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Effects of different hormones on organogenesis *in vitro* of some varieties of cassava (*Manihot esculenta* CRANTZ) grown in Senegal

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Cassava (*Manihot esculenta*) is a perennial euphorbiaceous shrub grown mainly for its starchy tubers and its leaves rich in protein. The most known method of propagation of this crop is the classical cuttings' planting. However, *in vitro* propagation appears most useful and permits to obtain high quantity of healthy vegetable material in a short period. In this work, it was to study the impact of different hormones on the organogenetic capacities *in vitro* of five cassava varieties cultivated in Senegal. Axillary uninodal sections were disinfected and cultured in Murashige and Skoog (MS) basal medium added or not of different concentrations (0.1, 0.5 and 1 mg/L) of auxin (α-naphthalene acetic acid (NAA)) or cytokines (benzyl aminopurine (BAP) and kinetin). Best shoot growth and rooting was observed in MS medium containing 0.1 mg/L kinetin with normal development of the leaves. Highest proliferation of shoots and leaves was obtained with medium MS + BAP 1 mg/L. Callus formation was observed in all media containing hormone but most in MS + NAA 1 mg/L. This work proposes a rapid and economic technique for cassava multiplication.

Key words: Organogenesis in vitro, cassava, varieties, hormones, Senegal.

INTRODUCTION

Manihot esculenta known as cassava is a plant-tubers grown mainly in tropical regions where it presents a high economic importance. According to FAO (1995) it is the most important locally-produced food in a third of the world's low-income and food-deficit countries, especially in Africa. Its starchy roots and high-protein leaves are consumed at home or sold, in fresh or in processed form. Because of its simple technology of culture and large flexibility, cassava is often grown in rural areas where other crops fail (Thro et al., 1998).

However, cassava propagation using cuttings classical method is not adequate for rapid and healthy

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multiplication. The annual production of vegetable material of this plant is very low (average 10 cuttings per plant per year) and yields are reduced by pests and diseases attack, essentially by the cassava african mosaic, the cassava bacterial blight due to *Xanthomonas campestris* and the floury cochineal (*Phenacoccus manihotis*).

An alternative method that proves to remediate the low coefficient of multiplication and infections of cassava is micropropagation *in vitro*. It consists of regenerating whole plants by cultivating explants in artificial medium, sterile conditions and controlled environment (light and temperature). Several plants conserving genetic identity are obtained from successive generations and can be multiplied to infinity. According to Guo and Liu (1995), hundreds of millions of plants derived from culture *in vitro* are produced annually in the world.

Micropropagation is therefore a rapid and powerful technique of multiplication that permits to obtain plants in sufficient quantity to satisfy continuous production independent of the seasons. Indeed, contrarily to the classical method that gives a single individual by seed or cutting, *in vitro* propagation may produce as many copies as liked from a single explant (Rancillac, 1981) and help to overcome constraints related to the availability of high quality of planting material (Wheatley et al., 2005; Vaillant et al., 2005).

The work of Fereol (1978) highlighted perfectly the interest of culture *in vitro* in cassava. The ability of vegetative propagation *in vitro* has been estimated in african clones, to a potential of production of a million plants a year from a single cutting (Lourd, 1981). Thus, with this method of propagation, research can make a significant contribution to food security and economic development in the areas of culture of cassava (Thro et al., 1998). This contribution is more interesting when it comes to propagate resistant or tolerant varieties to major constraints to the culture.

In this present study, it was made to study the effect of some hormones on organogenetic capacities *in vitro* of 5 varieties of cassava grown in Senegal and selected for their tolerance to termites ravaging cuttings *Odontotermes* sp. aff. *erraticus* (Faye et al., 2014). The general objective of the study was to determine the best medium for an optimal response of cassava to multiplication *in vitro*. Specific objectives were to assess the effects of different hormone concentrations on the growth and development (caulogenesis, rooting, shoot and root growth, phyllogenesis and callus formation) *in vitro* of the different varieties of cassava.

MATERIALS AND METHODS

Plant material

The plant material was composed of five cassava varieties cultivated in the Department of Tivaouane (Senegal) including 3 local: *Soya*, *Cololi* and *Niargi* and 2 Brazilian: *Cacau* and *Cacau roja*. Cuttings were collected from farms and transplanted into pots placed in a greenhouse. They were watered every two days at 1/2 L per cutting. Fungicide and insecticide treatments using respectively 50 mg/L mancozeb and 2 ml/L chlorpyrifos-ethyl were conducted in this culture. Plants obtained have provided explants which were used for material of introduction *in vitro*.

Sterilization

After a brief wash with soap followed by 3 successive rinses, explants have been soaked for 20 min in a solution of 80 g/L calcium hypochlorite with 2-3 drops of tween 20, then in bleach for 10 min. Each soaking was immediately followed by 3 successive rinses with sterile water.

Culture media and control

The basal nutrient used was the complete MS (Murashige and Skoog) (1962) medium. It was solidified with 8 $g.L^{-1}$ agar at pH adjusted 5.7 and contained 25 $g.L^{-1}$ of sucrose. This medium was or not supplemented with NAA (α-naphthalene acetic acid), BAP (benzyl aminopurine) or kinetin at different concentrations (0.1, 0.5 and 1 mg/L). The media were distributed in glass test tubes at 20 ml/tube and then sterilized by autoclaving at 110°C for 20 min. The surface disinfected explants were placed vertically on the media, in sterile conditions. Cultures were incubated in the dark for 24 h and then transferred to the culture chamber lighted with 4000 lux where they were maintained at 27 ± 1°C, under a photoperiod of 13 h day.

A sample of 12 tubes per medium per variety was considered for the parameters assessment. Weekly measures have been conducted on the 3rd generation plantlets during 30 days of culture. The parameters evaluated were the shoot and root numbers and growth, the number of leaves and the rate of callus formation.

Statistical analyses

Data collected on this study were entered on Excel and analyzed with software Costat. They have been subjected to analysis of variance of the Student, Newman and Keuls test at the 0.05 level of significance.

RESULTS

Effect of hormones on caulogenesis *in vitro* of the different varieties of cassava

Figure 1 shows that the addition of hormones in the basal medium MS favored multiple shoots formation in all the varieties of cassava. Indeed, average 5.3 shoots by plantlet were count in the variety *Soya* in the MS + BAP 0.1 mg/L medium after four weeks of culture. Highest numbers of shoots were observed in medium containing BAP or kinetin. Medium with NAA were however less favorable to caulogenesis. The variation of shoots number were significant ($F = 0.383$; $P = 0.05$) among the different hormonal concentrations experimented, according to statistics.

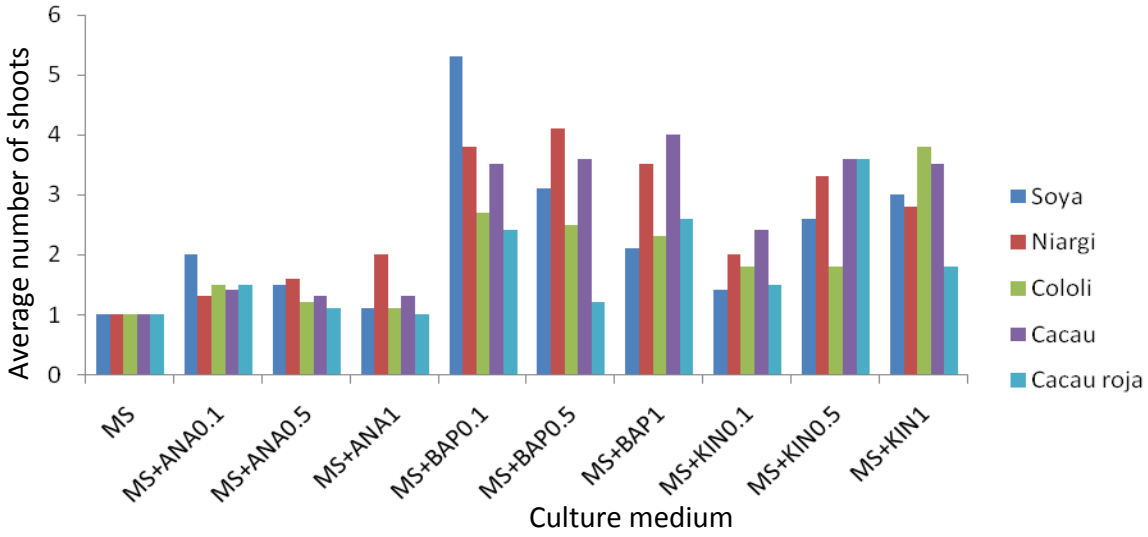

Figure 1. Influence of different hormonal concentrations (mg/L) and control on shoots' proliferation *in vitro* of 5 varieties of cassava after 1 month of culture.

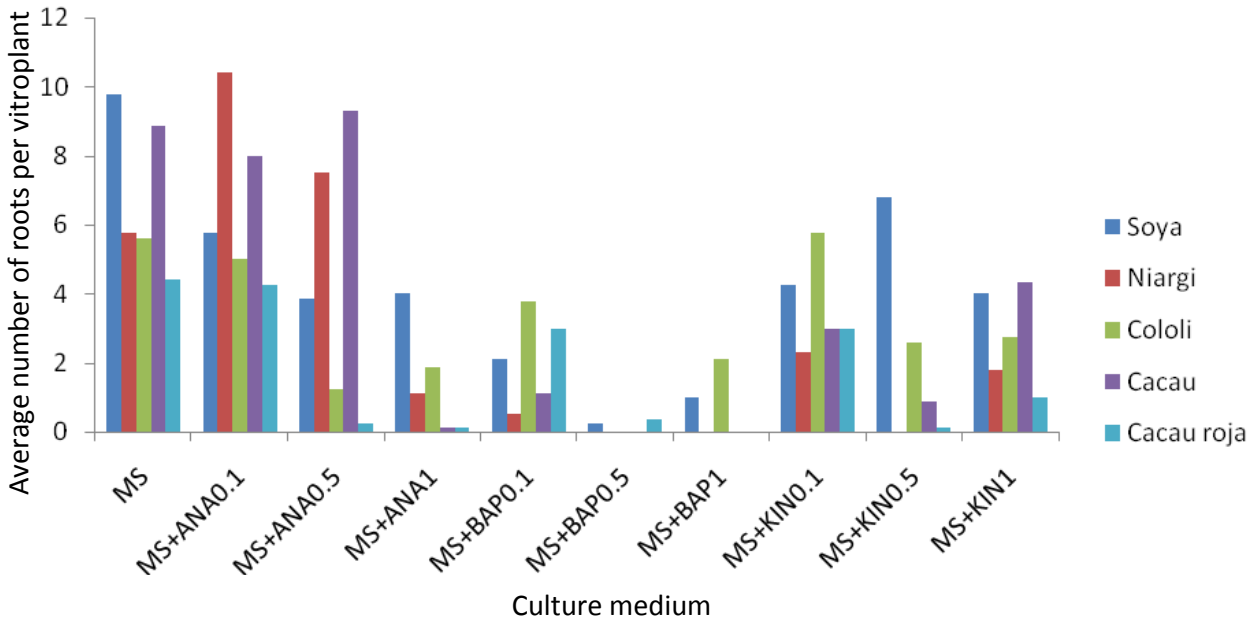

Figure 2. Influence of different hormonal concentrations (mg/L) and control on rooting *in vitro* of 5 varieties of cassava after 1 month of culture.

Effect of hormones on rooting *in vitro* of the different varieties of cassava

The extent of root formation *in vitro* in cassava depends on the culture medium. We noted that the addition of BAP, especially at high doses in the MS medium reduced strongly the formation of roots. Indeed, no rooting was observed among the varieties *Niargi*, *Cacau* and *Cacau roja* in the MS + BAP 1 mg/L medium during 1 month of culture. In contrast, highest numbers of roots (average 10.4 per plantlet in variety *Niargi*) were found in MS + NAA 0.1 mg/L after 4 weeks of culture (Figure 2). According to ANOVA, the number of roots in the 5 varieties of cassava varied significantly (F = 3.453; P =

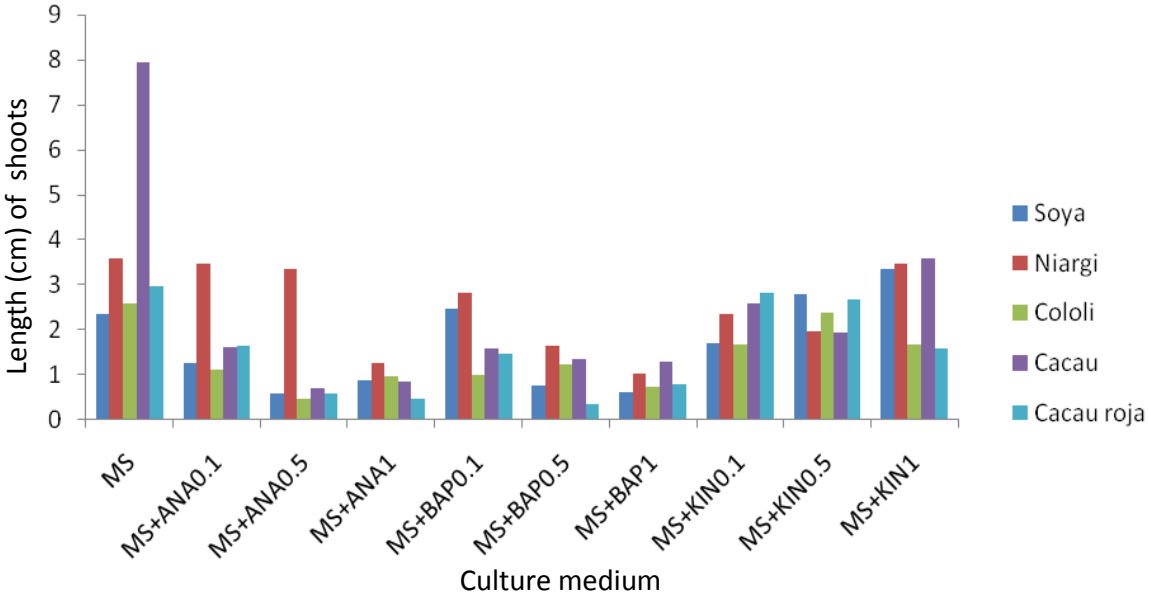

Figure 3. Influence of different hormonal concentrations (mg/L) and control on shoots' growth *in vitro* of five varieties of cassava after 1 month of culture.

Figure 4. Poor shoots growth in MS+BAP 0.1 mg/L after 30 days of culture.

0.05) among the hormonal concentrations tested.

Effect of hormones on shoots' growth *in vitro* of the different varieties of cassava

Explants cultured in media containing kinetin showed best growth of shoots. Respectively 3.56, 3.44 and 3.33 cm averages of shoots' lengths were observed among varieties *Cacau*, *Niargi* and *Soya* in MS + KIN 1 mg/L after four weeks of culture (Figure 3). However BAP and NAA were less favorable to shoots' growth. Therefore shortest shoots with averages 0.45 and 0.32 cm long, respectively have been observed among varieties *Cololi* and *Cacau roja* in the media MS + NAA 0.5 mg/L and MS + BAP 0.5 mg/L after 1 month of culture.

According to the analysis of variance, the variation of the shoots' length among the different culture media tested was significant (F = 0.639; P = 0.05).

Effect of hormones on roots' growth *in vitro* of the different varieties of cassava

The elongation of plantlets' roots varied depending on the culture medium. Thus, we have seen that the addition of hormone, especially in high concentrations, in the medium MS enhanced the roots growth. Kinetin proved however best elongation of roots in some varieties. Thus, we could record respectively 4.62 and 3.58 cm averages of roots' lengths among varieties *Cacau* and *Soya* in the MS + KIN 1 mg/L medium after 1 month of culture. In contrast, the lowest root growth occurred in media containing the NAA where very short roots not exceeding 0.06 cm average have been observed in the variety *Cacau roja* in the medium MS + NAA 1 mg/L after 4 weeks of culture (Figure 5). The variation of the roots' length was significant (F = 1.554; P = 0.05) among the culture media experienced, according to the ANOVA.

Effect of hormones on the phyllogenesis *in vitro* of the different cassava varieties

Figures 4 and 7 show that explants cultured in medium

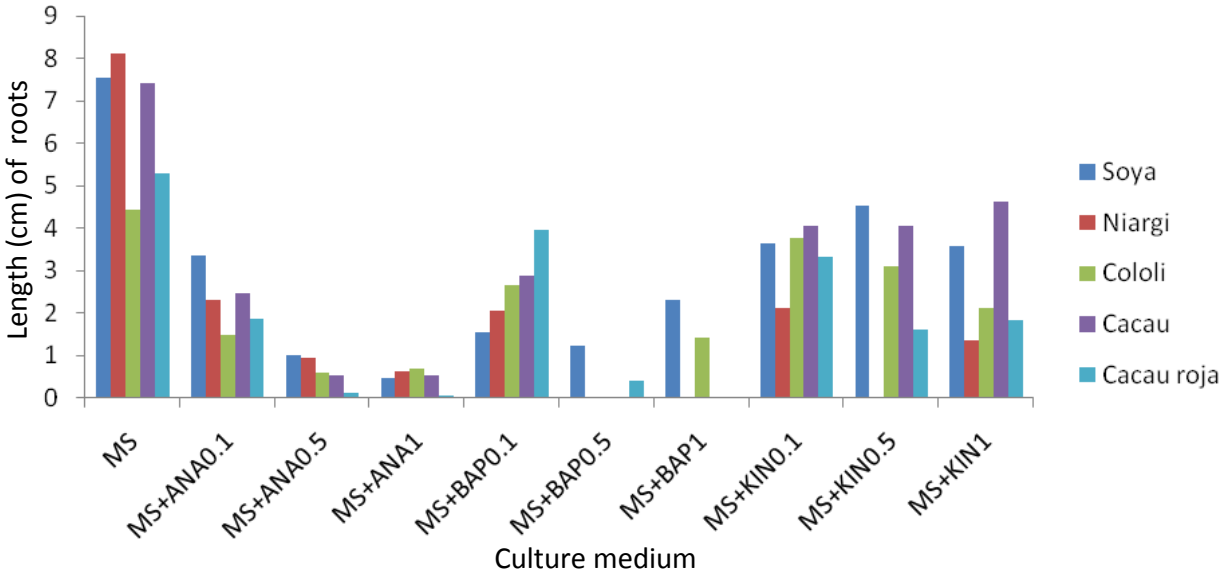

Figure 5. Influence of different hormonal concentrations (mg/L) and control on roots' growth *in vitro* of five varieties of cassava after one month of culture.

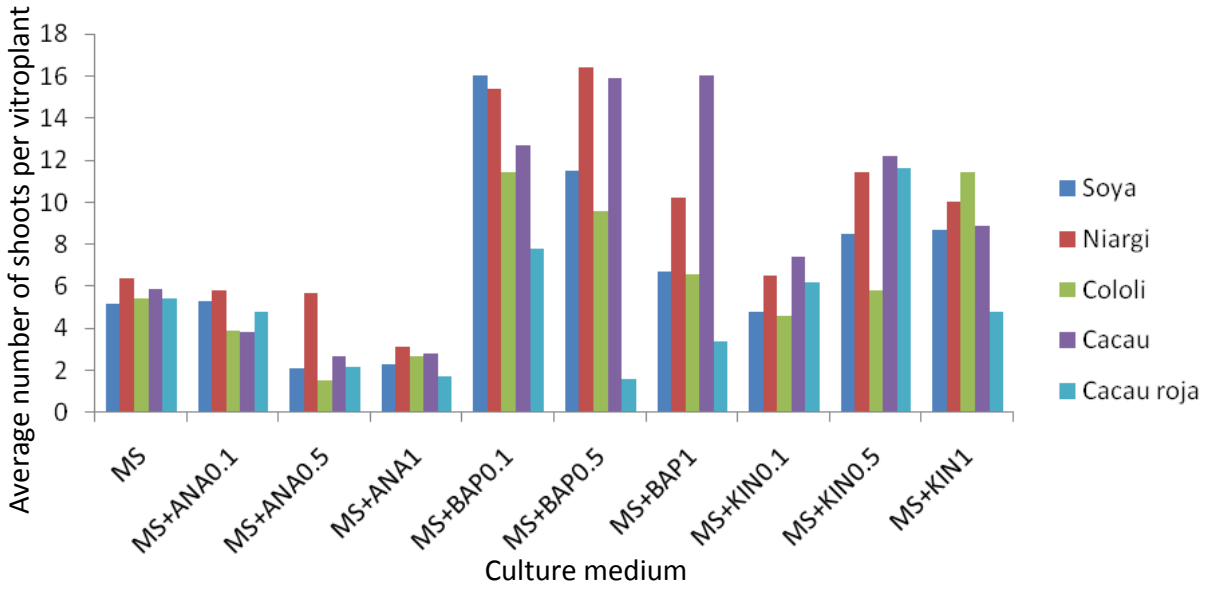

Figure 6. Influence of different hormonal concentrations (mg/L) and control on phyllogenesis *in vitro* of five varieties of cassava after 1 month of culture.

with BAP produced more leaves than those cultured in medium containing kinetin. Indeed, after four weeks of culture we could count up to 16.4 leaves average in plantlets of the variety *Niargi* in the MS + BAP 0.5 mg/L medium. Leaves produced in media addition of kinetin presented however the best development. In contrast, NAA appeared to be a hormone unfavorable to the phyllogenesis in all varieties. Thus the number of leaves per plantlet could fall up to 1.5 average after 4 weeks of culture in the medium MS + NAA 0.5 mg/L in the variety *Cololi* (Figure 6).

The number of leaves formed *in vitro* was significantly variable ($F = 10.310$; $P = 0.05$) among the different culture media tested, according to the analysis of

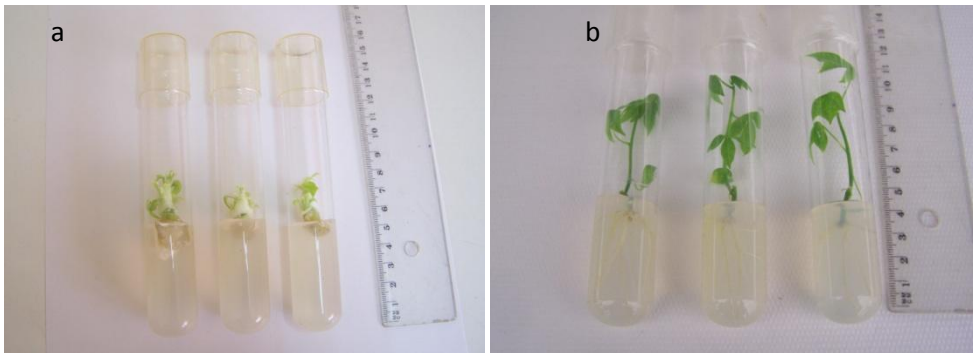

Figure 7. Shoots growth in MS+ANA0.1 mg/L (a) and MS+KIN0.1 mg/L (b) media after 30 days of culture.

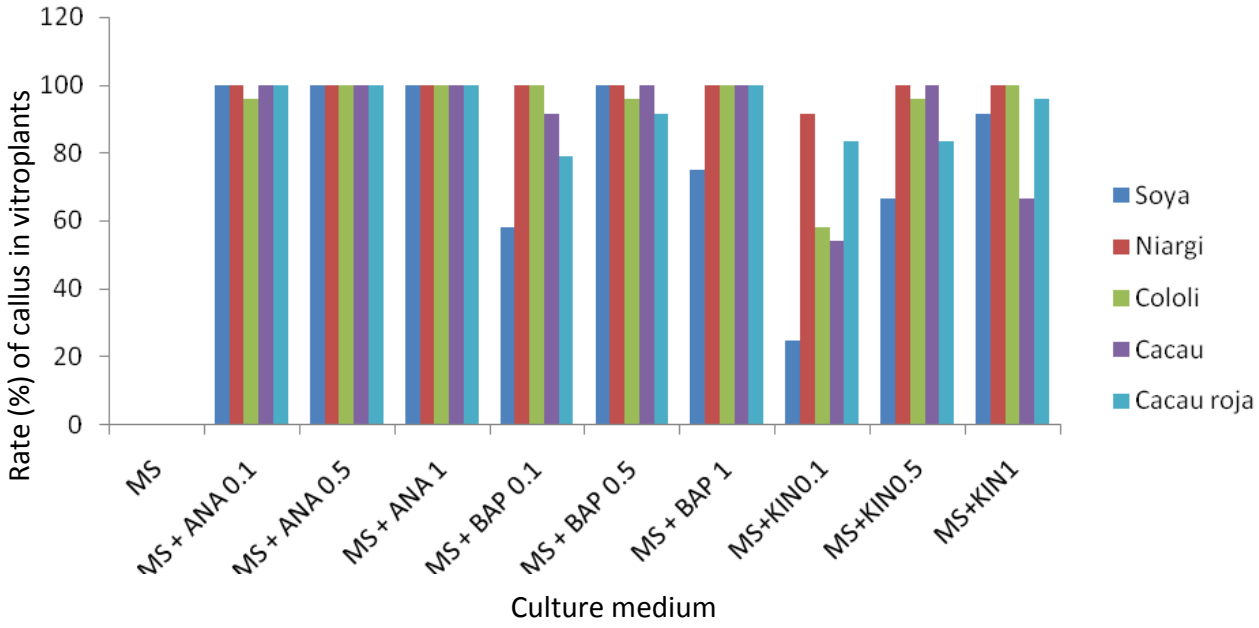

Figure 8. Influence of different hormonal concentrations (mg/L) and control on callus formation *in vitro* of five varieties of cassava after one month of culture.

variance.

Effect of hormones on the callus formation *in vitro* of the different varieties of cassava

Callus formation in plantlets appeared to be induced by the addition of growth regulators in the basal medium MS. Figure 8 shows that NAA, especially at high concentration, were more favorable to the callus formation, compared to the BAP and the kinetin which had the least. Indeed, after 4 weeks of culture, we recorded 100% of callus formation in all varieties in media MS + NAA 0.5 mg/L and MS + NAA 1 mg/L against 25 and 58.3%, respectively in varieties *Soya* and *Cololi* in the MS + KIN 0.1 mg/L medium. No callus formation was observed however in the basal medium MS (Figure 4). The analysis of variance showed that the callus formation's rate varied significantly ($F = 32.538$; $P = 0.05$) among the different media tested.

DISCUSSION

Our results showed that the response of cassava to micropropagation *in vitro* depends on the culture medium.

Thus, the addition of growth regulators at different concentrations in the basal culture medium MS oriented the organogenesis *in vitro* of this plant. In our experimental conditions, we could note that the application of a low concentration (0.1 mg/L) of NAA, BAP or kinetin favored more than high concentration (1 mg/L), best growth of organs except callus. Such observations are confirmed by the results obtained by Cacai et al. (2012) according to which the effect of kinetin on the shoots' growth varies depending on the applied concentration. Das et al. (2013) also found that the nature and the concentration of cytokinin determined significant growth' variations in some genotypes of Dioscorea sp. ; reflecting the results of Ondo et al. (2007) who, using high concentration (2 mg/l) of kinetin, have noticed a reduction of the length of roots in the *Dioscorea cayenensis – Dioscorea rotundata* complex.

In our experiment, explants cultured in media containing BAP produced highest numbers of shoots and leaves in all the cassava varieties. Kinetin resulted better growth of shoots, roots and leaves, while NAA induced more importantly the formation of callus, compared to the 2 cytokinins (BAP and kinetin). These results are confirmed by the works of Malaurie et al. (1995), Miller and Skoog (1957), James and Newton (1977), Navatel (1979), Ammirato (1984), Bennett et al. (1986), Lalmohanlal et al. (1990), Romano et al. (1992), Boniface (1992), Yepez et al. (2001), Kbiach (2002), Namwenje et al. (2003) and Ahanhanzo et al. (2008) reported by Cacai et al. (2012), which have shown that kinetin induces more roots than BAP. Indeed, these cytokinins, unlike auxin (NAA), would encourage more development and growth of the aerial organs (shoots and leaves) than of roots. Therefore, according to Phil et al. (1995) the variations of the ratio auxin/cytokinin have specific effects on the development of the explants and then determine the organogenesis tendency. These authors have shown that a high ratio (more auxin than cytokinin) resulted in differentiation of roots, while a low ratio (more cytokinin than auxin) resulted in differentiation of shoots. However, Ahanhanzo et al. (2008), using NAA (0.5 mg/l) + BAP (0.5 mg/l) on one hand and NAA (0.5 mg/l) + KIN (0.5 mg/l) on the other hand, have not observed callus formation in three varieties of cassava (RB 89509, BEN 86052 and TMS 30572). This would be explained by the fact that there was some interaction between growth regulators so that shoot or root differentiation would depend on the type of hormonal combination made.

Our results also show that the organogenesis *in vitro* of the different varieties of cassava were ideal in the culture medium MS, without any addition of hormone. Plantlets were well rooted and showed good vegetative development in this basal medium in all varieties. Such findings agree with results obtained by Boher (1988) and Lourd (1981) which could successfully multiply 65 cassava cultivars without using growth regulator. It appeared according to this researcher that the plantlets' growth *in vitro* could however be extremely variable depending on the cultivars or the type of explants of the same cultivar.

Therefore, according to our results, the variety would also have an influence on organogenesis *in vitro* of cassava. Thus, varieties *Soya*, *Cacau* and *Niargi* have presented best growth and development of organs, compared to varieties *Cololi* and *Cacau roja* in the culture medium MS. Such observations are confirmed by the work of Cacai et al. (2012) according to which the reaction of explants in different culture media is not the same from one variety to another. According to these authors, MS + KIN and MS + NAA media gave the highest shoots' length average in beninese cultivars *Agric Sazoue* (3.54 ± 0.4 cm) and *Gbeze* (6.36 ± 0, 3 cm) on one hand and *Ahouandjan* (8.62 ± 0.8 cm) on the other hand respectively.

In varieties *92/0057* (1.63±0, 1 cm), *BEN 86052* (4.20 ± 0.6 cm), *Sekandji* (6.6 ± 0.4 cm) and *Okoyao* (1.85 ± 0.3 cm) however, it was the NAA + KIN combination that gave the highest averages of shoot length. Similarly, work conducted by Ahanhanzo et al. (2010) on different genotypes of yam have confirmed that the response of the microcuttings to cytokinins' action depended on the genotype of the plant.

Conclusion

Organogenesis *in vitro* of cassava explants depends on the culture medium and the variety. The addition of different growth regulators (NAA, BAP and kinetin) at different concentrations (0.1, 0.5 and 1 mg/L) each in the basal Murashige & Skoog (MS) medium allowed to observe different tendencies in organs' development among 5 varieties *Soya*, *Niargi*, *Cololi*, *Cacau* and *Cacau roja* put in experience. The cytokinin BAP enabled to obtain more shoots, more leaves and fewer roots, while NAA has the most promoted callus formation in all varieties. Kinetin was found to be more favorable to best elongation of shoots and roots and the normal development of the leaves. In all media, rooting and growth of shoots and roots, conversely to number of shoots as well as the phyllogenesis and the callus formation, were more favored with low (0.1 mg/L) than with high (1 mg/L) hormonal concentration. However, the ideal response was observed with the basal culture medium MS in all the cassava varieties, so that the use of hormones appears not necessary for propagation *in vitro* of this plant.

Conflict of Interest

The authors have not declared any conflict of interest.

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