon-boya*. The leaf number of *Kounondakou* and *Gnon-boya,* was less than 1 leaf in the control medium and increased with an average of approximately 2 leaves on the medium containing Benzylaminopurin (Figure 2). The action of this growth regulator on variety *RB89579* is less accentuated with a leaf number from an average of approximately 1.2 leaves on the control medium to an average of 1.7 leaves on medium MC + BAP (Figure 2). Zeatin has a similar action on *Kounondakou* and *Gnon-boya* with an increase in half of the average of the leaf number compared to the MC. In addition, zeatin remained without any effect on RB89579. BAP induced the appearance of leaves more than zeatin for all varieties.

Effect on roots number

The effect of BAP and the zeatin results in a significant reduction in root number for the three varieties (Table 4). Root number has an average of 1 root on media MC + BAP and MC + ZEAT, whereas this average is 2 roots in the control medium.

Effect on roots length

BAP induced a significant decrease ($p < 0.05$) in roots length by the three varieties (Figure 3). The reduction in length observed is higher with the varieties *Kounondakou* and *Gnon-boya*, compared to variety *RB89579*. The highest average for roots length on the medium containing BAP is by 3.08 cm and is obtained for variety *RB89579*, whereas it is by 5.01 cm on the control medium for *Kounondakou*. Zeatin effect is similar to the one of BAP, except that the reduction observed is significant ($p < 0.05$) only for varieties *Kounondakou* and *RB89579*. The highest average value of roots length recorded on the medium containing zeatin is of 2.81 cm for *Kounondakou* and 5.01 cm for the same variety by the control medium (Figure 3).

Effect on shoot height

BAP produced a significant increase ($p < 0.01$) in the height of *vitro* plants for the three varieties. The effect of BAP was clearly dissociated from that of zeatin with all the tested varieties (Figure 4). The highest height was 5.42 cm for K*ounondakou* on medium with BAP against 0.7 cm on control medium and 4.1 cm on medium with zeatin. For *Gnon-boya*, plant height was about 4.22 cm on medium with BAP against 0.7 cm on control medium and about 3.2 on medium with zeatin; while it was about 5.02 cm on medium with BAP against 2.19 cm on control medium and about 3.5 cm on medium with zeatin for variety *RB89579*. Plant height has also been significantly increased under the effect of zeatin on all varieties and this increase is more important with variety *Kounondakou.*

DISCUSSION

The results recorded during the second week of culture showed that BAP improved better axillaries buds sprouting for all yam varieties studied and facilitated the appearance and the development of the stems and leaves of variety *Kounondakou*. These results confirm the conclusion on *Quercus suber* L. where BAP appears to

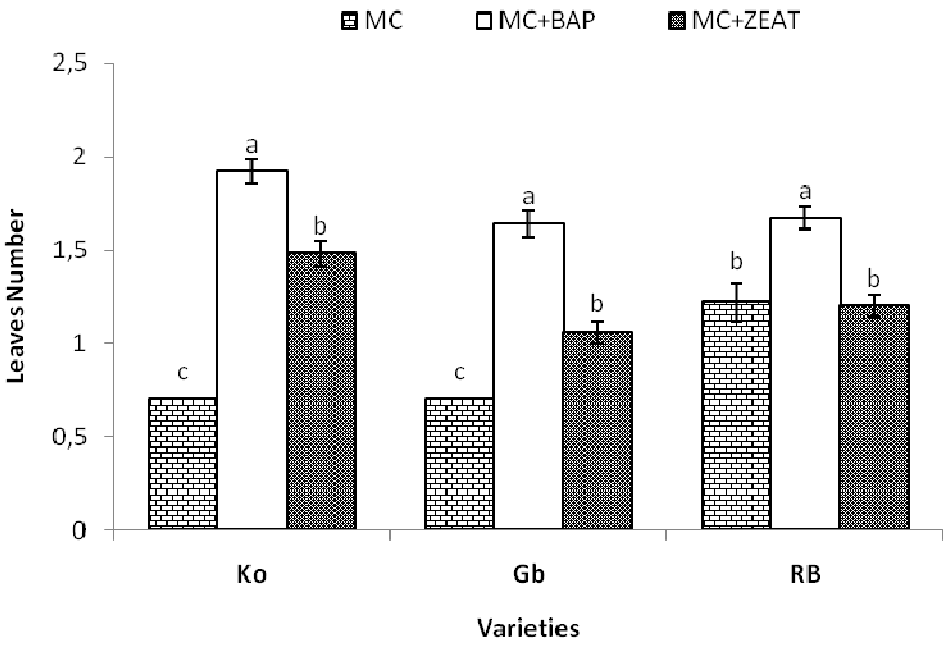

Figure 2. Effect of BAP (0.5 mg/l) and zeatin (0.5 mg/l) on leaves number of 3 genotypes of *Dioscorea* after 5 weeks culture on three different media. Values with different letters are significantly different ($p < 0.05$). MC: Control medium containing ANA and without cytokinin.

Table 4. Effect of BAP (0.5 mg/l) and zeatin (0.5 mg/l) on root number of 3 genotypes of *Dioscorea* after 5 weeks in culture on three different media.

Varieties	Culture medium	Roots number
Kounondakou	MC	2.99 ±0.16 a
	MC + BAP	1.03 ±0.09 b
	MC + ZEA	1.35± 0.12 b
	P>F	< 0.0001
	CV (%)	39.82
	Means	1.79± 0.12
Gnon-boya	MC	2.05 ±0.12 a
	MC + BAP	1.04 ±0.09 b
	MC + ZEA	1.21 ± 0.06 b
	P > F	< 0.0001
	CV (%)	35.54
	Means	1.44 ± 0.07
RB89579	MC	2.27 ±0.17 a
	MC + BAP	1.19 ±0.08 b
	MC + ZEA	1.21 ± 0.05 b
	P > F	< 0.0001
	CV (%)	39.,59
	Means	1.59 ± 0.08

Values with different letters are significantly different ($p < 0.05$).
MC: Control medium containing ANA and without cytokinin.

be the best growth regulator adapted to axillary buds sprouting (Kbiach et al., 2002). The absence of stems on the different control medium confirms the importance of cytokinins for the development of plant aerial organs, compared to auxins, (Kbiach et al., 2002). The stimulation of the development of the aerial part of *vitro* plants in the presence of cytokinins is followed by an inhibition of the development of root part. This observation was reported to be the classical effect of cytokinins on the aerial and root parts of plants *in vitro* (Zrÿd, 1988). Compared to the results for the fifth week, the effect of BAP and zeatin on *vitro* plants showed an improvement of various parame-ters such as mass of fresh matter, mass of dry matter, number of leaves and height of the plant. However, BAP generally showed its efficiency in comparison to zeatin. These results are similar to those obtained by Hunter et al. (1984) on strawberry plant where BAP leads to an increase in fresh matter weight. These results confirm also those recorded by Saleil et al. (1990) who noted that, the nature and the amount of the cytokinin had determined significantly, variations of growth on various genotypes of *Dioscorea* sp. Moreover, Vuylsteke and Delanghe (1985) also proved that BAP showed its efficiency when compared to zeatin with regards to the micropropagation of banana tree. Mantell and Hugo (1989) reported that cytokinins induced an increase in the mass of dry matter of the bulblets of *Dioscorea bulbifera* Will. Better, with the same species, Toklo (2000) obtained similar results with variety Afassie Blanche, a Togolese variety. Compared with kinetin, BAP showed the best shoot growth for other yam varieties of Benin (Montcho, 2004).

The response of the various varieties of yams tested in

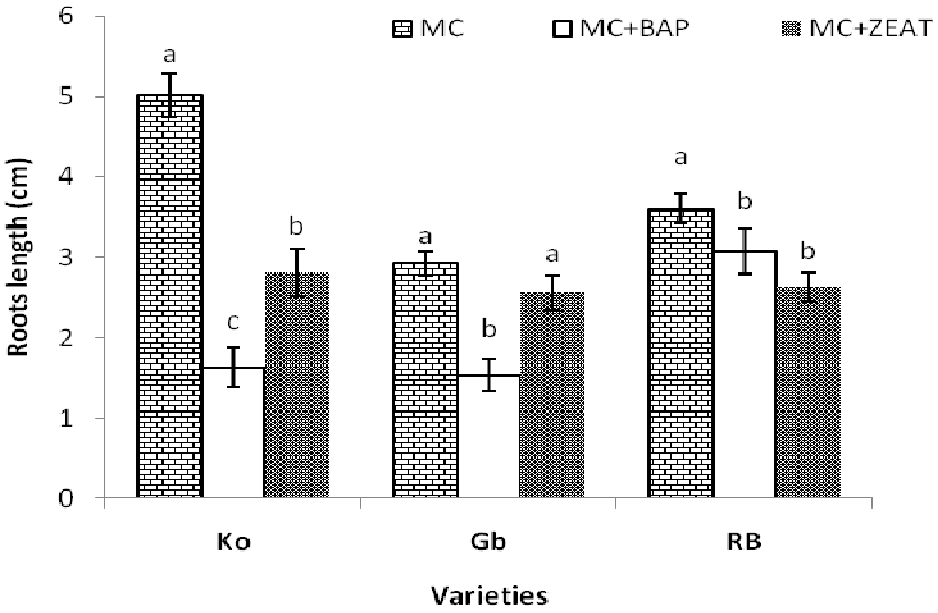

Figure 3. Effect of BAP (0.5 mg/l) and zeatin (0.5 mg/l) on roots length of 3 genotypes of *Dioscorea* after 5 weeks in culture on three different media. MC: Control medium containing ANA and without cytokinin. Values with different letters are significantly different ($p < 0.05$).

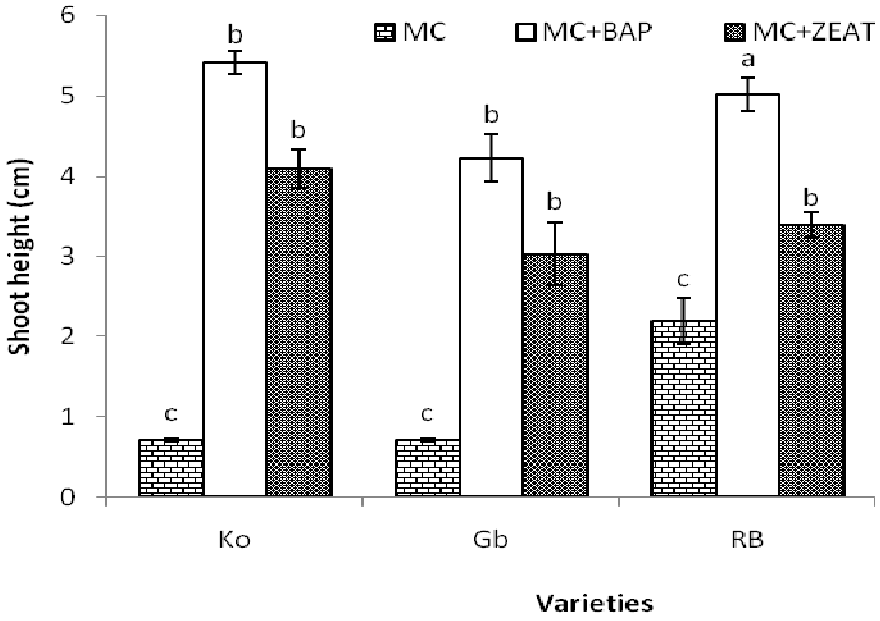

Figure 4. Effect of BAP (0.5 mg/l) and zeatin (0.5 mg/l) on shoot height of 3 genotypes of *Dioscorea* after 5 weeks in culture on three different media. MC: Control medium containing ANA and without cytokinin. Values with different letters are significantly different ($p < 0.05$).

the current study changes for the same type of growth regulator. Among these, the variety *Kounondakou* behaved better in the presence of BAP as well as in the zeatin. However, root length is more important in the presence of zeatin than in the presence of BAP with the variety *Gnon-boya*, as previously reported by Forsynth and Van Staden (1984) on *D. bulbifera* Will. In the same way, for the micropropagation of the olvier (*Olea europea*), zeatin seems to be the most suitable cytokinin (Rokba et al., 2000; Abdelhadi et al., 2005). Based on

this fact, the response of explants to the action of cytokinins depends in a large extent on the genotype of the plant.

Conclusion

This study shows that the media with BAP and zeatin presented a good development of yam plant aerial parts. BAP appeared to improve strongly the morphological parameters in comparison with zeatin. Thus, BAP is retained as the cytokinin having a good morphogenic aptitude when compared to zeatin for yam micropropagation. Among the tested varieties, *Kounondakou* showed the best response to the cytokinins action.

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