

In vitro Regeneration of Two Local Bambara Groundnut Landraces [*Vigna subterranea* (L.) Verdc.] in Kenya

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Abstract

Bambara groundnut [*Vigna subterranea* (L.) Verdc.] is a climate-resilient and nutrient-dense legume with limited genetic improvement in Kenya due to the absence of efficient *in vitro* regeneration systems. This study evaluated regeneration responses of two Kenyan landraces, Cream with Black Eye and Yellow with White Eye, using different explant sources and plant growth regulator regimes. Callus induction was achieved in all explants, with frequencies reaching 100% under 2,4-dichlorophenoxyacetic acid and 50-100% under naphthaleneacetic acid. Direct shoot regeneration from embryo axes cultured on Murashige and Skoog medium supplemented with 0.25 mg/L 6-benzylaminopurine produced the highest proliferation, yielding mean shoot numbers of 34.8 and 37.2 per explant in the two landraces. No shoot regeneration was observed from callus under any treatment. Rooting frequency reached 80-93% at 0.2 mg/L indole-3-butyric acid. Direct organogenesis proved more reliable than callus-mediated regeneration. These findings establish a reproducible regeneration protocol for Kenyan Bambara groundnut and provide a basis for propagation and future biotechnological applications.

Keywords: tissue culture, plant growth regulators, organogenesis, auxins, cytokinins, embryo explants

1. Introduction

One of the primary factors influencing crop production and contributing to food insecurity and poor livelihoods in sub-Saharan Africa (SSA) is the limited availability of improved crop varieties and modern agricultural technologies. Currently, only a small number of major crop species account for a large proportion of global food consumption, while many nutritionally rich indigenous crops remain underutilized and insufficiently researched (Majola et al., 2021; Okafor et al., 2022). This imbalance constrains diversification of agricultural systems and limits the potential to improve nutrition and resilience, particularly in marginal environments. Bambara groundnut [*Vigna subterranea* (L.) Verdc.] is an indigenous African legume recognized for its ability to perform under low-input conditions and its potential contribution to food and nutritional security (Khan et al., 2021; Mayes et al., 2019). Despite these attributes, the crop remains underutilized and weakly integrated into formal research and market systems, especially in Eastern Africa. In Kenya, Bambara groundnut is primarily cultivated at subsistence level using farmer-saved landraces, with limited institutional support for varietal development and value chain expansion (Chelangat et al., 2023). This reflects a broader limitation in the application of advanced crop improvement technologies to orphan crops.

Efforts to improve Bambara groundnut through conventional breeding have been constrained by genetic complexity and limited research investment, resulting in continued reliance on heterogeneous landraces rather than improved varieties (Ahmad et al., 2016; Alake & Ayo-Vaughan, 2016). These constraints have increased interest in alternative approaches such as plant tissue culture, which offers opportunities for rapid propagation, germplasm conservation, and genetic improvement (Alake & Ayo-Vaughan, 2016). However, the development of efficient regeneration systems in Bambara groundnut remains a significant challenge. Regeneration responses are highly dependent on genotype and explant source, leading to inconsistent outcomes across studies (Koné et al., 2009; Unigwe et al., 2017). In particular, indirect regeneration via callus is often unreliable, while direct organogenesis from embryo-derived tissues has been reported to produce more consistent results (Aliyu et al., 2014; Mubaiwa et al., 2016). These limitations highlight the need for optimized and reproducible regeneration protocols tailored to specific genetic materials. In the Kenyan context, there is limited empirical evidence on the *in vitro* response of locally adapted Bambara groundnut landraces. Although genotype-specific variation has

been documented elsewhere, the response of Kenyan germplasm to controlled tissue culture conditions remains insufficiently characterized (Koné et al., 2009; Mongomaké et al., 2009). This gap constrains the application of biotechnological tools for crop improvement and limits progress toward enhanced productivity and resilience.

Previous studies have demonstrated that regeneration in Bambara groundnut is strongly influenced by both genotype and explant type, with embryo axes frequently identified as the most responsive tissues for shoot induction (Koné et al., 2009; Lacroix et al., 2003). Auxins such as 2,4-dichlorophenoxyacetic acid and naphthaleneacetic acid are widely used for callus induction, although their effectiveness varies depending on concentration and explant source (Konaté et al., 2013). Cytokinins, particularly 6-benzylaminopurine, play a central role in promoting shoot organogenesis, but optimal concentrations differ across genotypes and experimental conditions (Koné et al., 2013). Despite these advances, most existing studies have focused on non-Kenyan germplasm, limiting the applicability of findings to local production systems. Bambara groundnut exhibits substantial genetic diversity across African landraces, and regeneration efficiency is frequently genotype dependent (Aliyu et al., 2016; Minnaar-Ontong et al., 2021). The limited evaluation of Kenyan landraces under controlled conditions has therefore constrained the development of standardized regeneration protocols suitable for local use.

To address these limitations, the present study evaluates the *in vitro* regeneration potential of two Kenyan Bambara groundnut landraces using different explant sources and plant growth regulator regimes. The study focuses on assessing callus induction, shoot regeneration, and root formation under controlled culture conditions. Specifically, the study aims to establish callus cultures from multiple explants and to determine the efficiency of plantlet regeneration through both direct and indirect pathways. The experimental design, based on controlled hormone treatments and replicated trials, enables comparison of regeneration responses across landraces, explant types, and growth regulator concentrations. This approach provides a structured basis for identifying optimal conditions for regeneration and supports the development of reproducible protocols applicable to Kenyan germplasm.

2. Methodology

2.1 Plant Material

Bambara groundnut seeds [*Vigna subterranea* (L.) Verdc.] from the Kenya Agricultural and Livestock Research Organization (KALRO), Kakamega, were used in the study. The seeds were verified and represented germplasm that was grown locally and tailored to the agro-ecological conditions of Kenya. Based on local availability and seed coat characteristics, two different landraces were chosen for this investigation. Many smallholder farmers cultivate these landraces, and farmer-saved seed systems play a major role in their continued existence. The testa of the first landrace, Cream with Black Eye (CBE), is cream in color and has a noticeable black hilum. With a pale hilum and a yellowish seed coat, Yellow with White Eye (YWE) is the second landrace (Figure 1). Seed color and visual morphology, which farmers frequently use to differentiate between Bambara kinds, are differences between the two landraces. These landraces were chosen in order to evaluate responses to *in vitro* culture conditions that are dependent on genotype. Seeds were kept at room temperature in a dry laboratory setting before being used in order to preserve their viability.

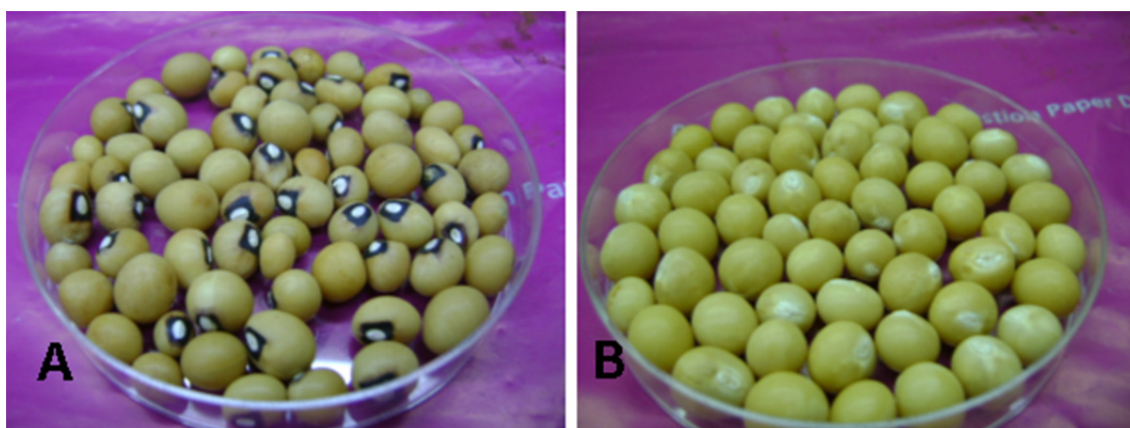


Figure 1. Bambara groundnut seeds, Land race A, CBE (Cream with Black Eye), Land race B, YWE (Yellow with White Eye)

2.2 Explant Preparation and Surface Sterilization

In this study, mature zygotic embryos, cotyledons, leaf explants, and petioles were the four types of explants used. After imbibition, mature embryos were removed straight from the seeds. Aseptically germinated seedlings were used to obtain cotyledons, leaves, and petioles. To reduce contamination, seedlings were surface sterilized according to a defined procedure. To get rid of surface dirt, the seeds were first rinsed under running tap water. After that, they were brought inside the lab and submerged for 30 seconds in 70% ethanol. A 40-minute treatment with a 100% commercial sodium hypochlorite solution came next. To get rid of any remaining disinfectant, seeds were rinsed three or four times with sterile distilled water after sterilization. To soften the seed coat for embryo explants, sterilized seeds were immersed in sterile distilled water for 48 hours.

Using sterile forceps and scalpel blades, the seed coat was removed and the embryo axes were carefully extracted under aseptic circumstances in a laminar airflow cabinet. The embryo base was protected from mechanical harm by taking precautions. Sterilized seeds were cultivated on hormone-free *Murashige* and *Skoog* media in sterile circumstances to produce cotyledon, leaf, and petiole explants. Cotyledons were removed from young seedlings once they had established themselves, and leaf and petiole explants were taken from fully grown but still immature leaves. Before inoculation, all explants were cut to uniform size. Strict aseptic procedures were followed during explant preparation, such as igniting equipment to sterilize them and using ethanol to disinfect work surfaces.

2.3 Culture Media and Growth Conditions

For all in vitro culture studies, *Murashige* and *Skoog* basal media served as the basis. The medium comprised vitamins, sugar as the carbon source, and typical macro and micronutrients. Agar was utilized as the gelling agent at a concentration of eight grams per liter, and sucrose was added at a concentration of thirty grams per liter. The medium's pH was adjusted to 5.8 prior to autoclaving. As needed, media were transferred onto Petri plates or culture bottles, and they were autoclaved at 121 degrees Celsius for 15 minutes under standard pressure to sanitize them. When autoclaving was required, heat-sensitive plant growth regulators were added to the medium after filter sterilization.

The cultures were kept in a controlled growth environment with a photoperiod of sixteen hours of light and eight hours of darkness, and a temperature of twenty-eight degrees Celsius. It was illuminated by cold white fluorescent lights. On growth shelves, culture vessels were positioned at random to reduce positional effects. Explants were checked every day for morphogenic reactions and contamination. Two-week intervals were used for subculturing unless excessive growth or browning required an earlier transfer to fresh medium.

2.4 Callus Induction Experiments

Experiments on callus induction were conducted to evaluate the impact of auxin type, concentration, explant source, and landrace on callus formation. Two auxins were assessed: 2,4-dichlorophenoxyacetic acid and naphthaleneacetic acid. Each auxin was evaluated at doses between 0.5 and 8.0 milligrams per liter. Explants of embryo, cotyledon, leaf, and petiole from both landraces were inoculated onto *Murashige* and *Skoog* media augmented with the corresponding auxin concentrations. The hormone-free *Murashige* and *Skoog* media functioned as the control. Explants were positioned with the cut surface in contact with the media to facilitate callus initiation. Each treatment comprised several explants allocated among replicate culture containers. The cultures were cultivated under the previously specified growth parameters. Callus induction was observed by the emergence of disorganized proliferative tissue at the cut surfaces of explants. The duration of callus start, callus texture, and coloration were documented. The frequency of callus induction was determined as the proportion of explants generating callus in relation to the total number of explants grown for each treatment. Subsequent the initial induction, calli were transferred to fresh media of identical composition to sustain development.

2.5 Shoot Regeneration

Shoot regeneration experiments focused on direct organogenesis from zygotic embryo explants. Indirect regeneration from callus was also evaluated, although the primary emphasis was on embryo-based regeneration due to its reported efficiency in legumes. For direct shoot induction, embryo explants were cultured on *Murashige* and *Skoog* medium supplemented with 6 benzylaminopurine. BAP was tested at concentrations of 0.25, 0.5, 1.0, and 2.0 milligrams per litre. Hormone free *Murashige* and *Skoog* medium was used as a control to assess normal embryo germination. Embryos were placed vertically on the medium with the embryo base in contact with the surface. Cultures were maintained under standard growth conditions. Shoot regeneration was assessed by the formation of shoot buds and elongated shoots from the embryo base. The number of shoots per explant was recorded at regular intervals. Explants producing multiple shoots were sub-cultured onto fresh

medium of the same hormonal composition every twenty days to promote further shoot development and elongation. Attempts were also made to regenerate shoots from calli derived from various explants by transferring calli onto Murashige and Skoog medium supplemented with BAP alone or in combination with low concentrations of auxins.

2.6 Root Induction and Acclimatization

Regenerated shoots of adequate length were excised and transferred to rooting media. Root induction was conducted on half strength Murashige and Skoog medium to reduce salt concentration and facilitate root formation. Shoots were initially cultured on hormone free medium for root initiation, followed by transfer to half strength Murashige and Skoog medium supplemented with 0.25 mg/L indole 3 butyric acid to promote root elongation. Rooting frequency, root number, and root morphology were recorded.

2.7 Experimental Design

All experiments were conducted using a completely randomized design. Each treatment comprised six replicates, with five explants per replicate. To enhance reliability and reproducibility, the experiments were repeated three times. The response variables measured included callus induction frequency, number of shoots per explant, and rooting frequency. Treatments were randomly assigned to explants to minimize positional and handling effects during culture.

2.8 Statistical Analysis

Percentage data were subjected to arcsine transformation prior to analysis to satisfy assumptions of normality and homogeneity of variance. Analysis of variance was performed to assess the effects of landrace, explant type, auxin concentration, and cytokinin concentration on the measured responses. Where significant differences were detected, means were separated using the Tukey honestly significant difference test at a five percent probability level. All statistical analyses were conducted using IBM SPSS Statistics software, and results are presented as mean values with associated variation.

3. Results

3.1 Callus Induction from Different Explants

3.1.1 Effect of 2,4-D on Callus Induction

Callus induction was observed in embryo, leaf, petiole, and cotyledon explants cultured on MS medium supplemented with 2,4-D. No callus formation occurred on basal MS medium lacking plant growth regulators. Table 1 summarizes the callus induction frequencies observed across embryo, leaf, petiole, and cotyledon explants cultured on MS medium supplemented with varying concentrations of 2,4 D in both Bambara groundnut landraces.

In embryo explants, callus induction occurred at all 2,4-D concentrations (0.5-8.0 mg/L) in both landraces. Callus initiation began on the third day of culture, originating from the region where the plumule was separated from the radicle. The induced calli were white to cream in colour and proliferated progressively. Induction frequencies ranged from 86.7% to 100% in CBE and from 90.0% to 100% in YWE. Maximum induction was recorded at 2.0 and 4.0 mg/L for CBE and at concentrations ≥ 1.0 mg/L for YWE. Leaf explants exhibited callus formation across all 2,4-D concentrations except the control. Callus initiation occurred between the seventh and ninth day of culture, restricted primarily to cut edges. Induction frequencies increased with concentration up to 2.0 mg/L, reaching 93.3% in CBE and 96.7% in YWE, followed by reduced frequencies at 8.0 mg/L. Petiole explants showed callus induction at all tested concentrations. Callus initiation occurred within six to ten days. Both landraces achieved 100% induction at concentrations from 0.5 to 2.0 mg/L. At higher concentrations (4.0-8.0 mg/L), induction frequencies declined slightly but remained above 90%. Cotyledon explants also produced callus at all 2,4-D concentrations. Initiation began between eight and ten days. Induction frequencies approached or reached 100% across most concentrations. Browning of calli was observed at higher 2,4-D concentrations during prolonged culture. Morphological characteristics of calli induced under 2,4-D are presented in Figure 2.

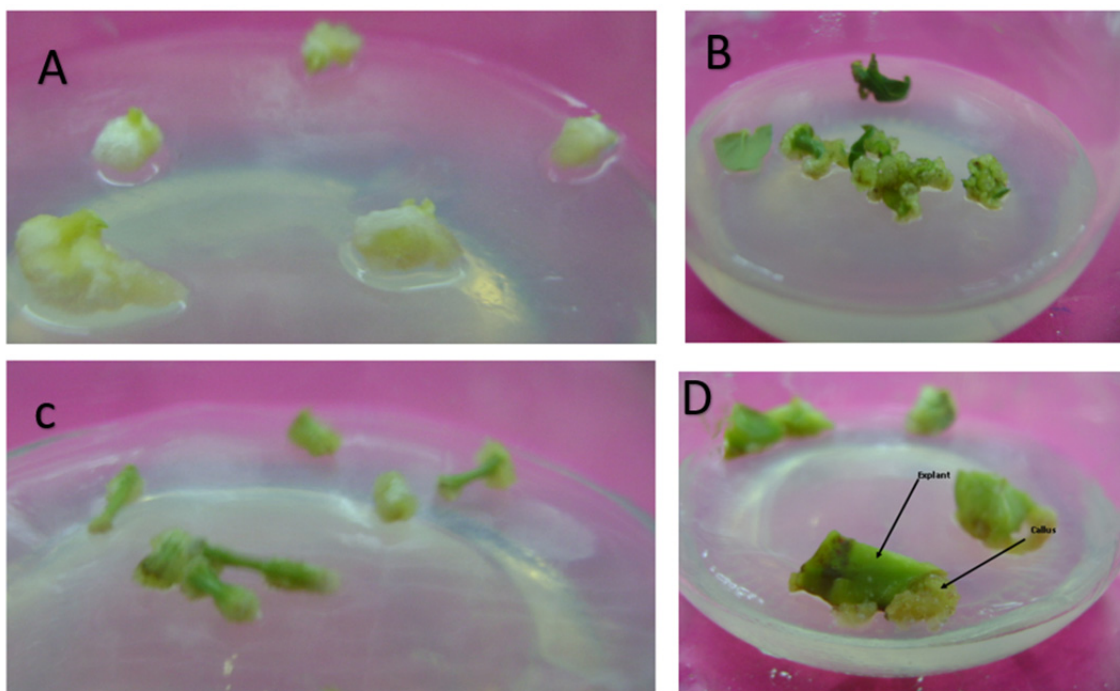


Figure 2. Bambara explants (A-embryo, B-Leaf, C-Petiole, and D-Cotyledon) showing callus induction on 2, 4 D MS medium after two culture weeks

3.1.2 Effect of NAA on Callus Induction

Callus formation was observed in all explant types cultured on MS medium supplemented with NAA. No callus induction occurred on basal MS medium. Table 2 presents the callus induction frequencies recorded for embryo, leaf, petiole, and cotyledon explants cultured on MS medium supplemented with varying concentrations of NAA in both Bambara groundnut landraces.

In embryo explants, callus induction occurred at all NAA concentrations (0.5-8.0 mg/L). Callus initiation began on the fourth day of culture at injury sites. The calli were white to cream and spongy in texture (Figure X). Induction frequencies ranged from 53.3% to 80.0% in CBE and from 60.0% to 83.3% in YWE. The highest induction frequencies were recorded at 2.0 mg/L in CBE and 4.0 mg/L in YWE. Leaf explants exhibited delayed callus formation under NAA treatments. Callus initiation occurred between ten and sixteen days. Induction frequencies ranged from 56.7% to 83.3% in CBE and from 50.0% to 86.7% in YWE. Maximum induction occurred at 2.0 mg/L in CBE and 8.0 mg/L in YWE. Petiole explants responded strongly to NAA supplementation. Callus initiation began on the fourth day of culture. Induction frequencies reached 100% at 1.0 and 2.0 mg/L in CBE and at 2.0 and 4.0 mg/L in YWE. At other concentrations, induction frequencies remained above 83%. Cotyledon explants showed callus induction across all NAA concentrations. Initiation occurred around the thirteenth day of culture. Induction frequencies reached 100% at concentrations from 1.0 to 8.0 mg/L in CBE, while YWE exhibited induction frequencies between 90.0% and 100%. Browning was not observed in NAA-derived calli. Morphological characteristics of calli induced under NAA treatments are presented in Figure 2.

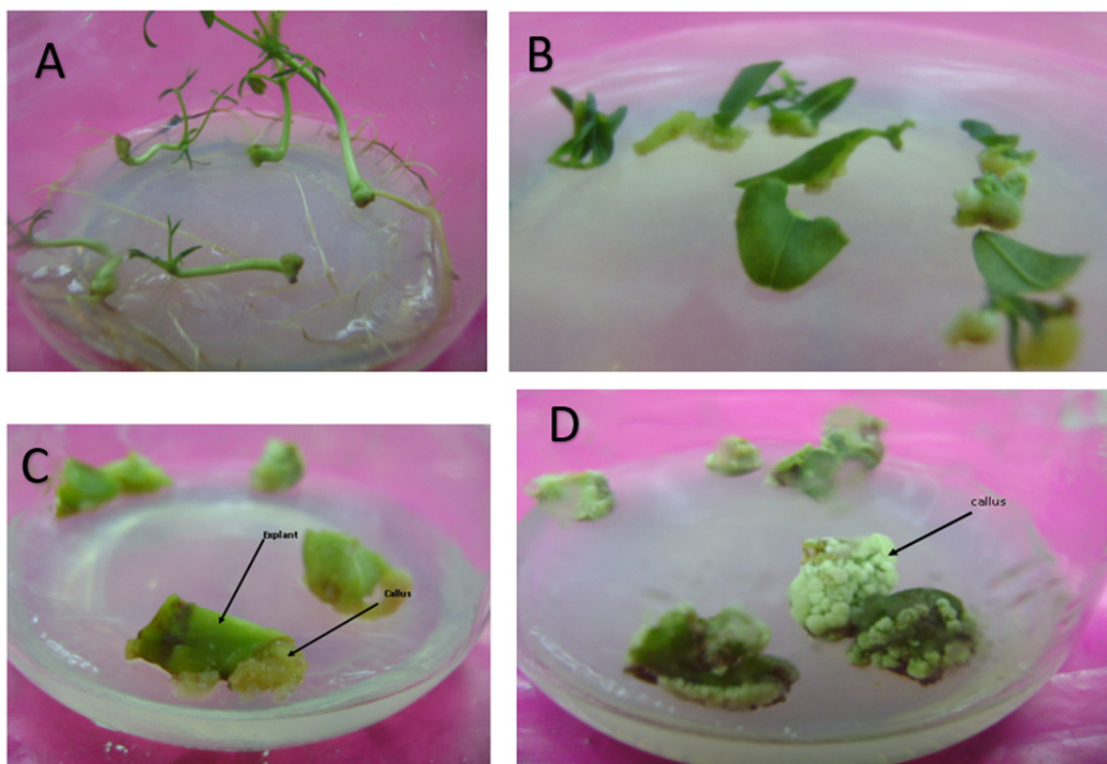


Figure 3. Bambara explants (A-embryo, B-Leaf, C-Petiole, and D-Cotyledon) showing callus induction on MS medium complemented with NAA

Table 1. Effect of 2,4-D on Callus Induction Frequency (%) from Different Explants of Bambara Groundnut Landraces CBE and YWE

Naa (mg/L)	No. of explants	Embryo explants Lr. CBE	Embryo explants Lr. YWE	Leaf explants Lr. CBE	Leaf explants Lr. YWE	Petiole explants Lr. CBE	Petiole explants Lr. YWE	Cotyledon explants Lr. CBE	Cotyledon explants Lr. YWE
0.0	30	0.0±0.0 ^a	0.0±0.0 ^a	0.0±0.0 ^a	0.0±0.0 ^a	0.0±0.0 ^a	0.0±0.0 ^a	0.0±0.0 ^a	0.0±0.0 ^a
0.5	30	63.3±6.1 ^{bc}	60.0±5.2 ^b	56.7±6.1 ^b	50.0±6.8 ^b	86.7±6.7 ^b	93.3±6.7 ^b	86.7±6.7 ^b	90.0±6.8 ^b
1.0	30	53.3±4.2 ^b	70.0±6.8 ^b	66.7±8.4 ^{bc}	73.3±6.7 ^{bc}	100±0.0 ^b	83.3±8.0 ^b	100±0.0 ^c	100±0.0 ^b
2.0	30	80.0±5.1 ^c	73.3±4.2 ^b	83.3±6.1 ^c	70.0±6.8 ^{bc}	100±0.0 ^b	100±0.0 ^b	100±0.0 ^c	100±0.0 ^b
4.0	30	73.3±8.4 ^{bc}	83.3±6.1 ^b	80.0±5.2 ^{bc}	63.3±6.1 ^{bc}	96.7±3.3 ^b	100±0.0 ^b	100±0.0 ^c	90.0±4.5 ^b
8.0	30	70.0±4.5 ^{bc}	63.3±8.0 ^b	60.0±5.1 ^{bc}	86.7±4.2 ^c	83.3±6.1 ^b	93.3±6.7 ^b	100±0.0 ^c	93.3±4.2 ^b

Note. Values are mean±SE. Percentage data were arcsine transformed prior to ANOVA. Means followed by different letters within each column differ significantly at $P < 0.05$ according to Tukey's HSD test.

Table 2. Effect of NAA on Callus Frequency (%) from Different Explants of Bambara Groundnut Landraces CBE and YWE

2,4-d (mg/L)	No. of Explants	Embryo Explants Lr. CBE	Embryo Explants Lr. YWE	Leaf Explants Lr. CBE	Leaf Explants Lr. YWE	Petiole Explants Lr. CBE	Petiole Explants Lr. YWE	Cotyledon Explants Lr. CBE	Cotyledon Explants Lr. YWE
0.0	30	0.0±0.0 ^a	0.0±0.0 ^a	0.0±0.0 ^a	0.0±0.0 ^a	0.0±0.0 ^a	0.0±0.0 ^a	0.0±0.0 ^a	0.0±0.0 ^a
0.5	30	86.7±6.7 ^b	90.0±4.5 ^b	70.0±4.5 ^{bc}	66.7±6.7 ^b	100.0±0.0 ^b	100.0±0.0 ^b	96.7±3.3 ^b	90.0±6.8 ^b
1.0	30	93.3±4.2 ^b	100.0±0.0 ^c	86.7±4.2 ^{bcd}	83.3±6.1 ^{bc}	100.0±0.0 ^b	100.0±0.0 ^b	100.0±0.0 ^b	100.0±0.0 ^b
2.0	30	100.0±0.0 ^b	100.0±0.0 ^c	93.3±6.7 ^d	96.7±3.3 ^c	100.0±0.0 ^b	100.0±0.0 ^b	100.0±0.0 ^b	96.7±3.3 ^b
4.0	30	100.0±0.0 ^b	100.0±0.0 ^c	90.0±4.5 ^{cd}	80.0±8.9 ^{bc}	93.3±4.2 ^b	96.7±3.3 ^b	100.0±0.0 ^b	100.0±0.0 ^b
8.0	30	96.7±3.3 ^b	100.0±0.0 ^c	66.7±8.4 ^b	63.3±6.1 ^b	90.0±6.8 ^b	96.7±3.3 ^b	100.0±0.0 ^b	100.0±0.0 ^b

Note. Values are mean±SE. Percentage data were arcsine transformed prior to ANOVA. Means followed by different letters within each column differ significantly at $P < 0.05$ according to Tukey's HSD test.

3.2 Shoot and Root Regeneration

3.2.1 Regeneration From Callus

Calli derived from embryo, leaf, petiole, and cotyledon explants cultured on media containing 2,4-D or NAA were subsequently transferred to regeneration media with varying BAP concentrations (0.1 to 0.8 mg/L) to evaluate shoot induction. Additional calli were cultured on MS medium supplemented with BAP (0.5 to 3.0 mg/L) in combination with NAA (0.1 to 0.5 mg/L). Across all treatments, no shoot regeneration from callus was observed. Plantlet formation detected in cotyledon explants originated from the cotyledonary node rather than callus tissue (Figure 4). Root regeneration occurred in cotyledon explants cultured on 2.0 mg/L 2,4-D after two to four weeks (Figure 5), whereas no rooting was recorded in NAA-derived calli.

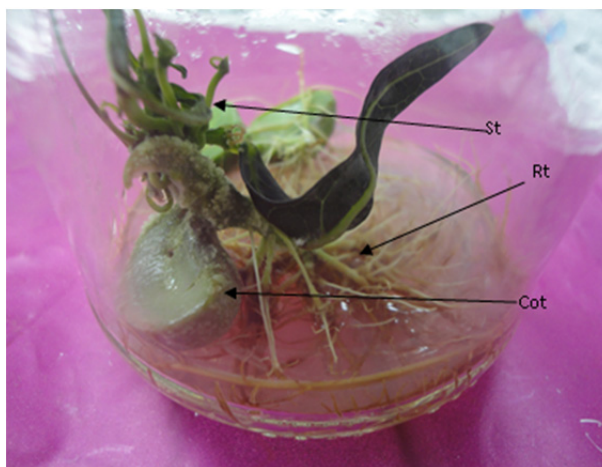


Figure 4. Plantlet regeneration from cotyledon node using 0.5 mg/L BAP on MS medium

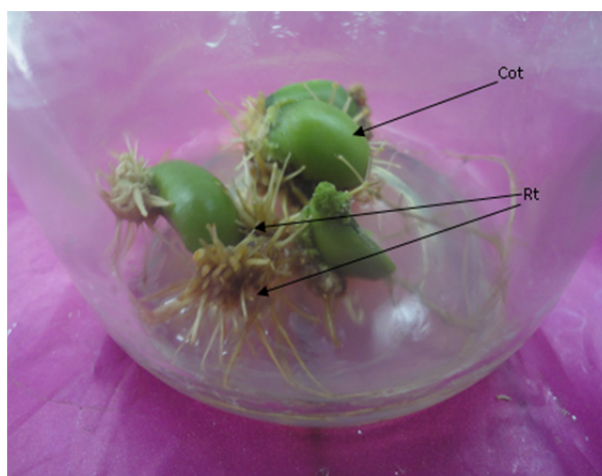


Figure 5. Root regeneration (rt) from Bambara cotyledons on 2 mg/L 2,4 D after 21 culture weeks

3.2.2 Shoot Regeneration From Zygotic Embryo Explants

Zygotic embryo axes cultured on basal MS medium germinated normally, with elongation of the plumule and no evidence of callus formation at the embryo base. Supplementation with 6-benzylaminopurine at 0.25 mg/L induced noticeable swelling at the embryo base by the third day of culture. The swollen region progressively enlarged, reaching an average diameter of approximately 0.5 cm by day 7. Multiple shoot buds emerged from this area by day 14 (Figure 6). By day 18, 40% and 36% of embryos had produced an average of three and four shoots in landraces CBE and YWE, respectively. Shoot proliferation continued, yielding mean values of 18 (CBE) and 20 (YWE) shoots by day 30, and 35 (CBE) and 37 (YWE) shoots by day 60. At 0.5 mg/L BAP,

swelling was also evident from day 3. Multiple shoots were observed from day 18 onward. By day 30, embryos produced averages of five shoots in CBE and nine shoots in YWE. Although browning of tissues occurred with prolonged culture, shoot bud formation persisted. By day 60, mean shoot numbers reached 11 in CBE and 15 in YWE.

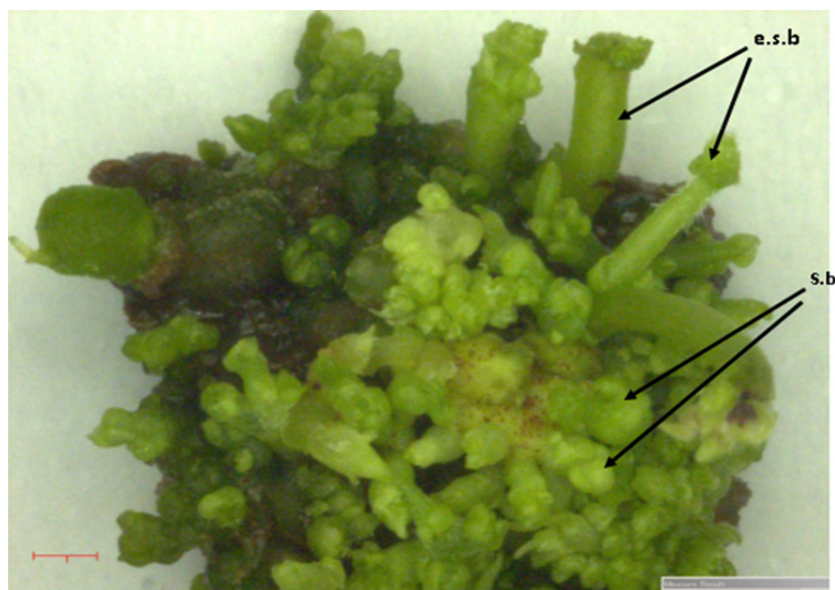


Figure 6. Shoot organogenesis from embryo base of Bambara on MS medium with 0.25 mg/L 6-Benzylaminopurine after 36 culture days

The shoot regeneration results from embryo explants in different concentrations of BAP were as presented in Table 3.

Table 3. BAP concentrations effect on shoot regeneration

BAP (mg/L)	No. of explants	Average shoots per embryo	
		Lr. CBE	Lr. YWE
0.0	30	1.00	1.00
0.25	30	34.8	37.2
0.5	30	11.3	14.7
1.0	30	5.80	7.30
2.0	30	6.70	6.20

Swelling responses induced by 1.0 mg/L BAP developed slowly at the embryo base. Although initial swelling was visible by day 3, subsequent enlargement progressed at a reduced rate. Shoot bud formation was first detected around day 23. By day 30, mean shoot numbers reached three and four per embryo in landraces CBE and YWE, respectively. Compared with lower BAP concentrations (0.25 to 0.5 mg/L), shoot elongation was markedly slower (Table 3). Swelling expansion also remained limited. By day 60, average shoot numbers increased modestly to six in CBE and seven in YWE. At 2.0 mg/L BAP, minimal swelling was observed by day 3. However, shoot elongation appeared suppressed. Many shoot buds initiated from the swollen base failed to develop into elongated shoots. By day 30, embryos produced averages of three shoots in CBE and four shoots in YWE. Slow tissue growth was associated with restricted swelling and reduced bud proliferation. By day 60, mean shoot numbers reached seven in CBE and six in YWE, indicating limited improvement under higher cytokinin concentration.



Figure 7. Regeneration of shoot from embryo explants on MS medium with 0.25 mg/L BAP after 48 culture days

3.2.3 Root Regeneration

Rooting was seen in shoots which were placed on $\frac{1}{2}$ MS lacking PGR after 13 days of culture. Little or no callus was formed at the shoots base anterior to root development on $\frac{1}{2}$ MS medium lacking any plant hormones. Roots formed as a single tap root with many short-lateral roots as displayed in Figure 8. By day 21 root induction frequency on $\frac{1}{2}$ MS basal was 33% and 37% in Lr. CBE and YWE respectively.



Figure 8. Root regeneration from shoots of Bambara on $\frac{1}{2}$ MS without PGRs after 20 culture days

The results of root regeneration from shoots derived from embryo explants in different concentrations of IBA were as presented in Table 4.

Table 4. IBA concentration effect on root regeneration in Lr. CBE and Lr. YWE of Bambara

IBA (mg/L)	No. of explants	% root regeneration frequency	
		Lr. CBE	Lr. YWE
0.0	30	36.7±6.1a	33.3±6.7a
0.1	30	66.7±6.7b	60.0±7.3b
0.2	30	80.0±7.3b	93.3±4.2c
0.4	30	83.3±3.3b	63.3±6.1b

Note. Data was arcsine remodelled before analysis of variance. Means ensued by constant figures within similar column are not considerably variant at $P < 0.05$ per Tukey's HSD test.

After 0.1 mg/L IBA was included in $\frac{1}{2}$ MS medium, root induction started much earlier by day 10 with little or no callusing from the base of the shoots. Roots appeared as single tap root with many lateral roots which continued to proliferate with time. By day 21, rooting frequency of 60% and 68% were achieved in Lr. CBE and YWE respectively. Rooting started on day 8 on 0.2 mg/L IBA as single tap roots with lateral buds. Minimal to no callusing was detected at the lower end of the shoot during induction process. By day 21, rooting frequency of 93% and 80% were achieved on Lr. CBE and YWE respectively. The optimum IBA concentration for rooting in Bambara Lr. CBE and YWE was 0.4mg/L and 0.2mg/L respectively (Table 4).

4. Discussion

The present study evaluated the regeneration potential of two Kenyan Bambara groundnut landraces using different explant sources and plant growth regulator regimes. The findings are interpreted in relation to callus induction behaviour, shoot organogenesis, and root formation, with emphasis on genotype-specific responses.

Callus induction occurred only in media supplemented with auxins, confirming that exogenous plant growth regulators were necessary to initiate dedifferentiation in both landraces. The absence of callus formation on basal Murashige and Skoog medium indicates that endogenous hormonal levels were insufficient to trigger cellular reprogramming. Similar observations have been reported in Bambara groundnut, where auxin supplementation is required for callus initiation (Konaté et al., 2013; Koné et al., 2009). In the present study, 2,4-dichlorophenoxyacetic acid consistently produced higher induction frequencies than naphthaleneacetic acid across all explants. For instance, embryo explants reached near-complete induction under 2,4-dichlorophenoxyacetic acid, whereas lower and more variable responses were observed under naphthaleneacetic acid. This difference suggests a stronger and more sustained dedifferentiation signal under 2,4-dichlorophenoxyacetic acid, likely linked to its higher stability and prolonged activity within plant tissues.

A clear genotype-related pattern was observed in callus induction. Although both landraces responded positively, Yellow with White Eye generally exhibited slightly higher induction frequencies and more consistent responses across explant types compared to Cream with Black Eye. This indicates differential sensitivity to auxin-mediated signalling between the two genotypes. Such variation has been widely reported in Bambara groundnut and other legumes, where regeneration efficiency is strongly genotype dependent (Koné et al., 2009; Jaiwal & Singh, 2003). The observed differences suggest that Yellow with White Eye may possess higher cellular responsiveness to dedifferentiation stimuli, which has implications for its suitability in callus-based applications.

Explant-specific responses further reinforced this pattern. Embryo and cotyledon explants showed earlier and more uniform callus initiation, whereas leaf explants exhibited delayed and lower induction frequencies. Between the two landraces, this contrast was more pronounced in Cream with Black Eye, where leaf responses were consistently lower. These differences reflect inherent physiological variation in tissue maturity and metabolic activity. Juvenile tissues, particularly embryos, contain active meristematic regions that support rapid cellular reprogramming, whereas more differentiated tissues such as leaves exhibit reduced plasticity (Jaiwal & Singh, 2003).

Despite successful callus induction, indirect shoot regeneration was not achieved under any treatment. Calli transferred to cytokinin-containing media failed to produce shoots, indicating a loss of morphogenic competence. This result aligns with previous reports that Bambara groundnut is recalcitrant in callus-mediated regeneration (Aliyu et al., 2014; Lacroix et al., 2003). The inability to regenerate shoots from callus suggests that dedifferentiation alone was insufficient to confer organogenic capacity, possibly due to genotype-specific constraints, hormonal imbalance, or oxidative stress during prolonged culture.

In contrast, direct shoot organogenesis from embryo axes was highly efficient. Low concentrations of 6-benzylaminopurine (0.25 mg/L) produced the highest shoot proliferation, with mean shoot numbers exceeding 34 in Cream with Black Eye and 37 in Yellow with White Eye. Increasing cytokinin concentration reduced both shoot number and elongation rate, indicating supra-optimal hormonal conditions at higher levels. Importantly, the superiority of Yellow with White Eye was consistently maintained across all cytokinin treatments, with higher shoot numbers and faster development compared to Cream with Black Eye. This confirms that genotype influences not only callus formation but also organogenic efficiency.

The observed genotype differences in shoot regeneration may reflect variation in hormonal sensitivity and developmental regulation. Bambara groundnut exhibits substantial genetic diversity across African landraces, and regeneration capacity has been linked to intrinsic differences in metabolic activity and signal transduction pathways (Aliyu et al., 2016; Minnaar-Ontong et al., 2021). The higher responsiveness of Yellow with White Eye suggests that it may be a more suitable candidate for regeneration-based improvement strategies.

Root induction was successfully achieved on half-strength Murashige and Skoog medium, with further enhancement under indole-3-butyric acid supplementation. Rooting frequency reached its highest levels at 0.2 mg/L indole-3-butyric acid, with Yellow with White Eye again showing a stronger response compared to Cream with Black Eye. This indicates that genotype-dependent differences extend to the rooting phase. Reduced salt concentration likely facilitated root initiation, while auxin supplementation promoted elongation and lateral root formation, consistent with established principles of rhizogenesis in tissue culture (Koné et al., 2013).

The results demonstrate that regeneration in Bambara groundnut is strongly influenced by both genotype and explant type, with direct organogenesis providing a more reliable pathway than callus-mediated approaches. The consistent superiority of Yellow with White Eye across callus induction, shoot regeneration, and rooting suggests that genotype selection is a critical factor in protocol optimization.

5. Conclusion

This study established an in vitro regeneration framework for two Kenyan Bambara groundnut landraces, CBE and YWE, using multiple explant types and defined plant growth regulator regimes. For shoot regeneration, Murashige and Skoog medium supplemented with 0.25 mg/L 6-benzylaminopurine produced the highest shoot proliferation from embryo axes and therefore represents the optimal cytokinin concentration among those tested. For rooting, shoots initiated roots on hormone-free medium, while half-strength Murashige and Skoog medium supplemented with 0.2 mg/L indole-3-butyric acid provided the highest rooting frequency and overall root quality. Although 0.4 mg/L indole-3-butyric acid supported root formation, it did not consistently improve rooting outcomes across both landraces. For callus induction, all explants required auxin supplementation, with petiole explants showing the strongest response, particularly on medium containing 2.0 mg/L 2,4-dichlorophenoxyacetic acid. The findings demonstrate that direct organogenesis from embryo axes is more reliable than callus-mediated regeneration and provide a reproducible protocol for propagation and future biotechnological applications in Kenyan Bambara groundnut.

6. Limitations of the Study

Several constraints should be considered. Only two landraces were included, limiting generalization across Bambara germplasm. The hormone regimes covered a defined concentration range; responses outside these windows were not tested. Culture conditions such as light spectrum, carbon source, antioxidants, and subculture intervals were not systematically varied, though they may influence morphogenesis. Indirect regeneration failed, yet physiological or molecular markers of callus competence were not assessed, restricting mechanistic interpretation. In addition, acclimatization was not evaluated, preventing conclusions on ex vitro survival and field performance. These factors indicate directions for refinement rather than weaknesses in experimental execution.

7. Practical Application

The optimized direct regeneration protocol from embryo axes offers a reliable platform for rapid multiplication of selected Bambara genotypes. It can support germplasm conservation, clonal propagation of superior landraces, and future transformation or gene editing workflows. Efficient shoot induction at lower BAP concentrations reduces culture duration and resource use, improving laboratory throughput. The rooting response on half strength medium with IBA enables production of well-developed plantlets suitable for downstream handling. Collectively, the protocol provides a technical foundation for breeding support, trait improvement, and scaling of planting material for climate resilient agriculture in Kenya.

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Authors Contributions

D.K.W. was responsible for data collection, laboratory experiments, data analysis, interpretation of results, and drafting of the manuscript. A.N.O. contributed to conceptualization of the study, supervision, manuscript editing, proofreading, and overall academic guidance. Both authors read and approved the final manuscript.

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