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Assessing the regenerability of selected Kenyan Cassava genotypes via Somatic Embryogenesis

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Abstract: *Cassava is a valuable source of calories in countries where malnutrition is widely spread. Despite its many uses as food, feed and in industries, it's constrained by biotic and abiotic stresses. Attempts to overcome challenges in cassava production by conventional breeding are limited. The application of genetic transformation to introduce agronomically useful traits would greatly compliment classical breeding approaches. The objective of this study was to determine the regenerability of selected Kenya cassava genotypes for use in genetic transformation studies. Three genotypes (Ex-ndolo, Karibuni and Shibe) were collected from coastal and eastern agro ecological zone based on their traits which were high yielding, early maturity and mealiness and maintained at the Kenyatta University Plant Transformation Laboratory glasshouse. Stokes were established in vitro and maintained on media containing Murashige and Skoog salts with vitamins, 30g/l sucrose and 3.0g/l gelrite and used as source of sterile explants. Picloram and 2, 4-dichlorophenoxyacetic acid were used to induce somatic embryos using leaf and stem explants under light and dark photo regimes. The differences in frequencies of somatic embryogenesis ranged between 31.95-81.48% for leaf explants and 19.65-42.83% for stem explants for all four concentrations under study while varying the photoperiod. Embryogenic calli was matured on media supplemented with different concentrations of 6-Benzylaminopurine, α-naphthaleneacetic acid and gibberellic acid before being transferred to regeneration media. Shoot development from somatic embryos had significant differences between genotypes. Ex-Ndolo was highly responsive to the maturation media and formed shoots when the embryos originated from leaf explants for both 2, 4-dichlorophenoxyacetic acid and picloram were matured. Root induction from shoots was relatively low, ranging between 10±2.82-26±1.41. Overall picloram emerged as the best auxin for somatic embryo induction while leaf explant was superior to stem in terms of embryogenic ability.*
Key Words*: explant, auxin, photo regimes, somatic embryo,*

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I. Introduction

Cassava (*Manihot esculenta* Crantz) is a root crop and it is the fourth source of food calories in tropical countries after maize, rice and wheat. It has the potential to improve food security in third world countries since it's consumed by 800 million people [1]. Nigeria is the largest producer of cassava in Africa at 54,831,600 Mt while Tanzania is the leading producer of cassava in East African region at 4,992,759 Mt. Cassava production in Kenya stands at 858,461tones according to [2]. In Kenya, cassava is mainly grown in Western, Coast and Eastern regions of the country while production in the other regions is relatively low.

Cassava is used as food, animal feed and a source of raw material in industries. The global demand for cassava is rapidly growing due to its starch content, which is attractive in the starch industry and it has a good potential for biofuel production [3].Farmers prefer cassava to other crops because;it gives adequate yields even when grown in marginal soils compared to other crops, is adapted to a wide range of climatic conditions due to its hardiness and tolerance to adverse environmental conditions [4]. Also, cassava tubers can be left in the soil for to three years after maturation without decay and harvested only when needed [5]. It is a source of income to farmers who sell to industries producing processed food, starch-based products, biofuels and animal feed [6] and [7]. Despite the many uses of cassava, production faces challenges such as susceptibility to insect, bacterial and viral transmitted diseases [8], [9] and [10] and weeds [11]. Others are accumulation of toxic cyanogenic glycosides [12], low nutritive value of cassava tubers[13] and low shelf life [14], which limit its palatability and marketability [15] and [16].

Conventional breeding has been used to overcome these challenges but it has limited success due to the unsynchronized flowering, high heterozygosity, allopolyploidy, limited seed set, and limited knowledge of inheritance traits that have agronomic importance [17]. Genetic engineering is an alternative and a complementary approach to conventional breeding. However, genetic transformation is constrained by lack of an efficient and robust regeneration system [18]. Previous studies on plant regeneration have shown that frequency of somatic embryogenesis is highly genotype-dependent. Plant regeneration is a prerequisite to successful genetic engineering since there exists no genotype independent protocol for practical use. Therefore, there is need to establish protocol for the locally available genotypes. There is need to explore other sources of explants such stem since the availability of leaves used for regeneration and transformation experiments is limited. Somatic embryogenesis is the most preferred method to induce regeneration from *in vitro* tissue culture [19].plant regeneration involves production embryos from explants, embryo maturation and germination, root induction and hardening and acclimatization. Regeneration through somatic embryogenesis is a two step process; callus induction, where embryos are formed in the presence of an auxin and embryo maturation and germination, where the auxin is removed or used in low concentration and a cytokinin (such as BAP) is used to promote shoot formation or generation mature cotyledons [20]. Since somatic embryogenesis is important aspects of plant regeneration, aspects of somatic embryogenesis have been investigated, which include types of hormones, hormone concentration, photoperiod regime and explants used [21],[22] and [23]. In this study, we report somatic embryogenesis in leaf and stem explants using 2, 4-D and picloram as auxins for embryo induction under different photo regimes. We also report embryo maturation, embryo germination from somatic embryos using BAP, NAA and GA_3 and plant recovery.

II. Materials and methods

2.1 Plant Material

Three Kenyan cassava genotypes *Ex-Ndolo, Karibuni* and *Shibe* were collected from coastal agro-ecological region of Kenya and maintained at Plant Transformation Laboratory at Kenyatta University. Mealiness, early maturity, and high yielding were key factors for genotypes selection. The nodal cuttings plants were surface sterilized with 10% (v/v) sodium hypochlorite (from household bleach) and two drops of Tween-20 for ten minutes, then placed in ethanol for five minutes and rinsed three times with autoclaved distilled water. The surface sterilized nodal cuttings were cultured in MS propagation media and maintained *in vitro* as shoot cultures on Murashige and Skoog medium with vitamins [24]supplemented with 30g/l sucrose, solidified with 3.0g/l gelrite, pH 5.8 at 28ºC under 16/8 h light and dark regime provided by cool white fluorescent lamps (40 µ mole/m^2/s) as described by [24]. The shoot cultures were sub-cultured after every 2-4 weeks for generation of sterile explants.

2.2 Induction of callus and generation somatic embryos

For induction of callus two week old leaves and stems from *in vitro* shoot cultures were used as explants. The stems and leaves were cut either transversely or cross sectionally and placed on MS medium supplemented with Gamborg's B5 vitamins [24], auxins (2, 4-D or picloram), 20 g/l sucrose, 100 mg/l myoinositol, 50 mg/l casein hydrolysate, 0.5 mg/l copper sulphate and 3.0 g/l gelrite, and pH adjusted to 5.7 then autoclaved at 121^{o}C for 15 minutes as described by [25]. The concentration of the two auxins added to the media were 4 mg/l, 6 mg/l, 8 mg/l and 10 mg/l. Photoperiod regimes were also varied with one set of explants incubated under 16/8 h light and dark regime while the other set was under 0/24 h light and dark regime. Explants forming callus were sub cultured onto fresh media after every two weeks and maintained on this media until formation of somatic embryos. The number of somatic embryos per explant was determined four weeks after callus induction and averages compared across the treatments. Ten stem explants and eight leaf explants per Petri plate were used for callus induction. The Petri plates were in five sets for each treatment (light and dark) photo regime for the all the four concentrations of 2, 4-D and four concentrations of picloram. The controls were stem and leaf explants cultured in hormone-free media.

2.3 Embryo germination

Calli showing presence of somatic embryos were transferred onto MS medium with Gamborg's B5 vitamins [26] supplemented 20 g/l sucrose, BAP, NAA, GA_3, (combination ratios for BAP, NAA and GA_3 are as shown in TABLE 1 100 mg/l myoinositol and 3.0g/l gelrite at pH 5.7 and later autoclaved at 121^{o}C for fifteen minutes. These were incubated at 28^{o}C under a 16/8 h light/dark regime. Sub-culturing was done after every two weeks until they formed shoots or greened.

Table 1: Hormone combination ratios for maturation media

Media	BAP	NAA(mg/l)	GA_3
Media 1	3	2	1
Media 2	3	1	2
Media 3	2	1	3
Media 4	2	3	1

Five calli per Petri plate were used, in five sets of Petri plates for all the concentrations of 2, 4 D and picloram under the two photo regimes. Five embryogenic calli per petri plates matured in light photo regime, in five sets for the four maturation media used in this study.

2.4 Root induction, acclimatization and hardening

The formed shoots were moved onto MS medium with Gamborg's B5 vitamins [25] supplemented with 20 g/l sucrose, 1 mg/l thiamine, 100 mg/l myoinositol, 7 g/l photo agar and 8% (w/v) activated charcoal. After a week of culture, the shoots were moved to a hormone-free medium (half MS salts with vitamins), 20 g/l sucrose and 2.8 g/l gelrite for regeneration and root development. Data on root induction frequency was taken. Once the roots developed, agar was washed off the roots using distilled water and plantlets transferred into 4 inch pots containing autoclaved peatmoss. The pots were covered with transparent plastic bags to maintain high humidity for three to seven days. Seven to ten days later, the plants were transplanted into bigger pots containing soil and transferred to the greenhouse. The plants were regularly watered until maturity.

2.5 Data analysis

Data were further subjected to analysis of variance to detect differences among treatments using a confidence level of 95% using Statistical Analysis System (SAS) software 9.1.3. The results were subjected to Tukey's post hoc test for mean separation and pairwise comparison, $P \leq 0.05$ was considered statistically significant.

III. Results

All the three cassava genotypes were able to form embryos at all concentrations of picloram and 2, 4-D (4mg/l-10mg/l) under the two photoperiod (light and dark) treatment using stem and leaf explants. For leaf explants, callusing began with curling of the tissue followed by swelling at the cut edges, while in the case of stems callusing was initiated at their terminals. The colour changes in the explants varied from green to yellow-brown (Fig. 1). Somatic embryos appeared between the 21st and 28th days depending on initial auxin used for callus induction and explants type. Embryogenic calli were characterized morphologically by appearance of nodular-like structures (Fig. 2). Whitish, smooth watery calli were considered non-embryogenic while compact brownish soft friable ones were considered embryogenic. Smooth watery calli were a common phenomenon in explants cultured under light regime. Explants under picloram formed embryos within the twenty one days while those under 2; 4-D took 28 days or longer.

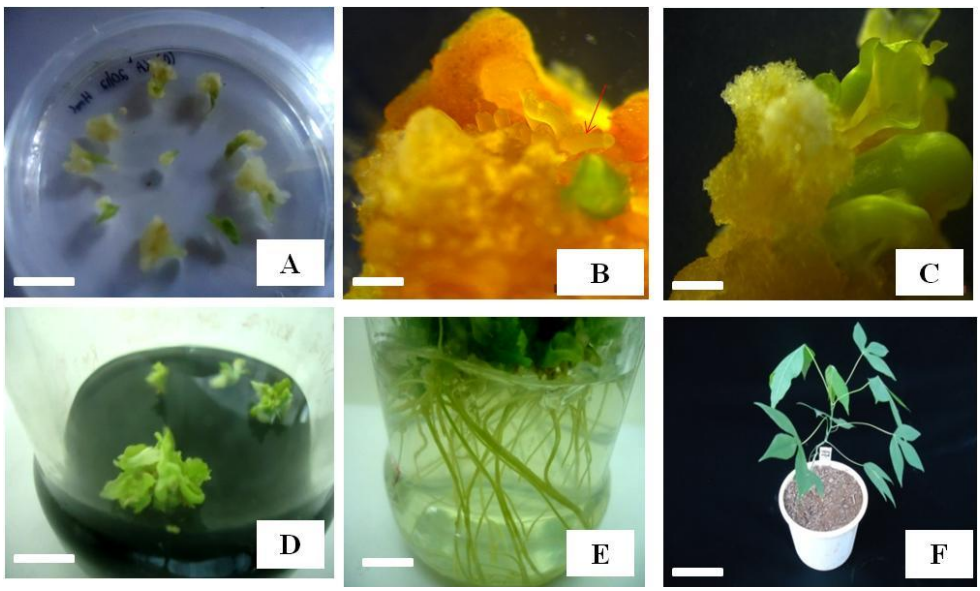

Figure 1: Profile of regeneration steps in cassava using leaf explants**.**

A. Two weeks old calli from leaf explants in genotype *Karibuni* in callus induction media(**bar = 2cm**).B. Calli from leaf in *Shibe* with somatic embryos. Somatic embryos are shown with the red arrow**(bar= 2mm)**. C. Shoots emerging from embryogenic calli cultured in maturation media in *Karibuni***(bar =2mm)**. D. Shoots from *Ex-ndolo* cultured in activated charcoal media to adsorb phenolics(**bar = 2cm**).E. Shoots from *Karibuni* in rooting media (**bar = 2cm**).F. A shoot from *Shibe* in peatmoss mixed with vermiculite during acclimatization and hardening**(bar =2mm)**

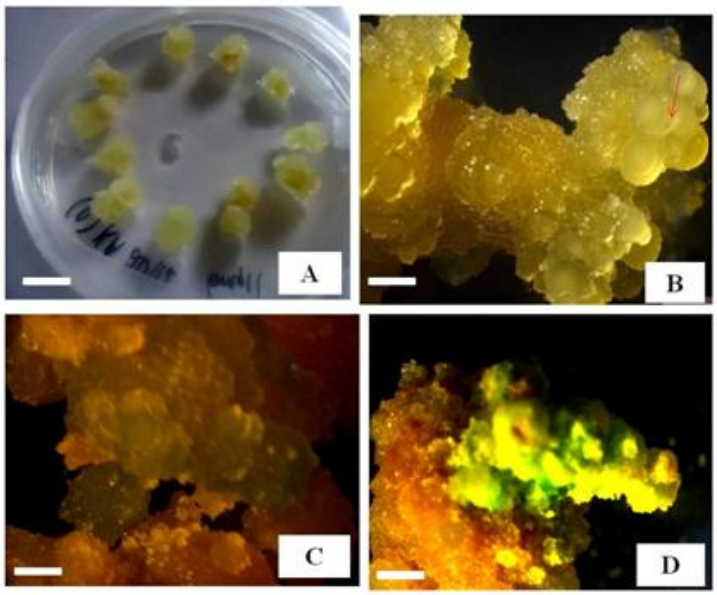

Figure 2: Profile of regeneration steps in cassava using stem explants.

A Two weeks old calli from stem explants in genotype *Karibuni*in callus induction media **(bar = 2cm)** B Callus from a stem in *Karibuni* with somatic embryos emerging shown with red arrow **(bar = 2mm)** C Microscopic representation of greening in calli from stem in *Shibe***(bar = 2mm)** D. The later stages of greening in embryogenic calli from stem in *Karibuni***(bar = 2mm)**

3.1 Effect of photoperiod regimes on somatic embryogenesis

Somatic embryos were formed under the photo regimes.There were no significant differences at $P \leq 0.05$ in somatic embryogenesis frequency across the three genotypes when media supplemented with picloram was used to induce somatic embryos under the two photo regimes (TABLE 2). *Ex-Ndolo* recorded the highest somatic embryogenesis frequency at 81.66±8.1 (darkness) and 77.78±6.9 (light). Lower frequencies were recorded when 2, 4-D was used to induce somatic embryos under the two photoregimes. *Karibuni* gave the highest somatic embryogenesis frequencies at 45.31±1.7and 42.31±13.6 for dark and light regimes respectively (TABLE 3).

Table 2: Frequencies of somatic embryogeniesis in cassava genotypes

Genotypes	4mg/l		6mg/l		8mg/l		10mg/l	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark
Ex-Ndolo	59.57±8.9[a]	68.05±7.3[a]	60.77±10.45[a]	67.55±10.14[a]	77.78±6.9[a]	81.66±8.1[a]	62.50±7.2[a]	66.35±9.4[a]
Karibuni	49.89±17.27[a]	42.17±18.59[a]	55.48±14.73[a]	43.35±19.32[a]	55.05±14.48[a]	54.72±14.28[a]	66.56±15.37[a]	62.84±13.2[a]
Shibe	40.62±16.9[a]	44.45±18.5[a]	45.10±18.30[a]	46.28±18.3[a]	47.39±17.68[a]	52.69±20.1[a]	51.15±17.6[a]	59.07±21.0[a]
LSD values	58.82	62.26	58.63	64.95	54.46	59.22	55.92	60.70
Alpha values	0.68	0.47	0.76	0.55	0.32	0.36	0.74	0.95

under light and dark photoperiod regimes using Picloram

Values are a mean of 3 replicates with their respective standard errors. Values followed by the same letter in each column are not significantly different at $P \leq 0.05$.

Table 3: Frequencies of somatic embryogenesis in cassava genotypes under light and dark photoperiod regimes using 2, 4-**D**

	4mg/l		6mg/l		8mg/l		10mg/l	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark
Ex-Ndolo	18.83±2.1^{ab}	37.76±1.3^{a}	25.28±4.8^{a}	31.00±5.2^{a}	27.00±4.8^{a}	26.42±2.4^{a}	33.47±6.6^{a}	26.66±4.0^{a}
Karibuni	35.89±2.1^{a}	45.31±1.7^{a}	42.31±1.7^{a}	35.39±6.5^{a}	27.76±1.3^{a}	39.50±11.8^{a}	37.01±4.9^{a}	26.54±3.9^{a}
Shibe	17.92±7.0^{b}	30.04±14.1^{a}	32.74±14.8^{a}	34.69±15.5^{a}	21.26±8.7^{a}	21.66±4.8^{a}	31.61±10.6^{a}	42.31±13.6^{a}
LSD values	17.53	40.18	39.10	40.32	22.99	29.73	30.73	33.65
Alpha	0.03	0.59	0.50	0.95	0.70	0.27	0.88	0.32

Values are a mean of 3 replicates with their respective standard errors. Values followed by the same letter in each column are not significantly different at $P \leq 0.05$

3.2 Effects of explants on somatic embryogenesis

The two explants were able to form somatic embryos under the two auxins used in this study. There were significant differences ($P \leq 0.05$) in somatic embryogenesis frequencies when media supplemented with 2, 4-D and picloram were used to induce embryos using the two explants (TABLE 4). Calli induced using leaf explants showed higher somatic embryo frequencies compared to those from stems. For instance, *Ex-Ndolo* recorded the highest frequencies in somatic embryogenesis in media supplemented with picloram for both leaf and stem explants at 92.27% and 67.17% respectively. When media supplemented with 2, 4-D was used for embryo induction. *Shibe* recorded the highest frequency for leaves at 59.93% while *Karibuni* had the highest for stem at 41.46% (TABLE 5).

Table 4 Frequencies of somatic embryogenesis in cassava genotypes using leaf and stem under Picloram

Genotypes	**4mg/l**		**6mg/l**		**8mg/l**		**10mg/l**	
	Leaf	stem	Leaf	stem	Leaf	stem	Leaf	stem
Ex-Ndolo	77.63±1.8^{a}	49.99±3.9^{a}	81.85±2.4^{a}	46.47±2.3^{a}	92.27±3.2^{a}	67.17±1.4^{a}	78.85±2.3^{a}	50.00±0.7^{a}
Karibuni	77.06±1.8^{a}	15.00±2.9^{b}	78.86±1.8^{a}	19.97±5.8^{b}	79.78±0.4^{b}	30.00±0.4^{b}	89.40±2.2^{a}	40.00±1.7^{b}
Shibe	73.32±1.9^{a}	11.95±0.5^{b}	77.32±1.7^{a}	14.06±0.7^{b}	82.75±2.7^{ab}	17.33±0.9^{c}	88.62±4.0^{a}	21.60±1.5^{c}
Lsd Values	7.47	11.20	8.05	14.36	9.77	4.11	11.78	5.22
Alpha values	0.26	<0.00	0.33	0.00	0.02	<0.00	0.07	<0.00

Values are a mean of 3 replicates with their respective standard errors. Values followed by the same letter in each column are not significantly different at $P \leq 0.05$.

Table 5 Frequencies of in somatic embryogenesis cassava genotypes using leaf and stem under 2, 4-D

Genotypes	**4mg/l**		**6mg/l**		**8mg/l**		**10mg/l**	
	Leaf	stem	Leaf	stem	Leaf	stem	Leaf	stem
Ex-Ndolo	38.88±9.7^{a}	17.70±1.4^{b}	36.58±2.5^{b}	19.70±1.4^{b}	32.50±2.1^{a}	20.92±1.4^{a}	38.88±3.8^{b}	21.25±1.3^{a}
Karibuni	39.74±3.9^{a}	41.46±2.4^{a}	50.60±2.6^{a}	27.37±2.1^{a}	43.63±9.4^{a}	23.63±2.9^{a}	39.39±3.9^{b}	24.16±2.6^{a}
Shibe	42.27±7.1^{a}	5.7±0.0^{c}	59.93±2.3^{a}	7.5±0.2^{c}	33.18±2.0^{a}	9.75±2.1^{b}	58.33±4.9^{a}	16.61±2.6^{a}
Lsd values	29.06	6.54	10.03	5.95	22.67	8.95	16.45	9.07
Alpha Values	0.39	< 0.00	0.00	<0.00	0.35	0.00	0.01	0.11

Values are a mean of 3 replicates with their respective standard errors. Values followed by the same letter in each column are not significantly different at $P \leq 0.05$.

3.3 Effects of auxin somatic embryogenesis

Regarding the effects of auxins (2, 4-D and Picloram) on somatic embryo frequency, there were significant differences ($P \leq 0.05$) across the three genotypes as shown in Fig 3. Generally, picloram induced higher frequencies of somatic embryogenesis as compared to 2, 4-D. *Ex-Ndolo* recorded the highest frequencies in somatic embryogenesis at 79.72% in picloram and *Karibuni* recorded 40.60% in 2,4-D. The lowest frequencies among the three genotypes was recorded in *Shibe* at 42.57% in picloram and at 21.46% in 2, 4-D.

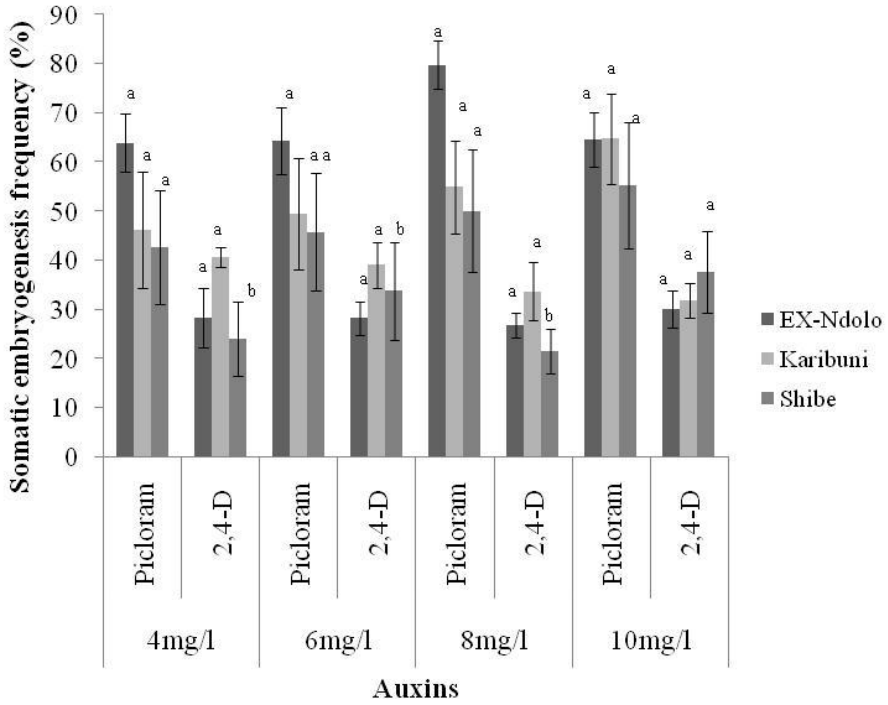

Figure 3: Effect of different auxins on frequencies of somatic embryogenesis across the three genotypes.

Data was recorded days after culture on callus induction media and represents a mean of three replicates. Vertical bars represent standard error of the mean while letters above data show separation
Bars with same letters above each error bar are not significantly different $P \leq 0.05$

3.4 Embryo maturation and germination

Embryo maturation was described by appearance of shoots, greening of cotyledons or a combination of the two. Embryogenic calli from leaf explants formed shoots (Fig. 1) while those from stem explants formed green cotyledon that later failed to convert into shoots (Fig. 2). Maturation of embyrogenic calli was carried out under the light regime (16h/8h). *Ex-ndolo* was highly responsive to maturation compared to the other two genotypes. The highest maturation frequency was recorded in media 2 at 40% for embryogenic calli from leaf from *Ex-Ndolo* that had been cultured under darkness using picloram while the highest maturation frequency for embryogenic calli from leaf that had been cultured in light in media 2 at 50% (Fig 4). *Karibuni* recorded the highest maturation frequency for embryogenic calli from leaf that had been cultured under light in media 1 and the highest maturation frequency at 20.93% under dark in media 4. No shoots were formed for genotypes *Karibuni* and *Shibe* for embryogenic calli that had been cultured in 2, 4-D despite the presence of embryogenic calli. The highest shoot formation was recorded in media 3 for embryogenic calli cultured in 2, 4-D under dark in *Ex-Ndolo* as shown in Fig. 5.

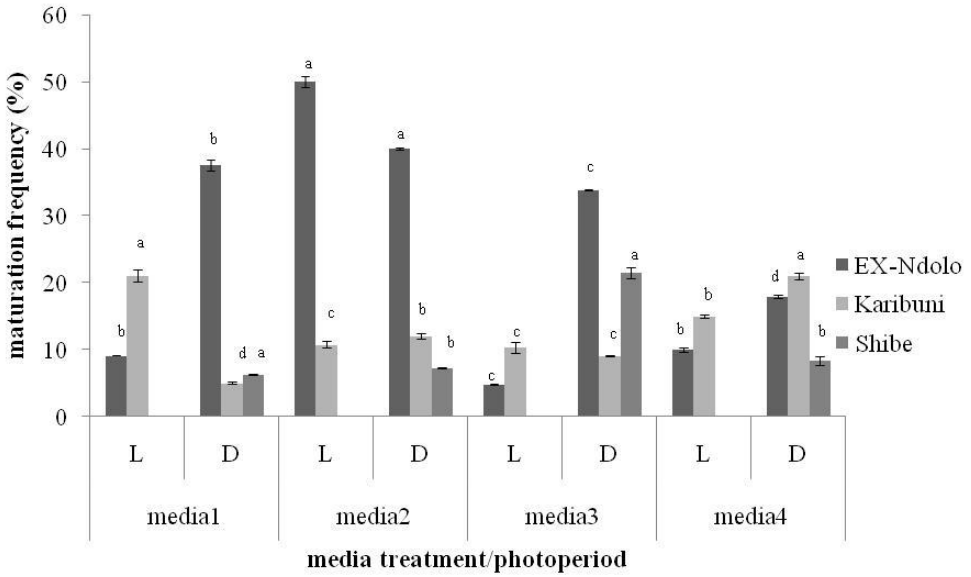

Figure 4 Effects of varying concentrations of different hormone combination ratios on the frequency of embryo maturation in calli from cassava leaves induced using picloram.

Data was recorded days after culture on maturation media and represents a mean of three replicates. Vertical bars represent standard error of the mean while letters above data show separation.
Bars with same letters above each error bar are not significantly different $P \leq 0.05$ per photo regime
L light D dark

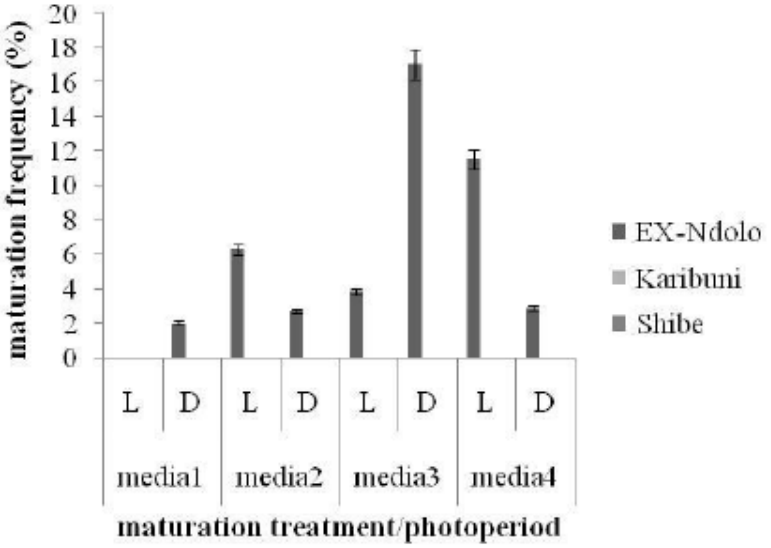

Figure 5: Effects of varying concentrations of different hormone combination ratios on the frequency of embryo maturation in calli from cassava leaves induced using 2, 4-D.

Data was recorded days after culture on maturation media and represents a mean of three replicates. Vertical bars represent standard error of the mean while letters above data show separation.

L- Light D Dark.

Embryos originating from stem explants did not form shoots but instead remained at the cotyledonary stage of the somatic embryogenesis. The highest maturation frequency for stems was recorded in media 1 when genotype *Karibuni* cultured under light in media supplemented with picloram produced 53.19% frequency. On the other hand, those cultured under darkness had the highest maturation frequency in media 2 at 53.33% for *Ex-Ndolo* (Fig. 6). The highest maturation frequency for embryogenic calli from stems cultured under light using 2,

4-D was recorded under media 3 with 86.67% frequency in *Karibuni* while the highest for embryogenic calli from stem cultured under dark was recorded in media 1 at 72.72% (Fig. 7). For embryogenic calli cultured in picloram, higher GA_3 concentration seemed to favour the maturation while in 2, 4-D higher concentration of BAP favoured maturation in calli from stem explants.

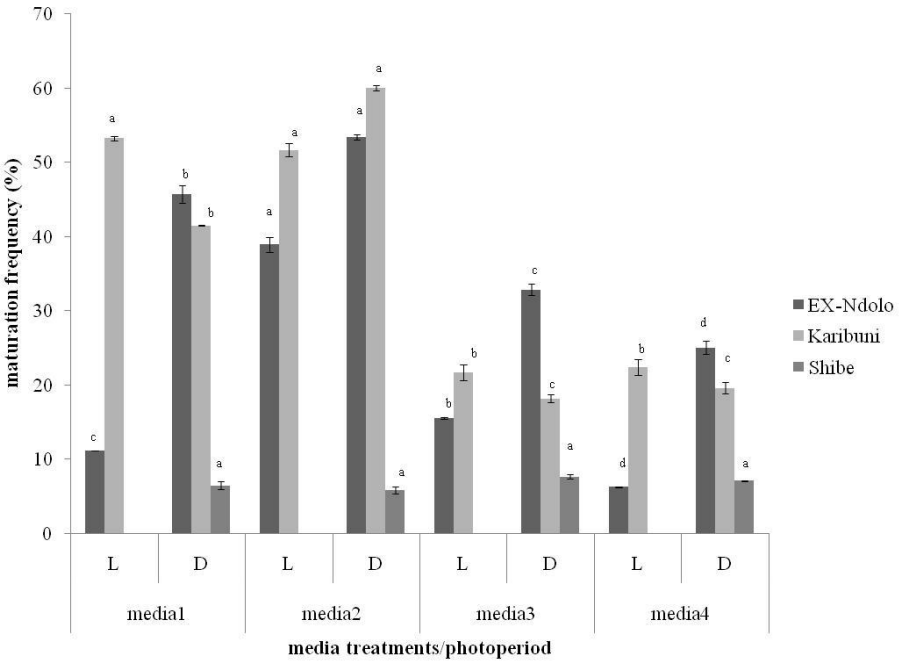

Figure 6: Effects of varying concentrations of different hormone combination ratios on the frequency of embryo maturation in calli from cassava stem induced using picloram.

Data was recorded days after culture on maturation media and represents a mean of three replicates. Vertical bars represent standard error of the mean while letters above data show separation.
Bars with same letters above each error bar are not significantly different $p \leq 0.05$ per photoregime
L - Light D- Dark

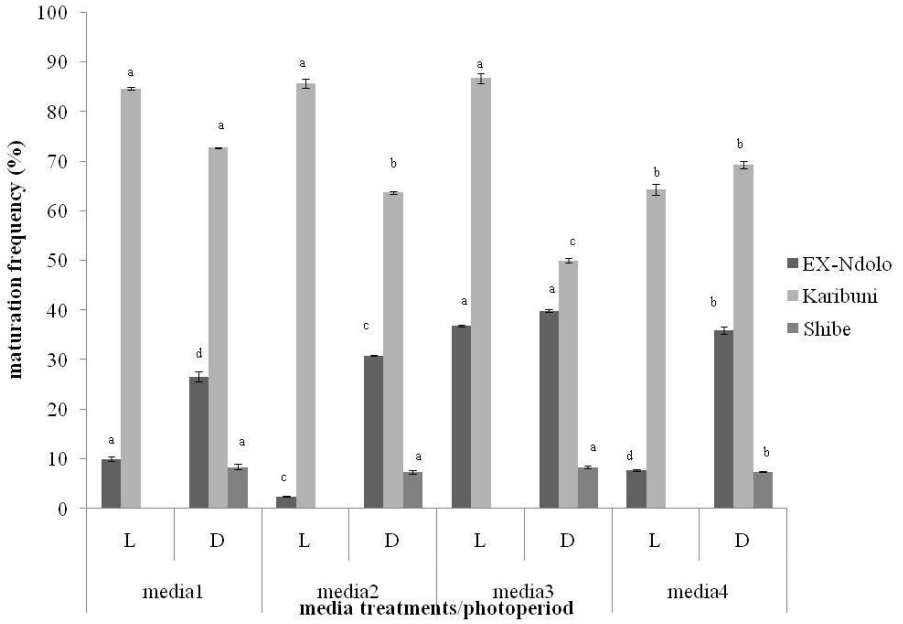

Figure 7: Effects of varying concentrations of different hormone combination ratios on the frequency of embryo maturation in calli from cassava stem induced using 2, 4-D.

Data was recorded days after culture on maturation media and represents a mean of three replicates. Vertical bars represent standard error of the mean while letters above data show separation.
Bars with same letters above each error bar are not significantly different $P \leq 0.05$ per photoregime

L - Light D – Dark

Shoots were transferred to MS media supplemented with sucrose, BAP and 8mg/l activated charcoal for a week then moved to rooting media (with half strength MS, sucrose and gelling agent). Some shoots didn't form roots as required. Root induction frequency was highest in *Ex-Ndolo* at 26% and lowest in *Shibe* at 10% (TABLE 6). Plantlets (shoots with roots) were transplanted into pots with a mixture of peat moss and vermiculite and kept under high humidity conditions by use of a plastic bag under a shade to avoid direct sunlight (Fig 1). The survival percentage of these plants at the acclimatization stage was determined six weeks after the hardening process. *Ex-Ndolo* had a highest survival rate at 16% while*Shibe* recorded the lowest survival rate 3% (TABLE 6).

Table 6: Root induction frequency and survival rate of cassava shoots

Genotypes	Root Induction %	Survival rate %
Ex-Ndolo	26 ± 1.41^{a}	16.00 ± 1.41^{a}
Karibuni	18 ± 2.82^{b}	10.00 ± 1.41^{b}
Shibe	10 ± 2.82^{c}	3.00 ± 1.41^{c}
Lsd Values	10.24	5.90
Alpha values	0.02	0.01

Values followed by the same letter in each column are not significantly different at $P \leq 0.05$.

IV Discussion

Tissue culture provides an alternative means for crop improvement and with previous works on plant species such as cotton [27], sugarcane [28] (and maize[29] showing that responsiveness to tissue culture is highly genotype dependent, it was necessary to optimize a regeneration protocol for cassava genotypes for any anticipated improvement programs. This study revealed differences among the three genotypes regarding somatic embryogenesis and this could be due to variations that occur among different genotypes as has been previously reported in cassava from South America [30], Africa [31] [32] and [25]and in several other plant species such as maize[29].

In the current study, leaf explants were superior to stem explants in terms of their embryogenic capacity as shown by somatic embryogenic frequencies which were higher in both picloram and 2, 4-D compared to stem counterparts. A similar study by [33] investigated effects of explants stem, leaf and auxillary buds under different concentrations of 2, 4-D on callus induction using different cultivars and found 8mg/l of 2, 4-D was the best concentration for callus induction in Ghanaian cassava varieties. Furthermore, leaf explants were shown to be more totipotent compared to stem petioles and auxiliary buds. Under the current study, a high embryogenic frequency translated to high number of embryos which increased chances of embryo germination therefore agreeing with [33] who further found leaves to be more totipotent, easily programmed and less lignified hence allowing phytohormone penetration. On the other hand, contrary reports [34] have shown that frequency of somatic embryos is not always affected by type of explants and tissue culture conditions may vary within tissues within the same plant and therefore explants obtained from the same plant could give different results [35].

There were no significant differences between explants cultured under light and dark concerning the embryogenic capacity. Related studies have reported that growth under white light has been associated with elevated phenolic compound production and an increased level of abscisic acid which could affect development of resulting embryos [36].[21]found that three out of seven cultivars screened were responsive in limited light, which agreed with our findings that some cassava genotypes form embryos in light (16 hour light/8hours in dark) while other form embryos in dark (0 hour in light/24 hour in dark). Other studies [25] [32] have reported contrary findings where more embryos are formed under dark regime.

Maturation media with higher concentrations of a BAP seemed to favour shoot formation is testament of the importance of the cytokinin to promotion of shoot formation.However, earlier reports have reported that it is a combination of cytokinin, auxin and gibberellins in appropriate combinations that boosts shoot formation in cassava[23],[30]. Gibberellic acid promotes shoot elongation while NAA boosts maturation of primary embryos to secondary embryos. A positive effect of BAP and Gibberellic acid in shoot formation and elongation has been reported and showed that when BAP and GA3 were used solely, fewer plantlets were observed, but when the two hormones were combined in media, efficient embryo germination was observed [37]. These synergistic effects of cytokinin and gibberellin in promoted the conversion of embryos to plantlets has also been reported in a number of species [38], [39] and [40].

Our data further revealed that shoot formation was genotype dependent with *Ex-Ndolo* being highly responsive to all the four maturation treatments. The greening of embryos from stem explants was an indication that with right manipulation of tissue culture conditions in terms of phytohormone concentration, it is possible to obtain shoots. Somatic embryos from both leaf and stem explants from *Karibuni* and *Shibe* could not be converted into shoots when 2, 4-D was used as the initial hormone for callus induction and somatic embryo production. This is in line with [34] who showed that shoot apical meristems did not form in the embryos originating from the culture media containing 2, 4-D. Histological studies on embryos derived from 2, 4-D revealed incomplete formation of the protoderm and it is postulated that this might have affected the full development of the epidermis, stalks and roots [34]. In the current study, embryos originating from 2, 4-D could be associated with malformations leading to failure to form shoots.

Various studies have demonstrated the recalcitrant nature of cassava to tissue culture. Using twenty one cultivars, [23] tested the regeneration potential of twenty one cultivars and found that eight cultivars formed plants, three reached the globular stage of somatic embryogenesis and one cotyledonary and the rest only formed callus. A similar trend was observed when fourteen cultivars were used to test plant regeneration using FECs. Eight of these cultivars formed plantlets, four achieved the cotelydonary stage the rest formed globular stage and others remained as friable embryogenic callus [41]. This demonstrates the recalcitrant nature of cassava to tissue culture procedures. Plant regeneration is important for regeneration system that can regenerate transformed explants.

V. Conclusion

Overall, this study showed that the leaf explant was superior to the stem regarding embryogenesis. Also, Picloram was a superior auxin to 2, 4-D andthat light and dark photoregimes can be used for induction of somatic embryos. Embryo maturation in embryogenic calli originating from leaf formed shoots while those from stem formed green cotyledons. Notable, only embryogenic callli from leaf in genotype *Ex-Ndolo* cultured under 2, 4-D formed shoots while the other genotypes didn't form shoots.Shoots emerged from embryogenic calli of leaf explant cultured in picloram in all the three genotypes. Further optimization maturation media and use of other cytokinins can be used to achieve a higher maturation frequency especially in stem explants.

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References

[1]. **FAO(2014).**FAO Statistical Yearbook 2014 - Africa Food and Agriculture. . http//:www.fao.org.
[2]. **FAOSTAT(2014).** Agricultural Statistics. Food and Agricultural Organization of the United Nations., Rome. http://faostat.fao.org.
[3]. **Jansson, C., Westerbergh, A., Zhang, J., Hu, X., and Sun, C. (2009).** Cassava, a potential biofuel crop in the People's Republic of China. Applied Energy 86, 95-99.
[4]. **Nweke, F., Spencer, D., and Lynam, J. (2009)**The cassava transformation. Africa's best kept secret. Michigan State University, Press.
[5]. **Lebot, V. (2009).** Tropical root and tuber crops: cassava, sweet potato, yams and aroids, Center for Agriculture and Biosciences International (CABI), 434.
[6]. **Dixon, A. G., Bandyopadhyay, R., Coyne, D., Ferguson, M., Ferris, R. S. B., Hanna, R., Hughes, J., Ingelbrecht, I., Legg, J., and**
[7]. **Mahungu, N. (2003).** Cassava: From poor farmers' crop to pacesetter of African rural development. Chronica Horticulturae **43**, 8-15.
[8]. **Tonukari, N. J. (2004).** Cassava and the future of starch. Electronic journal of biotechnology 7, 5-8.
[9]. **Hillocks, R., and Jennings, D. (2003).** Cassava brown streak disease: a review of present knowledge and research needs. International Journal of Pest Management 49, 225-234.
[10]. **Patil, B. L., and Fauquet, C. M. (2009).** Cassava mosaic geminiviruses: actual knowledge and perspectives. Molecular Plant Pathology 10, 685-701.
[11]. **Boher, B., and Verdier, V. (2009).** Cassava bacterial blight in Africa: the state of knowledge and implications for designing control strategies. African Crop Science Society 2, 505-509.
[12]. **Silva, D. V., Santos, J. B., Cury, J. P., Carvalho, F. P., Silva, E. B., Fernandes, J. S. C., Ferreira, E. A., and Concenço, G. (2013).** Competitive capacity of cassava with weeds: Implications on accumulation of dry matter. African Journal of Agricultural Research 8, 525-531.
[13]. **White, W. L., Arias-Garzon, D. I., McMahon, J. M., and Sayre, R. T. (1998).** Cyanogenesis in Cassava. The Role of Hydroxynitrile Lyase in Root Cyanide Production. Plant Physiology 116, 1219-1225.
[14]. **Sayre, R., Beeching, J. R., Cahoon, E. B., Egesi, C., Fauquet, C., Fellman, J., Fregene, M., Gruissem, W., Mallowa, S., and Manary, M. (2011).** The BioCassava plus program: biofortification of cassava for sub-Saharan Africa. Annual review of plant biology 62, 251-272.
[15]. **Wenham, J. E. (1995).** "Post-harvest deterioration of cassava: A biotechnology perspective," Food and Agriculture Organization.
[16]. **Beeching, J. R., Han, Y., Gomez-Vasquez, R., Day, R. C., and Cooper, R. M. (1998).** Wound and defense responses in cassava as related to post-harvest physiological deterioration. Phytochemical Signals and Plant-Microbe Interactions, 231-248.
[17]. **Reilly, K., Han, Y., Tohme, M., Joseph, B., and John, R. (1998).** Oxidative stress-related genes in cassava post-harvest physiological deterioration. Cassava biotechnology: Proceedings: International Scientific Meeting Cassava Biotechnology Network Brasil,

[18]. **Nassar, N. M., and Ortiz, R. (2009).** Cassava Genetic Resources: Manipulation for Crop Improvement. Plant Breeding Reviews **31**, 247.

[19]. **Nyaboga, E., Joshua Njiru, Nguu, E., Gruissem, W., Vanderschuren, H., and Tripathi, L. (2013).** Unlocking the potential of tropical root crop biotechnology in East Africa by establishing a genetic transformation platform for local farmer-preferred cassava cultivars. Frontiers in Plant Science | Plant Biotechnology 4, 1-12.

[20]. **Jiménez, V. M. (2001).** Regulation of in vitro somatic embryogenesis with emphasis on to the role of endogenous hormones. Revista Brasileira de Fisiologia Vegetal 13, 196-223.

[21]. **Hankoua, B., Taylor, N., Ng, S., Fawole, I., Puonti-Kaerlas, J., Padmanabhan, C., Yadav, J., Fauquet, C., Dixon, A., and Fondong, V. (2006).** Production of the first transgenic cassava in Africa via direct shoot organogenesis from friable embryogenic calli and germination of maturing somatic embryos. African Journal of Biotechnology 5. 1700-1712.

[22]. **Atehnkeng, J., Adetimirin, V., and Ng, S. (2006).** Exploring the African cassava (Manihot esculenta Crantz) germplasm for somatic embryogenic competence. African Journal of Biotechnology 5, 1324-1329.

[23]. **Rossin, C. (2008).** Cassava axillary bud transformation and production of somatic embryos of selected cassava cultivars, Msc thesis, University of the Witwatersrand.

[24]. **Elibariki, G., Lupembe, M., Hosea, K., and Ndunguru, J. (2014).** Evaluation of regeneration potentials of farmer-preferred cassava (Manihot esculenta Crantz) landraces to unlock cassava transformation barriers. International Journal of Agriculture and Crop Sciences 7, 560.

[25]. **Murashige, T., and Skoog, F. (1962).** A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia plantarum 15, 473-497.

[26]. **Ngugi, M., Oduor, R., Omwoyo, R., Njagi, J., and Mgutu, A. (2015).** Regeneration of Kenyan Cassava (Manihot esculenta Crantz) Genotypes. Journal Plant Biochemistry Physiology 3, 2.

[27]. **Gamborg, O. L. c., Miller, R. A., and Ojima, K. (1968).** Nutrient requirements of suspension cultures of soybean root cells. Experimental cell research 50, 151-158.

[28]. **Rajeswari, S., Muthuramu, S., Chandirakala, R., Thiruvengadam, V., and Raveendran, T. (2010).** Callus induction, somatic embryogenesis and plant regeneration in cotton (Gossypium hirsutum L.). Electronic Journal of Plant Breeding 1, 1186-1190.

[29]. **Gill, N. K., Gill, R., and Gosal, S. (2004).** Factors enhancing somatic embryogenesis and plant regeneration in sugarcane (Saccharum officinarum L.). Indian Journal of Biotechnology 3, 119-123.

[30]. **Mgutu, A. J., Anami, S. E., Taracha, C. O., Coussens, G., Karimi, M., Hilson, P., and Lijsebettens, M. V.(2009)**Somatic embryogenesis and plant regeneration of tropical maize genotypes, Plant Cell Tissue and Organ Culture.

[31]. **Szabados, L., Hoyos, R., and Roca, W. (1987).** In vitro somatic embryogenesis and plant regeneration of cassava. Plant Cell Reports 6, 248-251.

[32]. **Konan, N., Sangwan, R., and Sangwan, B. (1994).** Somatic embryogenesis from cultured mature cotyledons of cassava (Manihot esculenta Crantz). Plant cell, tissue and organ culture 37, 91-102.

[33]. **Ihemere, U. E. (2003)**Somatic embryogenesis and transformation of cassava for enhanced starch production, Doctoral diss., The Ohio State University.

[34]. **Fletcher, E. K., Amoako, T. N., and Twumasi, P. (2011).** Effect of 2, 4-D, explants type and cultivar on the callogenesis expression of cassava (Manihot esculenta Crantz) in Ghana. African Journal of Biotechnology 10, 9396-9401.

[35]. **Vidal, Á. M., Costa, M. A. P. d. C., Souza, A. d. S., Almeida, W. A. B. d., and Souza, F. V. D. (2014).** In vitro regeneration and morphogenesis of somatic embryos of cassava. Revista Ciência Agronômica 45, 558-565.

[36]. **Ezhova, T. (2003).** Genetic control of totipotency of plant cells in an in vitro culture. Russian Journal of Developmental Biology 34, 197-204.

[37]. **Smith, D. L., and Krikorian, A. D. (1990).** Somatic proembryo production from excised, wounded zygotic carrot embryos on hormone-free medium: evaluation of the effects of pH, ethylene and activated charcoal. Plant cell reports 9, 34-37.

[38]. **Emmanuel, S., Ignacimuthu, S., and Kathiravan, K. (2000).** Micropropagation of Wedelia calendulacea Less., a medicinal plant. Phytomorphology 50, 195-200.

[39]. **Martin, K. (2004).** Efficacy of different growth regulators at different stages of somatic embryogenesis Eryngium foetidumL.—A rare medicinal plant. In Vitro Cellular & Developmental Biology-Plant 40, 459-463.

[40]. **Paramageetham, C., Prasad Babu, G., and Rao, J. (2004).** Somatic embryogenesis in Centella asiatica L. an important medicinal and neutraceutical plant of India. Plant cell, tissue and organ culture **79**, 19-24.

[41]. **Junaid, A., Mujib, A., Bhat, M., Sharma, M., and Šamaj, J. (2007).** Somatic embryogenesis and plant regeneration in Catharanthus roseus. Biologia Plantarum 51, 641-646.

[42]. **Taylor, N. J., Munyaradzi, M. V., Rosa, C., Thao, H., Christian, S., and Claude, F. M. (2001).** Production of embryogenic tissues and regeneration of transgenic plants in cassava (Manihot esculenta Crantz). Euphytica 120, 25–34.