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EFFECT OF BENZYLAMINOPURINE ON *IN VITRO* REGENERATION OF BAMBARA GROUNDNUT (*VIGNA SUBTERRANEA* [L.]VERDC.)

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ABSTRACT

Bambara groundnut is a drought tolerant underutilized grain legume that has not received significant attention in terms of genetic improvement. This is probably due to the small sized nature of its flower, which was found to be difficult to hybridize. However, improvement of the crop can be achieved through genetic engineering as a tool for genetic enhancement. Development of an efficient in vitro regeneration system is a prerequisite for genetic engineering. The study was undertaken to study the influence of 6-Benzylaminopurine (BAP) on in vitro shoot regeneration in two Bambara groundnut landraces designated L-1 and L-2. Embryonic axes were used as explants, which were obtained from the seeds of the two landraces under aseptic conditions. The embryonic axes were further cultured on Murashige and Skoog (MS) media supplemented with different concentrations of BAP (0.5mg/L, 1.0mg/L, 1.5mg/L, 2.0mg/L) and compared with a control (hormone free medium). Results obtained from the study showed that maximum shoot formation (9.5 number of shoots/explant) was observed on MS medium supplemented with 1.0mg/L BAP, while the longest shoots (5.1cm) was observed on the hormone free medium. Significant variation was observed between the two landraces with respect to number of shoots per explant and shoot length. L-1 produced the highest number of shoots (7.8) while L-2 produced the tallest shoots (2.4cm). In conclusion, MS media supplemented with 1.0mg/L BAP can be adopted for shoot multiplication in in vitro regeneration of Bambara groundnut, while hormone free MS media could be used for shoot elongation using these landraces.

INTRODUCTION

Bambara groundnut (*Vignasubterranea* [L.] Verdcourt) also known as earth pea, is a member of the *Fabaceae* family. The center of origin of Bambara groundnut is perceived to be 'Bambara' near Timbuktu in Mali, West Africa. Its name is derived from a tribal name in Mali, the tribe called "Bambara" (Holm and Marloth, 1940; Murevanhema and Jideani, 2013). The crop is mainly cultivated in sub-Saharan Africa's warm tropics. Bambara groundnut has the potential for the attainment of food security and poverty alleviation in Africa, as it is drought tolerant and highly nutritious (Boateng et al., 2013). The nut can be used as food, in cosmetics and pharmaceutical industries. Flour obtained

from the nut can also be used as a partial replacement of wheat in cereal-based confectionaries such as biscuits, cake, bread, while the oil can be used in soap making (Afolabi, 2012; Ibrahim et al., 2016). Bambara groundnut is useful in crop rotation, because it contributes to the nitrogen pool of the soil (Ncube and Twomlow, 2007). Bambara groundnut is cultivated throughout tropical Africa and in Madagascar. About 45-50% of world, production comes from West Africa (Baudoin and Mergeai, 2001). Reliable production figures for the crop are difficult to obtain, because the crop is grown mainly for home consumption and sale at local markets (Brink et al., 2006).

World production increased from 29,800 tonnes in 1972 to 79,155 tonnes in 2005, and most recently, world production rose to 243,620 tonnes (FAOSTAT, 2015). Several factors have been reported to limit its wide adoption and production, particularly the presence of some anti-nutritional factors, like tannins and oxalate, which lowers protein availability and seed quality (Odumodu, 1992). The susceptibility of the crop to pests and diseases (Gwekwerere, 1995) and the unpredictable low yield (Massaweet *al.*, 2003) are also limiting factors. The yields are low because production and improvement of Bambara groundnut has been neglected for many years by researchers, even though the crop is important on small-scale production, due to its considerable commercial potentials (Bamishaiyeet *al.*, 2011). Improvement of Bambara groundnut can be achieved by genetic recombination and selection via artificial hybridization, but reports in this are limited (Lacroix *et al.*, 2003). The crop is self-pollinating, and due to the positioning of the flowers and the flower morphology, natural crossing has so far not been reported (Adu-Dapaah and Sangwan, 2003), while hybridization was found to be difficult to achieve fast genetic enhancement. Recent advances in biotechnology in the area of genetic transformation and embryo rescue was supposed to offer the opportunity to facilitate the development of improved genotypes by overcoming the difficulties faced during artificial hybridization. The utilization of this technology largely depends on the availability of efficient plant regeneration protocols. Although, there have been few reports on successful *in vitro* regeneration in Bambara groundnut (Koneet *al.*, 2009; 2015), however tissue culture is strongly genotype-dependent. Hence, the present study aimed at determining the effect of 6-Benzylaminopurine (BAP) on shoot induction and multiplication in two Bambara groundnut landraces to ascertain their amenability to tissue culture.

MATERIALS AND METHOD

Two (2) landraces of Bambara groundnut landraces designated L-1 and L-2 were used in the study. Seeds of the landraces were obtained from a local market in Kura, Kano State. The origin, description and passport data of the landraces are given in Table 1 and Plate 1, respectively. Seeds were surface-sterilized sequentially with 70% ethanol for 5min, and 2% Clorox (commercial bleach containing 3.5% NaOCl) plus 2-3 drops of Tween 20 for 20min with occasional stirring under the laminar hood. The seeds were then rinsed thrice with sterile distilled water, and were further soaked in sterile distilled water for 24hr. The seeds were then subjected to another sterilization sequence by immersing in 1% Clorox plus 2-3 drops of Tween 20 for 10min with occasional stirring and then rinsed thrice with sterile distilled water. Embryonic axes were dissected from sterile seeds and used as explants. The embryonic axes were cultured on MS media containing 30g/L sucrose, supplemented with BAP at varying concentrations (0, 0.5, 1.0, 1.5, 2.0 mg/L) and solidified with 1.5g/L of gelrite. Zero (0) mg/L BAP (hormone free) was used as control. The pH of the media was adjusted to 5.8 before autoclaving for 15 min at 121°C and 15psi. Cultures were maintained in growth chamber at 26±2°C under 16hr light/8hr dark photoperiod provided by cool white fluorescence tubes. Five test tubes were used per treatment and these were laid out in a completely randomized design replicated three times. Data were collected on regeneration frequency (%), plant height (cm) and number of shoots per explant. The data collected were subjected to analysis of variance (ANOVA) using the general linear model statistical analysis system (SAS institute Inc., 1990). Means were separated using least significant difference (LSD) at 0.05 probability level.

Table 1: Origin and Description of Bambara groundnut Landraces used in the study.

Landrace	Origin	Seed Colour	Eye
L-1	Obtained from a local market in Kura, Kano	Cream	Light greyed
L-2	Obtained from a local market in Kura, Kano	Cream with purple	Black-broad



Plate I: Bambara groundnut landraces used in the study. L-1: seeds with cream coat and light brown eye; and L-2: seeds with cream-striped seed coat and black-broad eye

RESULTS AND DISCUSSION

Effect of BAP and Landrace on Shoot Length in Bambara groundnut

Plant growth regulators are important in inducing signal for shoot organogenesis. A high cytokinin to auxin ratio promotes shoot meristem formation, while callus or root meristem is formed when the cytokinin to auxin ratio is low. Bambara groundnut can be regenerated using BAP singly, or in combination with NAA, Kin, IBA, TDZ (Konéet *al.*, 2009). Shoot regeneration was observed in all the treatments within 4 weeks of culture in responsive explants (Data not presented here). Results showed that the presence of BAP in the medium significantly ($P \leq 0.05$) affected shoot length. The control (hormone free medium) treatment produced the longest shoot length (5.12cm). A decrease in shoot length was observed with increasing concentration of BAP from 0.5mg/L; with medium supplemented with 2.0mg/L BAP recording the lowest shoot length, (0.77cm) (Fig. 1; Plate 2). Abdulmalik (2015), reported the inhibitory effect of BAP on shoot length in groundnut. Cytokinins are known to suppress apical dominance in plants (Taiz and Zeiger, 2003). This could be responsible for the decrease in shoot length observed in this study with increased concentration of BAP in the culture media. The two landraces tested in this study showed significant difference ($P \leq 0.05$) in terms of shoot length. These variations could be attributed to genotypic influence.

Effect of BAP and Landrace on Shoot Multiplication in Bambara groundnut

BAP is essential for shoot induction and multiplication in Bambara groundnut landraces as observed in this study. In the control (hormone free media) only single shoots were formed without any additional shoot bud development from the embryonic axes. The development of a single shoot from embryonic explant cultured on hormone-free medium has also been observed in groundnut (Abdulmalik, 2015). Supplementation of BAP in the media induced multiple shoot formation. MS medium supplemented with 1mg/L of BAP proved most effective for multiple shoot induction as maximum number of shoots per explant (9.5) was achieved in this medium (Fig. 3). On the contrary Konéet *al.*, (2009) observed maximum number of shoots per explant (3.7) with hypocotyls of Bambara groundnut on MS medium supplemented with 2mg/L BAP. The stimulatory effect of BAP on multiple shoot formation has been reported in cotton (Ganesanet *al.*, 2006). BAP is a cytokinin and cytokinin has been implicated in the enhancement of lateral bud formation in plant (Taiz and Zeiger, 2003). This could be responsible for the increase in multiple shoot formation with increased concentration of BAP in the media. Significant difference was also observed among the landraces evaluated in respect to multiple shoot formation. The landrace L-1 produced the highest number of shoots (7.8) than the landrace L-2 (4.4) (Fig. 4). The observed variation among the landraces could be attributed to genotypic influence.

Koné *et al.*, (2009) also reported that average number of shoots per explant differed among six landraces of Bambara nut evaluated for regeneration potential under tissue culture. This suggests that regeneration in Bambara nut is genotype dependent.

CONCLUSION

Results of the present study showed that maximum shoot multiplication in Bambara groundnut was obtained on MS medium

supplemented with 1.0mg/LBAP. Both the two landraces evaluated in this study proved amenable to tissue culture with L-1 recording maximum shoot multiplication (7.8 shoots per explant), while the landrace L-2 produced the tallest shoots (2.4cm). Further studies can be made to update this report, while comparing more Bambara groundnut genotypes using the protocol developed in this study.

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Plate 2: Effect of BAP on shoot length in Bambara groundnut

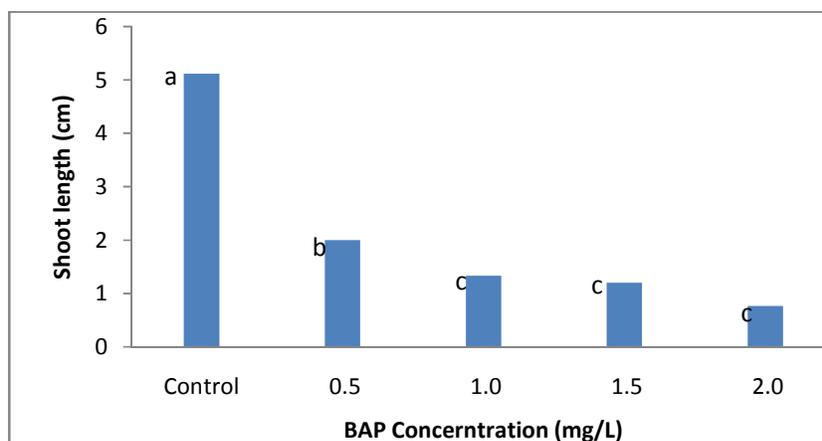


Figure 1: Effect of BAP treatment on shoot length in Bambara groundnut.

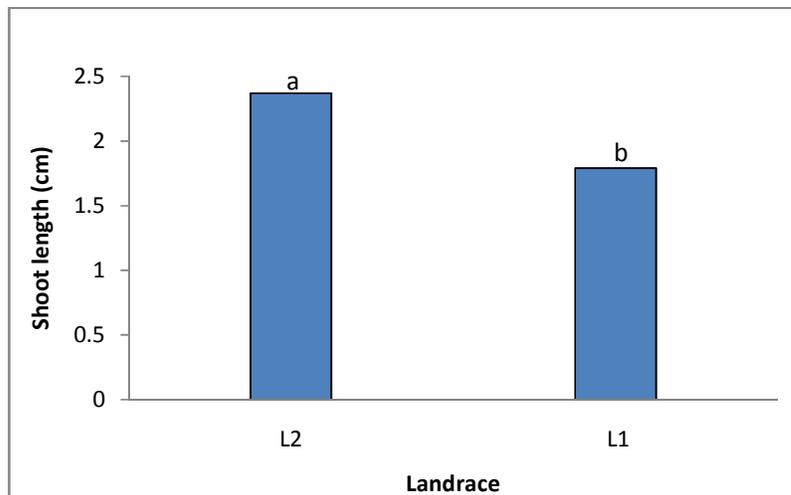


Figure 2: Effect of landrace on Shoot length in Bambara groundnut.

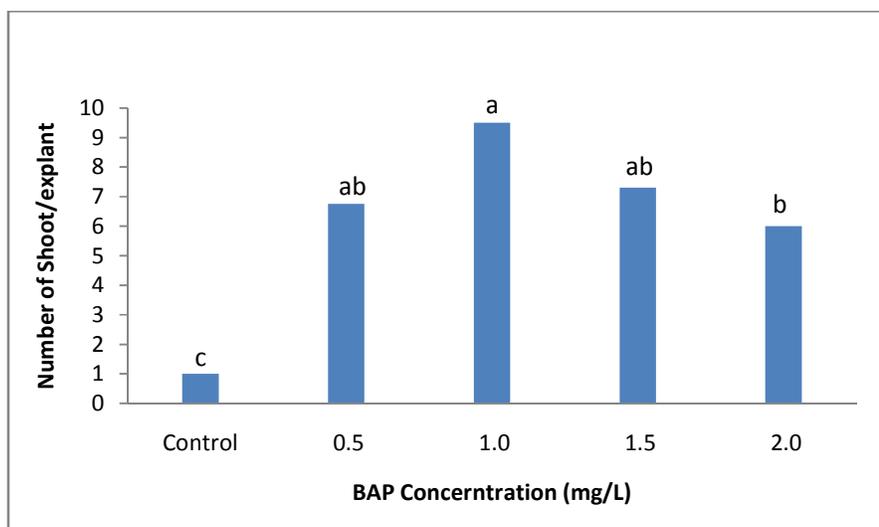


Figure 3: Effect of BAP treatment on multiple shoot formation in Bambara groundnut

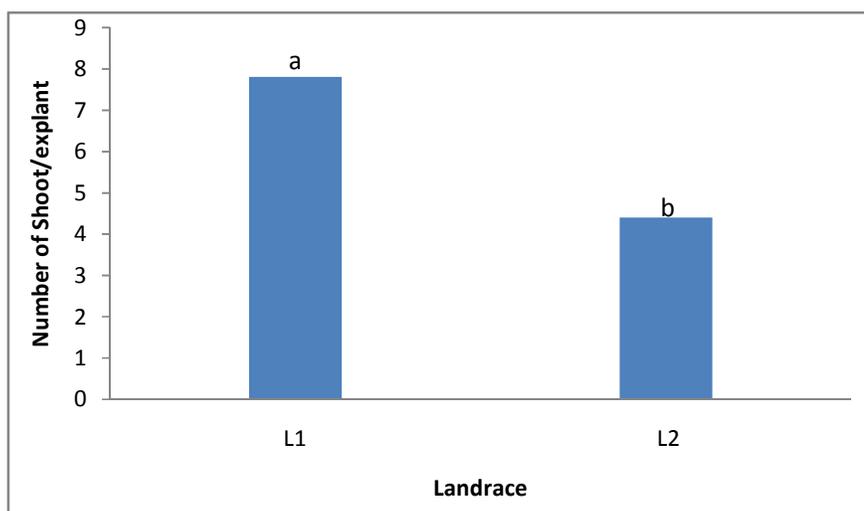


Figure 4: Effect of Land race on Multiple Shoot formation in Bambara groundnut.