J Plant Growth Regul (2011) 30:11–19
DOI 10.1007/s00344-010-9163-y

Effects of Plant Growth Regulators and Saccharide on In Vitro Plant and Tuberous Root Regeneration of Cassava (*Manihot esculenta* Crantz)

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Received: 1 March 2010 / Accepted: 20 May 2010 / Published online: 30 June 2010

Abstract A faster system to get tuberous roots from in vitro cultured cassava plants may enhance the process of exploring the function and practical application of some root-specific expressed genes. The effects of cytokinin, auxin, sucrose, maltose, and glucose on development of shoots and tuberous roots and plantlet regeneration of in vitro cultured cassava were investigated in this study. The cytokinin N-benzyladenine (BA) (0–2.0 mg l^{-1}) was effective on shoot regeneration. The auxin α-naphthalene acetic acid (NAA) (0–2.0 mg l^{-1}) proved to be effective on root development. Plantlets with fibrous roots easily generated tuberous roots in vitro. The tuberous roots were induced only when both BA and NAA were used in combination. MS medium supplemented with sucrose at 175 mM (or 6% w/v) resulted in the highest frequencies of shoot induction (71.43%) and average fresh weight (0.61 g) of tuberous roots when BA (0.5 mg l^{-1}) and NAA (0.5 mg l^{-1}) were also added to the MS medium.

Keywords Cassava · Glucose · Maltose · Storage root · Sucrose · Tuberous roots

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Introduction

Cassava (*Manihot esculenta* Crantz) is a perennial woody shrub with an edible root that grows in tropical and subtropical areas of the world. It is the major staple food for nearly one billion people in tropical and subtropical regions. Its importance is next to rice, maize, and sorghum in these areas (Puonti-Kaerlas 1998). Besides being a major staple food in the world, cassava is also increasingly used for the production of animal feed and starch-based products. In China, cassava is produced in more than ten provinces, and the annual production of cassava starch is about 5 million tons, which has resulted in a short supply of cassava fresh tuberous root in recent years. Therefore, cassava breeding for high root yield and improved quality is urgent. Molecular tools for crop improvement have become popular (Capell and others 2004; Liu and others 2005; Du and others 2009), so molecular cassava breeding is becoming a trend in genetic improvement. Of the goals for agronomic trait improvement in cassava, increased tuberous root yield and lysine content, improved physiological post-harvest deterioration, and reduced cyanogenic glycosides content in the root are the major concerns.

All cassava tissues, with the exception of seeds, contain the cyanogenic glycosides which are a major group of nitrile-containing plant secondary compounds that yield cyanide following their enzymatic breakdown. Various health disorders are associated with the consumption of cassava, which contains residual cyanogens. These disorders include hyperthyroidism, tropical ataxic neuropathy, and konzo (Osuntokun 1981; Tylleskar and others 1992). However, the enzymatic breakdown of the cyanogenic glycosides in the non-root organs like leaves, shoots, and stems helps the plant to avoid the damages caused by pests and diseases. These metabolic secondary compounds would

be agronomically important if we could reduce their levels in the roots but maintain normal levels in the shoot. But reality is just the opposite of the ideal design because these compounds accumulate at a lower level in the leaf compared with that in the root. A previous report indicated that the accumulation of acetone cyanohydrins in roots is due to the absence of hydroxynitrile lyase (HNL), not to inhibition of the enzyme (White and others 1998). It was reported that HNL, which catalyzes the conversion of acetone cyanohydrin to cyanide, is expressed predominantly in the cell walls and laticifers of leaves, whereas in contrast, roots have very low levels of HNL expression (Siritunga and others 2004). Therefore, transgenic cassava plants overexpressing HNL would be expected to increase HNL activity and hence to provide a safer food product. Yet, for ideal pest and disease control, the expression of HNL in the leaf organs is not expected to be increased. So, root-specific expression of HNL is the aim of HNL gene application.

The lifetime of cassava tuberous root production is usually about 1 year under field conditions, so it would take quite a long time to study the function of tuberous root-specific expressed genes like HNL. As an alternative, if a tissue-cultured cassava plant formed a tuberous root in the test tube in a short period of time, it would be very helpful in the study of the function and practical application of the above-mentioned genes. There are several reports on cassava tuber formation (Cabral and Carvalho 2000; Batista de Souza and others 2004). The tuberous roots of cassava need 6–8 months to mature, which is still not efficient enough to be practical. Therefore, a faster system to get tuberous roots from tissue-cultured cassava plants would be very important for biological, physiological, and biotechnological studies. It could save time in the study of the biochemical and nutrient characters of tuber roots. Most studies on microtuber formation have focused on potato (Barker 1953; Coleman and others 2001), with a few studies on *Dioscorea nipponica* Makino (Chen and others 2007). Tuberous root growth rates were dependent on sucrose availability and limited by the process of sucrose hydrolysis to glucose and fructose (Yu and others 2000).

In vitro tuberous root generation of cassava has rarely been studied. Kartha and others (1974) mentioned briefly the tuberous roots in rapid propagation of cassava virus-free plantlets. Cabral and others (1993) reported that tuberous roots were observed in developing a regeneration system via organogenesis and somatic embryogenesis. Medina and others (2007) described a storage root formation system in developing a tuberization model of cassava. Factors affecting the formation of tuberous roots, such as genotypes of cassava, segment primary position, cytokinins, and auxins were explored systematically. Yet, tuberization of cassava in vitro culture was affected significantly by genotypes and hormones that limited its practical application of this model. The aim of this study was to establish an improved protocol for generating in vitro tuberization of cassava so that it could be used to increase understanding of the mechanism of tuberous root production and to increase awareness of their potential role on in vitro screening of mutations and conservation of cassava germplasm.

Materials and Methods

Cassava plants of a genotype (WCE) distributed and cultivated in the San Ya region of Hainan Island in China were grown in soil pots. The nodal segment in the middle section of the stem with one axillary bud from 30–40-cm-high plants was taken as an explant to induce in vitro plants in a glass bottle containing 30 ml of MS medium. The regenerated shoot segments were then cut into single-node explants about 1–3 cm long. The cassava stem cuttings were defoliated and thoroughly washed with tap water for 30 min. Then their surfaces were sterilized with 75% alcohol for 60 s and 0.1% mercuric chloride solution (approximately 1% active chlorine) for 5 min and then rinsed five times with sterile distilled deionized water. The stem cuttings were inoculated vertically in a flask (100 ml) with 30 ml of initiation medium containing MS (Murashige and Skoog 1962) salts supplemented with macroelements, microelements, and vitamins at full concentration, 3% (w/v) sucrose, and 0.7% (w/v) agar according to a standard yam nodal segment culture protocol (Mantell and others 1978; Mantell and Hugo 1989). There were five nodal explants per flask. The explants were grown in flasks to about 40–60 days old, then were cut into single-node explants about 0.5–1.5 cm long for further incubation for various experiments.

In experiments to test the effect of various concentrations of different plant growth hormones, the initiation medium mentioned above was supplemented with BA alone at various concentrations (0, 0.5, 1.0, 2.0, and 4.0 mg l^{-1}) or in combination with NAA (0, 0.2, 0.5, 1.0, and 2.0 mg l^{-1}). Whole plantlets developed on this medium after 28 days, with and without tuberous roots, and were transferred intact onto a second medium for secondary development culture. The secondary induction medium based on MS medium was supplemented with 3% sucrose plus NAA (0.5 mg l^{-1}) and BA (0.5 mg l^{-1}). The mature medium was supplemented with 3, 6, and 9% (w/v) of sucrose, maltose, and glucose, respectively. In all cases, the pH of the medium was adjusted with 1 mol l^{-1} KOH to 5.8 before being autoclaved at 121°C for 15 min. All cultures took place at 18–26°C under 60 μmol m^{-2} s^{-1} light provided by a white, cool fluorescent tube at a photoperiod of 16/8 h (light/dark).

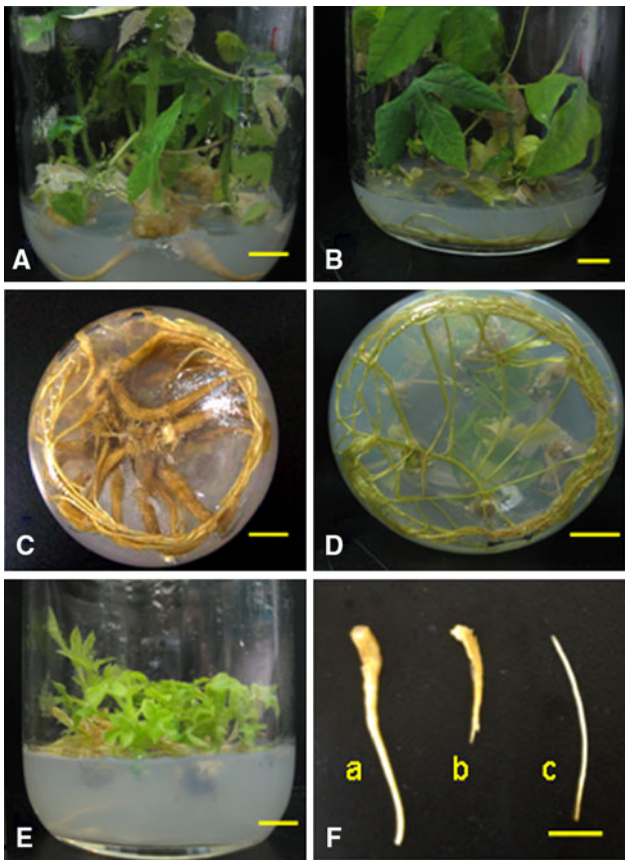

Fig. 1 Plant regeneration of cassava via nodal segment cutting. **A** Tuberous roots growing on MS medium supplemented with 3% (w/v) sucrose, 0.5 mg l^{-1} NAA, and 0.5 mg l^{-1} BA after 14 days. **B** Rooting in MS medium supplemented with 3% (w/v) sucrose after 14 days. **C** Root of panel A after 28 days of culture. **D** Tuberous roots of panel B after 28 days of culture. **E** Multiple buds and shoots developed gradually after nodal explants were cultured on MS medium supplemented with 3% (w/v) sucrose and 0.5 mg l^{-1} BA after 28 days of culture. **F** Root status: (*a*) tuberous roots growing on MS medium supplemented with 3% (w/v) sucrose, 0.5 mg l^{-1} NAA, and 0.5 mg l^{-1} BA after 28 days of culture; (*b*) tuberous roots growing on MS medium supplemented with 3% (w/v) sucrose, 0.5 mg l^{-1} NAA, and 0.5 mg l^{-1} BA after 14 days of culture; (*c*) fibrous root on MS medium supplemented with 3% (w/v) sucrose after 28 days of culture. Scale bar = 1 cm

Results

Tissue Culture of Nodal Explants from Tender Stem Cuttings

Nodal explants from tender stem cuttings of cassava were cultured on MS basal media supplemented with hormone combinations at different concentrations. After 7–14 days of culture, generated buds (Fig. 1E) and tuberous roots (Fig. 1A) displayed visible growth, and some of them grew into 0.2–7.5-cm-long shoots and more tuberous roots were generated (Fig. 1C) within 4 weeks. In contrast, nodal explants cultured on MS basal media without a hormone supplement did not generate tuberous roots (Fig. 1B, D). After 14 days of culture on MS basal media with or without hormone supplements, the roots generated from nodal explants were clearly distinguished as tuberous roots (Fig. 1F(b)) and fibrous roots (Fig. 1F(c)). Multiple buds growing on a suitable initiation medium developed into plantlets, with part of the plantlet producing tuberous roots. Thicker and more tuberous roots formed gradually on culture media with hormones. Thin light-brown or yellowish-white stripes appeared on the surface of the tuberous roots. There were a lot of buds that would potentially develop into plantlets. The inner parts of the tuberous roots were yellow (Fig. 1F).

Shoot regeneration frequency and shoot height as well as tuberous root formation were investigated from nodal segments cultured on media containing BA and/or NAA for 28 days (Figs. 2, 3, 4, 5, 6, 7). Figure 2 shows that in media without NAA but with a BA supplement, the frequency of shoot induction was the highest except in the medium without BA. The frequency of shoot induction showed a decreasing trend with increased BA concentrations (0–4 mg l^{-1}). The concentration of BA at 1 mg l^{-1} was the most optimal for shoot induction. The average shoot induction rate ranged from 9.5% (2 mg l^{-1} BA plus 0.5 mg l^{-1} NAA) to 85.7% (1 mg l^{-1} BA only). Similar to shoot induction rate, cassava shoot height also showed a decreasing trend with increased BA concentrations (0–4 mg l^{-1}), except for the culture medium without both BA and NAA (Fig. 3). The average of shoot height (AOSH) ranged from 0.35 cm (culture medium with 1 mg l^{-1} NAA and 2 mg l^{-1} BA) to 5.7 cm (medium with 0.5 mg l^{-1} NAA only). The result indicated that the culture medium with 0.5 mg l^{-1} NAA was optimal for

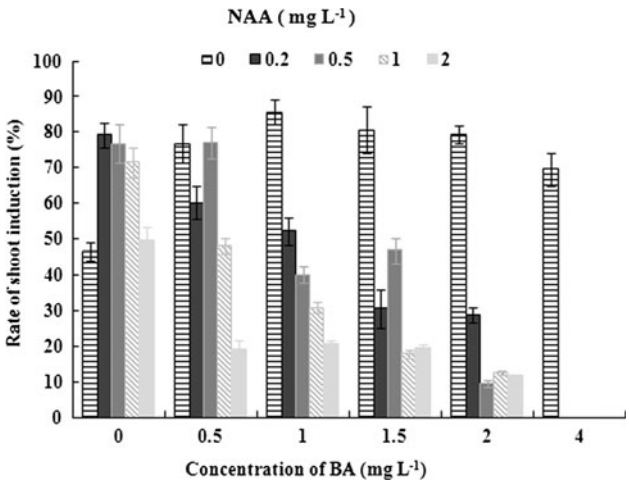

Fig. 2 The effect of different BA and NAA concentrations in the culture media on shoot induction frequency. For each combination of BA and NAA, 30 nodal segments were tested for shoot induction. Error bar indicates mean ± standard deviation

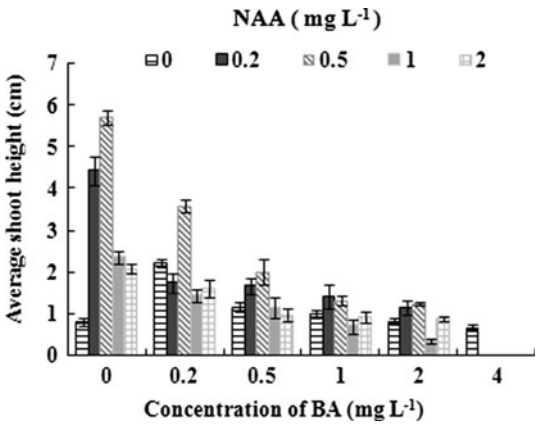

Fig. 3 The effect of different BA and NAA concentrations in the culture media on average shoot height of cassava regenerated from nodal segments. For each combination of BA and NAA, 30 nodal segments were tested for shoot induction. Error bar indicates mean ± standard deviation. The experimental condition was the same as for Fig. 2

shoot growth (Fig. 4). Combining the effects of different BA and NAA concentrations in the culture medium, low BA and NAA concentrations were better for shoot growth (Fig. 4).

The data presented in Fig. 5 indicate that the highest tuberous root induction frequency was obtained on culture medium with 0.5 mg l^{-1} BA and 0.5 mg l^{-1} NAA (45.2%). Tuberous root production decreased with increased BA concentration (Figs. 5, 6). The heaviest average fresh weight of tuberous roots (0.5 g) was obtained on culture medium with hormonal supplements of 0.5 mg l^{-1} BA and 0.5 mg l^{-1} NAA.

During the process of in vitro culture of cassava nodal explants, fibrous roots were regenerated in addition to tuberous roots. The highest fibrous root induction frequency was obtained on medium with 0.5 mg l^{-1} NAA (98.3%) (Fig. 7). Similar to shoot and tuberous root induction, the cassava fibrous root regeneration rate also showed a decreasing trend with increased BA concentrations from 0 to 4 mg l^{-1} (Figs. 2, 5, 7).

At any BA concentration, increased NAA concentration tended to inhibit shoot elongation and tuberous root production (Figs. 3, 6). According to the results of this study, the optimal hormonal combination for shoot induction (0.5 mg l^{-1} NAA) was different from that for tuberous root production (0.5 mg l^{-1} BA and 0.5 mg l^{-1} NAA).

Rooting and Growth of Plantlets After Transplanting

When vigorous plantlets via subculture grew to 28 days, some of the plantlets generated fibrous roots. Then cassava plantlets with or without fibrous roots were transplanted to newly made MS basal medium supplemented with 3% sucrose plus 0.5 mg l^{-1} NAA and 0.5 mg l^{-1} BA for the secondary development culture. After 28 days of secondary

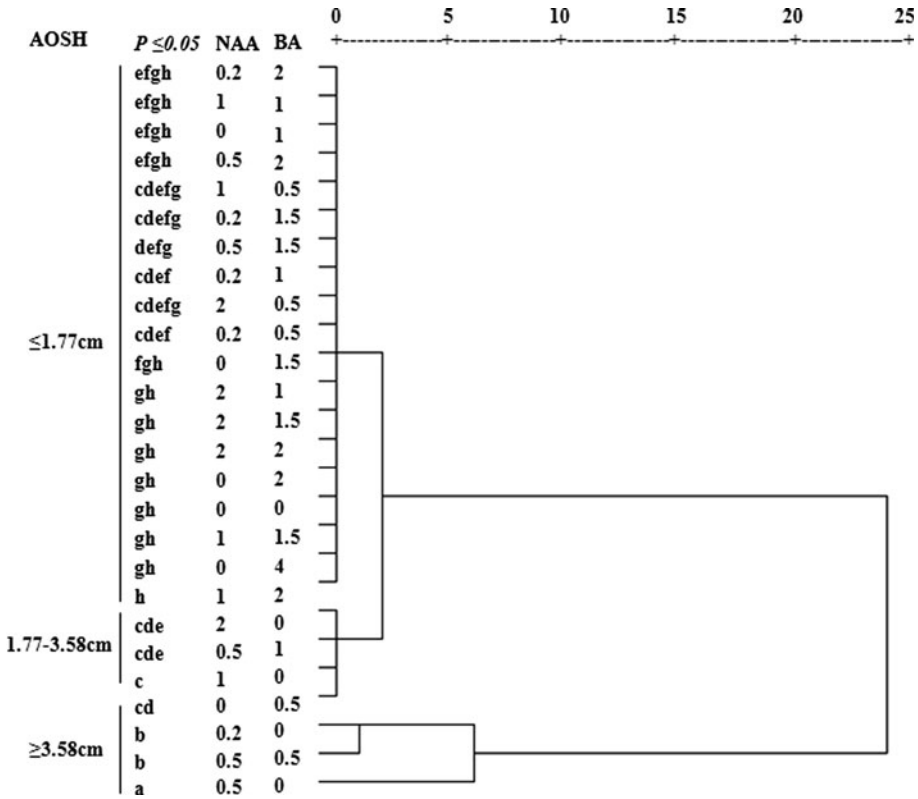

Fig. 4 Average of shoot height (AOSH) resulting from SPSS cluster analysis. Means were compared by using the least significant difference (LSD) test ($P \leq 0.05$). Data within each column followed by different letters differ significantly at $P \leq 0.05$. The coefficient used was the squared Euclidean distance

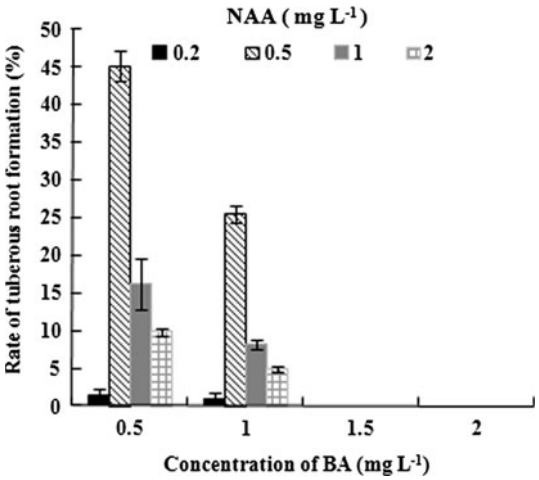

Fig. 5 The effect of different BA and NAA concentrations in the culture media on rate of tuberous root formation. Error bar indicates mean ± standard deviation. The experimental condition was the same as for Fig. 2

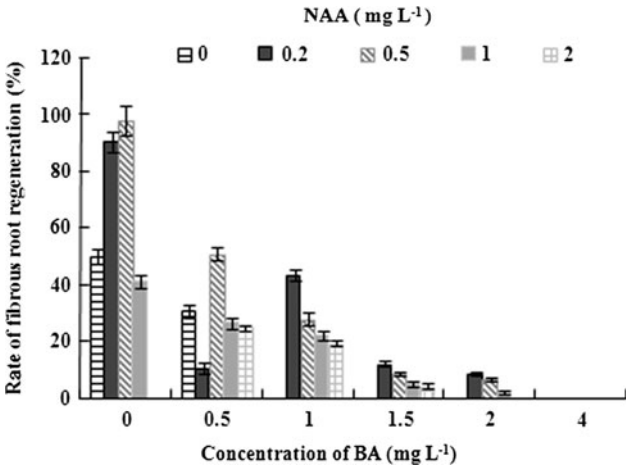

Fig. 7 The effect of different BA and NAA concentrations in the culture media on rate of fibrous root formation. Error bar indicates mean ± standard deviation. The experimental condition was the same as for Fig. 2

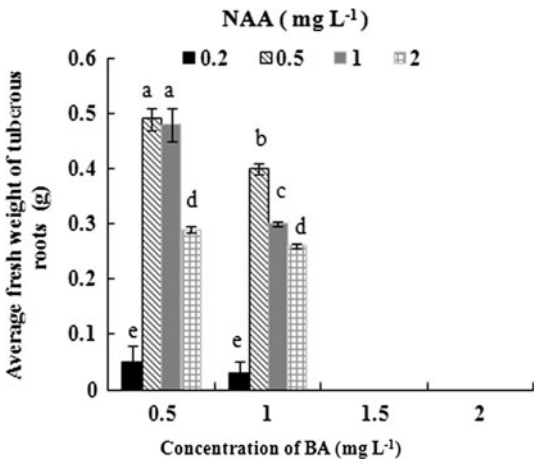

Fig. 6 The effect of different BA and NAA concentrations in the culture media on average fresh weight of tuberous root cultured for 28 days. Error bar indicates mean ± standard deviation. The experimental condition was the same as for Fig. 2

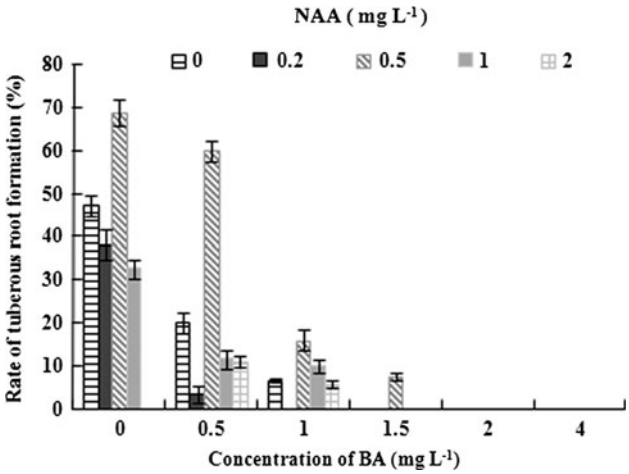

Fig. 8 The effect of different BA and NAA concentrations in the culture media on rate of tuberous root formation after 28 days of the secondary development culture. Error bar indicates mean ± standard deviation. The experimental condition was the same as for Fig. 2

culture, the tuberous root induction frequency and tuberous root production decreased with increased BA concentrations (Figs. 8, 9). Compared with the plantlet without fibrous roots, the plantlets with fibrous roots generated tuberous roots more easily, with the highest tuberous root induction rate of 68.57% (in the primary culture, plantlets were cultured on medium with 0.5 mg l^{-1} NAA). The heaviest tuberous root (1.30 g) was from the plantlets that were cultured on a medium with 0.5 mg l^{-1} NAA in the primary culture. Compared with the control, lower concentrations of BA and NAA in the secondary culture could increase the tuberous root regeneration frequency, and 0.5 mg l^{-1} BA was optimal for plantlets already with tuberous roots after the primary culture (Fig. 10).

Rooting and Growth of Plantlets on MS Basal Medium Enriched with Sucrose, Maltose, or Glucose

Cassava nodal segments were cultured on MS basal media enriched with 2, 3, 6, and 9% of sucrose, maltose or glucose, NAA (0.5 mg l^{-1}), and BA (0.5 mg l^{-1}) for 28 days.

Shoot induction frequency, shoot height, tuberous root induction frequency, and tuberous root production decreased with glucose concentration but increased with enriched maltose or sucrose (3–6% w/v) concentration (Figs. 11, 12). However, 9% (w/v) of either glucose, or maltose or sucrose was not fit for shoot induction and shoot growth (Figs. 11, 12). All the MS basal media enriched

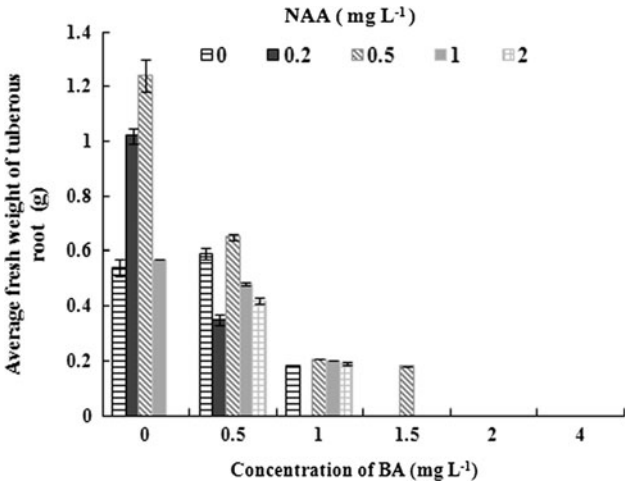

Fig. 9 The effect of different BA and NAA concentrations in the culture media on average fresh weight of tuberous roots after 28 days of the secondary development culture. Error bar indicates mean ± standard deviation. The experimental condition was the same as for Fig. 2

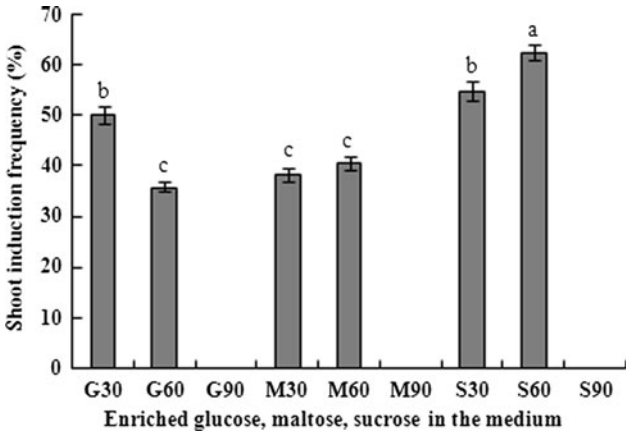

Fig. 11 The effect of different concentrations of sucrose, maltose, and glucose in the culture media on shoot induction frequency. The media contain 0.5 mg l^{-1} NAA and 0.5 mg l^{-1} BA. G30, 3% (167 mM) glucose; G60, 6% glucose; G90, 9% glucose; M30, 3% maltose; M60, 6% maltose; M90, 9% maltose; S30, 3% sucrose; S60, 6% sucrose; S90, 9% sucrose. Error bar indicates mean ± standard deviation. The experimental condition was the same as for Fig. 2

with glucose or maltose or sucrose were simultaneously supplemented with 0.5 mg l^{-1} BA and 0.5 mg l^{-1} NAA. Results indicated that medium enriched with 6% (w/v, or 175 mM) sucrose turned out to be the best among different kinds of sugar enrichment, with the rate of shoot induction being 62.3%, shoot height 5.6 cm, the rate of tuberous root generation 71.4%, and average tuberous root fresh weight 0.61 g (Figs. 11, 12, 13, 14).

With respect to the effects of saccharide on tuberous root generation, two more local cultivars, WCF and WCEF, and phenotypes COL 22 and COL1505 from CIAT were used in the complementary experiments (Fig. 15). Results indicated that the same trend as shown in experiments with the Sanya local cultivar (WCE) was observed (Figs. 11, 12, 13, 14, 15). Sucrose turned out to be better than maltose and glucose in tuberous root induction and growth in the similar molar concentrations.

Discussion

Auxins and cytokinins are necessary to get tuberous roots in vitro, and the absence of either of them in the medium could lead to less tuberous root induction or no tuberous roots generated at all (Loomis and Torrey 1964; Torrey and Loomis 1967; Webster and Radin 1972). Auxin (NAA) regulates vegetative growth and organ growth, and cytokinin (BA) facilitates not only cell sprouting but also cell division (Pan 2001). Different concentrations of BA and NAA had different abilities to induce microtuber and

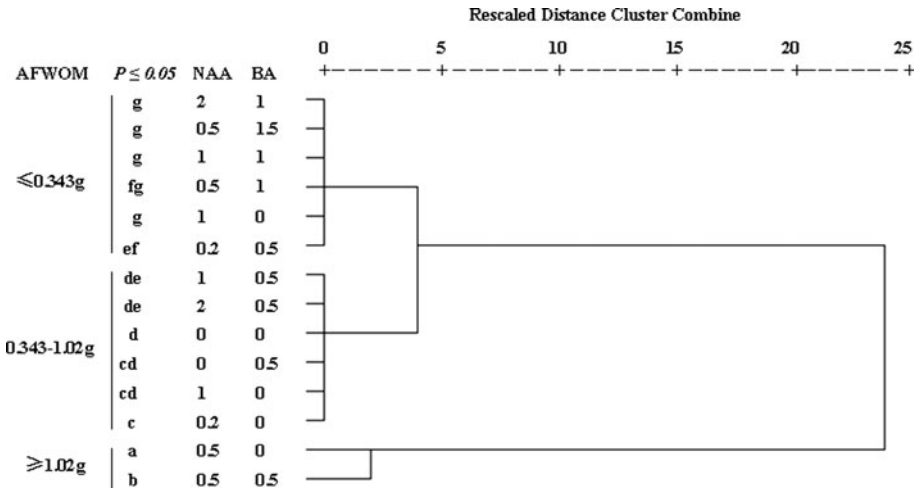

Fig. 10 Average fresh weight of tuberous roots (AFWOM) resulting from SPSS cluster analysis. Means were compared by using the least significant difference (LSD) test ($P \leq 0.05$). Data within each column followed by different letters differ significantly at $P \leq 0.05$. The coefficient used was the squared Euclidean distance

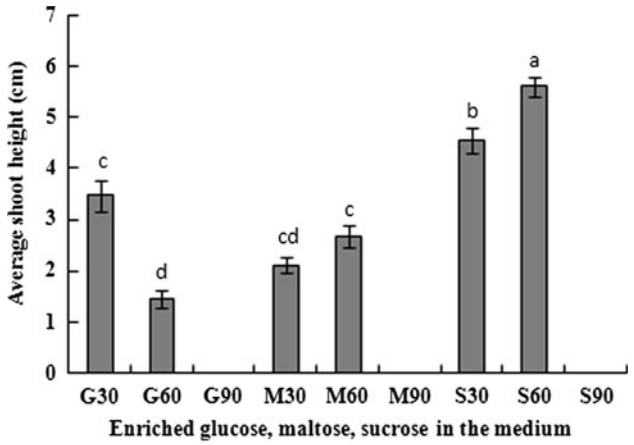

Fig. 12 The effect of different concentrations of sucrose, maltose, and glucose in the culture media on shoot growth expressed in shoot height. The media contain 0.5 mg l^{-1} NAA and 0.5 mg l^{-1} BA. G30, 3% glucose; G60, 6% glucose; G90, 9% glucose; M30, 3% maltose; M60, 6% maltose; M90, 9% maltose; S30, 3% sucrose; S60, 6% sucrose; S90, 9% sucrose. Error bar indicates mean ± standard deviation. The experimental condition was the same as for Fig. 2

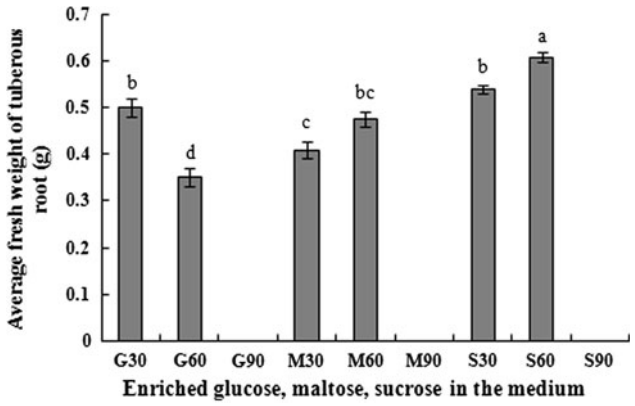

Fig. 14 The effect of different concentrations of sucrose, maltose, and glucose in the culture media on growth of generated tuberous roots. The media contain 0.5 mg l^{-1} NAA and 0.5 mg l^{-1} BA. G30, 3% glucose; G60, 6% glucose; G90, 9% glucose; M30, 3% maltose; M60, 6% maltose; M90, 9% maltose; S30, 3% sucrose; S60, 6% sucrose; S90, 9% sucrose. Error bar indicates mean ± standard deviation. The experimental condition was the same as for Fig. 2

plantlet regeneration (Medina and others 2007). In the present study, BA and NAA at different concentrations were added to MS basal medium to test their ability to affect the frequency of tuberous root induction in cassava and to optimize the medium composition for plantlet regeneration. On MS media containing 3% (w/v) sucrose and BA without NAA or NAA without BA, no tuberous roots were induced. The result indicated that the growth regulators BA and NAA could ensure in vitro regeneration of cassava and that the induction efficiency depends on their concentrations in the medium. It also revealed that the

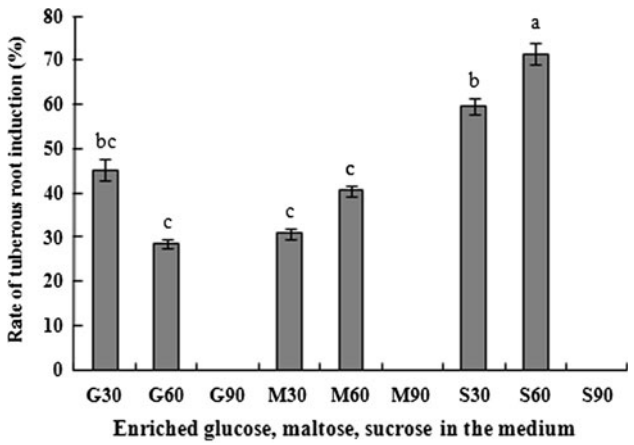

Fig. 13 The effect of different concentrations of sucrose, maltose, and glucose in the culture media on tuberous root-regenerating frequency. The media contain 0.5 mg l^{-1} NAA and 0.5 mg l^{-1} BA. G30, 3% glucose; G60, 6% glucose; G90, 9% glucose; M30, 3% maltose; M60, 6% maltose; M90, 9% maltose; S30, 3% sucrose; S60, 6% sucrose; S90, 9% sucrose. Error bar indicates mean ± standard deviation. The experimental condition was the same as for Fig. 2

synergism of BA and NAA in their proper concentration in the medium was extremely favorable for tuberous root growth of cassava. NAA was more suitable for in vitro regeneration of cassava nodal segments than BA. Our experimental results were in concordance with the findings that the interaction of auxin and cytokinin is necessary for plant in vitro organogenesis, and cytokinin of low concentrations and auxin of low concentrations were prerequisites for differentiation of adventitious buds (Skoog and Miller 1957). According to the current investigation on tuberous root induction in cassava in vitro culture, the effect of NAA on induction frequency of tuberous roots was obvious. However, the effect decreased gradually with increased NAA concentration in both treatments with NAA alone and NAA with BA.

Our investigation indicated that the absence or excessively high concentrations of hormones was not optimal for cassava to generate shoots and produce tuberous roots. Suitable combinations of hormones at different concentrations are very important for cassava culture. As in tissue culture of other plants, the case mentioned above relates to physiology, multiformity of plant acceptors, and metabolism and synthesis of endogenous hormones (Song 1985; Ni and Deng 1992). As for micropropagation and tuberization of cassava, auxin (NAA) and cytokinin (BA) are very important factors. Whether these hormones promote vegetative growth or tuberous root growth is determined by their cross interaction. It was clearly observed in potato (Trippi 1982) and dahlia (Halevy and Biran 1975) that factors promoting vegetative growth inhibit tuberization in vitro. Similarly in our study, treatments with low NAA (0.2–0.5 mg l^{-1}) and without BA were better for plantlet growth, showing greater shoot height and a higher rate of

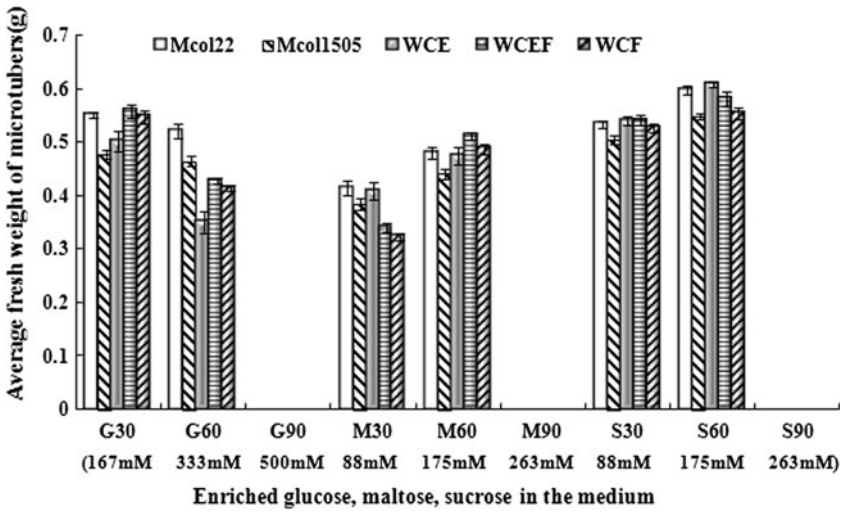

Fig. 15 The effect of different concentrations of sucrose, maltose, and glucose in the culture media on fresh weight of generated tuberous roots from five genotypes of cassava. WCW is the phenotype used in all the experiments and WEF, WCEF, COL 22, and COL 1505 are four additional cultivars used in this experiment. The media contain 0.5 mg l^{-1} NAA and 0.5 mg l^{-1} BA. G30, 3% (w/v, the same for the following, or 167 mM) glucose; G60, 6% (333 mM) glucose; G90: 9% (500 mM) glucose; M30, 3% (88 mM) maltose; M60, 6% (175 mM) maltose; M90, 9% (263 mM) maltose; S30, 3% (88 mM) sucrose; S60, 6% (175 mM) sucrose; S90, 9% (263 mM) sucrose. Error bar indicates mean ± standard deviation. The experimental condition was the same as for Fig. 2

shoot induction, fibrous root induction, and fibrous root production. When BA (0.5 mg l^{-1}) was added to MS medium with 0.5 mg l^{-1} NAA and 3% (m/v) sucrose, more tuberous roots were generated.

Sucrose, maltose, and glucose act primarily as a carbon frame and energy for shoot induction and tuberization in plantlets cultured in vitro. These saccharides are a kind of osmotic pressure agent. They can maintain a stable osmotic pressure environment for most plants cultured in vitro (Kuhlmann and Foroughi-Wehr 1989; Finnie and others 1989) and support vigorous growth (Lu 1981), so they were widely used as carbon sources in plant tissue culture. Sucrose provides a favorable osmolarity for the development of tuberous roots in potato (Khuri and Moorby 1995). And the growth rate of tuberous roots was limited by sucrose hydrolysis to glucose and fructose (Yu and others 2000).

In this study, high tuberous root induction levels (near 70%) were observed when 6% (w/v, or 175 mM) sucrose was used in culture of cassava nodal segments. This concentration of sucrose was equivalent to 3% glucose in molar concentration (167 mM), but the tuberous root induction rate on medium enriched with 3% glucose was only 45% (Fig. 13). The present investigation indicated that a high concentration of sucrose promoted the production of heavier tuberous roots of cassava (Fig. 14). Other experimental results (Figs. 11, 12) in this study proved that sucrose provided a favorable osmolarity for tuberous root regeneration, which is in agreement with an earlier study in potato by Khuri and Moorby (1995). In reference to the in vitro shoot induction and tuberous root formation of several *Dioscorea* species, some authors have pointed out the need to increase sucrose and decrease the photoperiod for tuberous root production of yam. For example, when 8 or 10% sucrose was supplied with 2.5 mM kinetin, the percentage of tuberization in *D. rotundata* was only 37.5 and 12.5%, respectively (Ng 1988). Furthermore, in in vitro culture of cassava, sucrose as a carbon source and osmotic pressure agent was needed.

Cassava is a food plant, but traditional breeding methods aimed at creating cultivars with heavy-producing and high-grade cassava tuberous roots take considerable time. A combination of tissue culture and genetic engineering techniques is likely the process to use for genetic improvement and selection of cassava, especially for tuber root-specific expressed traits. The key is to establish a plant regeneration system to suit genetic transformation. The present study experimentally established a regeneration system for cassava by inducing shoots and tuberous roots in vitro that will certainly benefit the improvement of root-specific expressed traits. This system could also be potentially important for immediate propagation of elite high-yielding varieties and for the conservation of cassava germplasm.

Acknowledgments This study was supported by FAO/IAEA joint research program CPR-13189 and grants (2005) 1-7 and (2006) 4-3 from the Agriculture Committee of Shanghai Municipal Administration, the National Basic Research Program (grant 2007CB815701). The authors thank Prof. Roberto Tuberosa and Ms. Kay Tuberosa for comments and revisions on an early version of the manuscript.

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