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ORIGINAL ARTICLE

Plant fruit extracts enhance the *in vitro* propagation of cowpea (*Vigna unguiculata*) on Murashige and Skoog media

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Abstract

Cowpea (*Vigna unguiculata*) is a versatile legume with diverse nutritional and nutraceutical properties that serve as a food security and medicinal crop for millions of households across Africa. An efficient protocol was developed to propagate shoot tip and cotyledonary node explants from six cowpea breeding accessions *in vitro* on Murashige and Skoog (MS) basal media supplemented with either banana extract, coconut water, orange or tomato juice. Micropropagation performance was compared to MS medium supplemented with B5 vitamins. A total of 500 plantlets were obtained *in vitro* across treatments and MS basal media supplemented with tomato juice had the highest micropropagation performance (154 plantlets), followed by banana extract (112 plantlets), orange juice (107 plantlets), and coconut water (82 plantlets). Three accessions (AGRAC 216, TA, and Asontem) were found to be the most amenable to *in vitro* propagation using plant-derived extracts. Overall, this study successfully established that plant-derived extracts can support *in vitro* cowpea propagation in the absence of synthetic plant growth regulators.

Key message

in vitro propagation of cowpea using plant fruit extracts as cheap substitutes for synthetic plant growth regulators allows for large scale production and transformation of cowpeas against environmental stress.

Keywords Cowpea · Micropropagation · Plant-derived extracts · Shoot tip · Cotyledonary node

Introduction

Cowpea (*Vigna unguiculata*) is an annual, herbaceous, warm-season crop that belongs to the Fabaceae family (Maréchal et al. 1978; OECD 2016). Globally, the total land area estimated for cowpea cultivation is about 12.3 million hectares with an annual dry grain production of about 7.2 million metric tons (FAOSTAT 2020a, b). Africa accounts for 95.2% of the total acreage production, of which Nigeria leads as the world's leading producer and consumer (FAOSTAT 2020a, b). Cowpeas play an essential role in most farming systems as a result of their ability to curb erosion, fix atmospheric nitrogen, and contribute to soil fertility via decay of its residues especially for subsequent cereal crop rotations (Coba de la Peña 2012; Ron 2015). In terms of health, they are mainly cheap sources of dietary protein, relatively low in fat and rich in minerals and vitamins (Carneiro da Silva et al. 2018). Cowpeas can be boiled and eaten as whole meals, or ground into whole

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or composite flours for the preparation of baby foods, or used as garnishes (Gómez 2003). Its leaves and green pods are used for treatment of diseases such as ulcers and measles among several others (Abebe and Alemayehu 2022). Consequently, this legume has been endorsed as "high-quality proteins" with the sole purpose of decreasing high incidences of nutritional malnutrition to "shift the world unto a more sustainable path" (UNDP 2020). In Sub-Saharan Africa, millions of people rely on cowpea as a principal component of their daily meals and is widely cultivated by small-scale farmers (Enyiukwu et al. 2018; Langyintuo et al. 2003; Singh et al. 1997).

Recently, the supply of cowpea has been unable to meet high consumer demands due to an inadequate supply of clean (specifically disease-free) seeds as planting materials for farmers, as well as limited mass propagation of improved cowpea cultivars for consumers. To curb these problems, researchers worldwide have identified tissue culture technology as an efficient tool to enable the mass propagation of improved cowpea cultivars and provide clean planting materials for use by farmers (Aragao and Campos 2007; Hussain et al. 2012; Sani et al. 2015; Suman 2017).

To upscale the production of cowpea using tissue culture, especially in Sub-Saharan Africa, several challenges need to be addressed, including the high cost of synthetic plant growth regulators, delays in product importation, and inaccessibility of these products in local markets. These serve as major drawbacks for laboratories with limited resources especially in developing countries (Datta et al. 2017).

To address these barriers, we aimed to develop new protocols for reducing costs without compromising on the quality of cowpea propagules propagated (Datta et al. 2017; Klerk et al. 2008). We tested the use of readily available, low-cost plant-derived extracts as substitutes for synthetic plant growth regulators and compared their propagative performance. Here we report results from the *in vitro* propagation of six cowpea breeding accessions using shoot tip and cotyledonary node explants cultured on MS media supplemented with four different plant-derived extracts (coconut water, orange juice, tomato juice, and banana extract). The objective of the study was to determine which plant-derived extract best supports cowpea *in vitro* propagation and which genotypes and explant types was best-suited to *in vitro* propagation with organic additions. This can be used for mass propagation of clean cowpea planting materials and plant genetic transformation.

Materials and method

Plant material and culture conditions

Six (6) cowpea accessions (REC 64, Asontem, AGRAC 216, Tintinwa A, Tintinwa B, Songotra) were obtained from the WACCI gene bank, University of Ghana for use in this study.

One hundred and seventy-five (175) seeds per accession were surface sterilized with 0.1% mercuric chloride and two drops of Tween-20 for 5 min, then rinsed three times with sterile distilled water. The surface sterilized seeds were incubated for 1 week on the germination medium of Murashige and Skoog basal salts (Phytotechlab) and 30 g/l sucrose (Central Drug House) after which the shoot tip and cotyledonary node explants were excised and cultured on the different propagation media under a laminar flow hood. A Completely Randomized Design (6 accessions × 5 treatments) was used and 5 seeds per treatment were cultured and replicated six times.

Explants were transferred to different propagation media constituted of 100 ml/l of the blended and sieved plant-derived extracts (coconut water, banana extract, orange juice, or tomato juice) prepared from fresh fruits in the laboratory (Table 1), sucrose, and Murashige and Skoog basal medium (Sigma). Murashige and Skoog supplemented with B5 vitamins and sucrose served as the control propagation medium. All prepared media was solidified with 6 g/l phytagel and adjusted to pH 5.8 ± 0.5 before autoclaving at 121 °C for 20 min at 15 PSI. All culture conditions were set at a 16-h photoperiod with a temperature of 25 ± 1 °C and light intensity of 3000 Lumens.

After 14 days, well-rooted plantlets were removed from culture vessels and any media adhering to them washed off with tap water. They were then placed in 350 cc containers containing sterile potting soil complete with additional fiber (Primasta) in the acclimatization chamber, and well misted with water before being covered with propagator lids. Over the subsequent 2 weeks period, misting was reduced gradually, and the chamber opened fully before plantlets were transferred to the greenhouse in containers with dimensions

Table 1 Description of plant-derived extracts

Plant-derived extract	Degree of maturity	Brix Range	pH Range of extract
Banana (hybrid cultivar)	Fully ripe with no brown patches	8.0–15.0	4.83–5.36
Tomato (Pectomech)	Fully ripe	2.4–3.6	3.97–4.24
Orange (Sweet orange)	Fully ripe	7.4–10.3	3.78–5.1
Coconut	Semi-matured	4.7–6.1	5.05–5.8

of 30 cm × 20 cm × 25 cm for full maturation and production of seeds.

Data collection and analysis

The days to shoot formation, number of shoots per explant, shoot length, number of leaves per explant, days to root formation, root number per explant, root length, plant height, and stem girth were recorded daily between the hours of 7:00 and 16:00 GMT for two weeks after the culture of explants. All statistical analysis was conducted using the GenStat Analytical Package Twelfth edition. The significant differences between the observed means per plant-derived extract treatment and genotype were determined using analysis of variance (ANOVA) and Duncan's Multiple range test and where significant differences were present, the F-protected least significant difference (F-protected LSD) was used to separate them.

Results

Varying responses of genotypes and explants cultured on different media

The six accessions tested responded differently to *in vitro* culture on the germination media. At the end of the first week, it was observed that five (AGRAC 216, REC 64, Asontem, TB, and TA) out of the six accessions were amenable to *in vitro* growth. At the end of two weeks, the rate of germination for the cowpea cultivar Songotra was 17.14%. See germination data in supplementary material.

in vitro propagation on media supplemented with plant-derived extracts

Explants of the germinated accessions also responded differently to the MS media fortified with different plant-derived extracts. It was observed that full plantlet regeneration of the various explants on MS medium supplemented with tomato juice and banana juice was obtained on the third day of *in vitro* culture (Fig. 1). At the end of week one, explants cultured on MS medium supplemented with orange had regenerated into full plantlets whiles those on MS medium supplemented with coconut lasted 14 days. It was observed that injuries caused at excision on the shoot tip explant and cotyledonary node explant of the six cowpea accessions resulted in callus formation amidst the formation of roots for the explants cultured on MS media supplemented with coconut juice.

Acclimatization of *in vitro* propagated cowpea plantlets

At the end of the experiment, 500 clean cowpea plantlets were fully propagated. These plantlets were placed in acclimatization chambers for two weeks. The acclimatization survival rate was 95%. Total humidity inside the chambers was maintained for 1 week after which it was gradually reduced before the transfer of plantlets to the greenhouse where they produced normal growth (Figs. 2, 3). The table below shows in further detail the genotypes, type of media, and percentage of cowpea plantlets obtained from each of the explants (Table 2).

The performance of each cowpea accession was determined by comparing the survival rate of the various explants

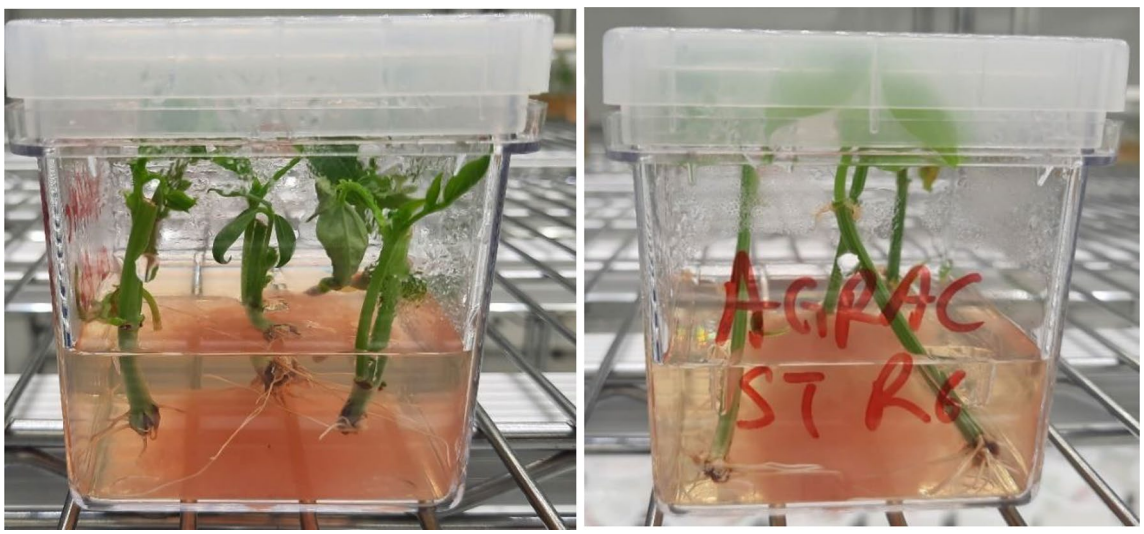


Fig. 1 Image of the cotyledonary node (left) and shoot tip explant (right) of the cowpea accession AGRAC 216 on the third day of culture on MS propagation media supplemented with tomato juice

Fig. 2 Image of the acclimatized plantlets during the first week of acclimatization

Fig. 3 Image of the acclimatized plantlets before and after transfer to the greenhouse

Table 2 Percentage of *in vitro* propagated plantlets produced from Cotyledonary node (2A) and Shoot tip (2B) explants

Cowpea germplasm	Micropropagation medium additive				
	Tomato juice (%)	Banana extract (%)	Orange juice (%)	Coconut water (%)	MSB5 (%)
(A)					
AGRAC	86.67	63.33	66.67	40.00	40.00
TA	66.67	76.67	33.33	36.67	6.67
TB	16.67	33.33	33.33	16.67	3.33
ASONTEM	80.00	33.33	23.33	33.33	23.33
REC064	26.67	3.33	26.67	NR	NR
SONGOTRA	NR	NA	26.67	NR	6.67
(B)					
AGRAC	100	30.00	60	36.67	30.00
TA	83.33	46.67	40	56.67	3.33
TB	13.33	50.00	6.67	20	NR
ASONTEM	40.00	26.67	33.33	16.67	33.33
REC064	NR	10.00	NR	6.67	NR
SONGOTRA	NA	NR	6.67	10	3.33

in the MS media supplemented with different plant-derived extracts against the control. The total survival rate = total number of survived explants/ total number of explants cultured) × 100%. NR equals no regeneration. A total of 30 explants were tested for micropropagation in each genotype × media treatment. Thus, five (5) explants were cultured per dish and a total of six replicates for each explant. The following were observed:

Overall, the cotyledonary node explant recorded the highest survival rate than the shoot tip explants cultured (see survived plantlets in supplementary material). In addition, it is deduced from the analysed data that the shoot tip and cotyledonary node explants of AGRAC 216 were the most amenable to growth in the MS media supplemented with varying plant-derived extracts. Collectively, Tintiwa A came second after AGRAC 216 in terms of the total number of explants regenerated on the different media for both shoot tips and cotyledonary node explants. This was followed by Asontem, Tintiwa B, Songotra, and Rec 064 for shoot tips, with Rec 064 having greater micropropagation than Songotra for cotyledonary nodes (Table 2).

Additionally, significant differences for selected growth parameters number of leaves, number of shoots, plant height, root number, and root length were observed between the explants of the various cowpea accessions cultured on the different propagation media respectively (Fig. 4). AGRAC 216 cultured on MS media supplemented with tomato juice recorded the highest plant height average for shoot tip and TB did the same for cotyledonary node explants on MS media supplemented with coconut water. Both explant types, however, recorded Songotra cultured on the control medium as having the least plant height average. TB and AGRAC 216 were the most efficient in terms of the number of roots produced for the different explants type cultured on MS media supplemented with banana extract. There was no direct correlation observed for root number and root length for explants of the various genotypes. The absent bars for the genotypes indicates no regeneration (Fig. 4).

Furthermore, significant differences were observed for the performance of the various propagation media based on selected parameters (Fig. 5). Tomato juice recorded the least significant difference for days to shoot formation,

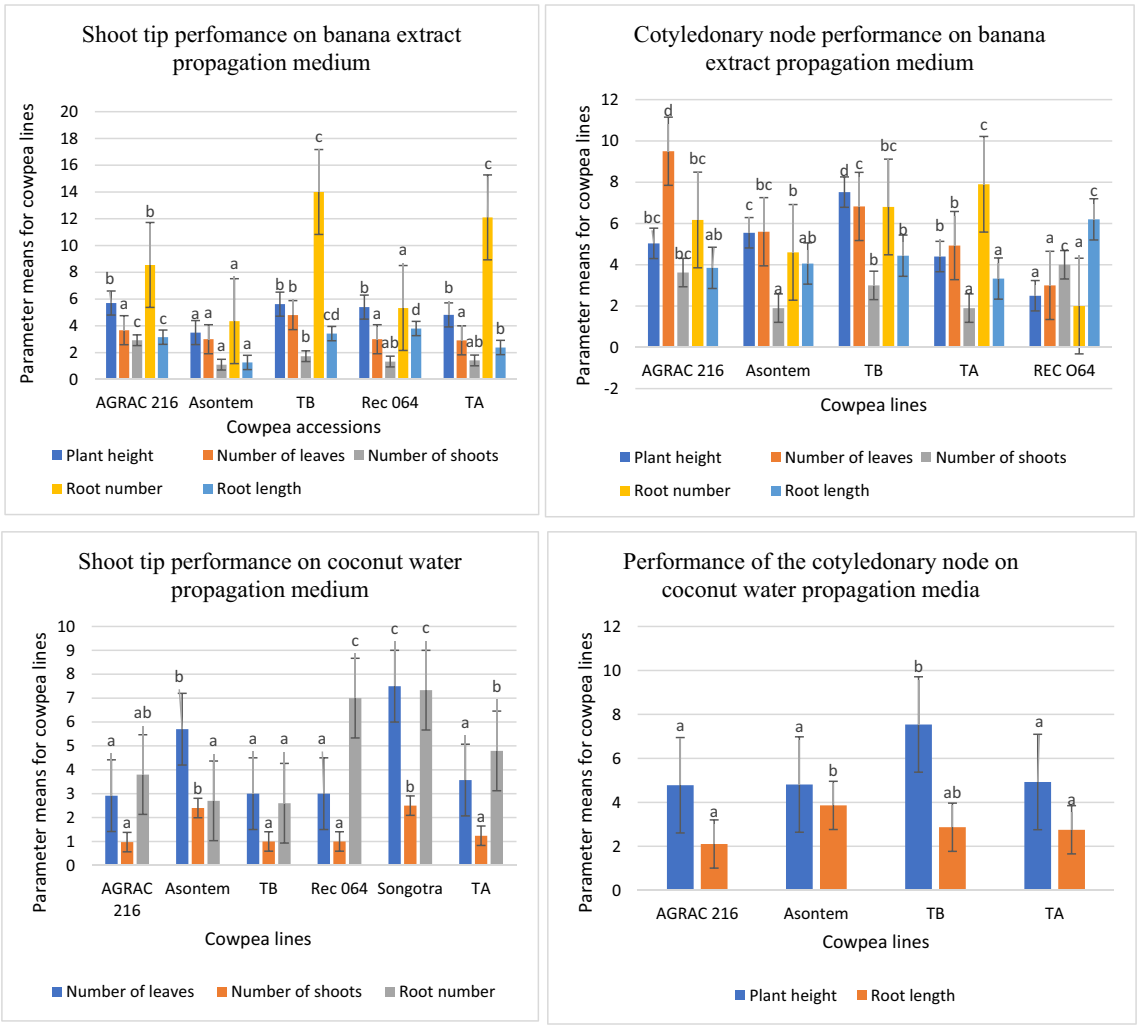


Fig. 4 Mean values for genotype performances on the different propagation media based on the growth parameters plant height, root number, root length, shoot number and shoot length of the shoot tip and cotyledonary node explants. All measurements in cm, error bars represent standard error of mean values

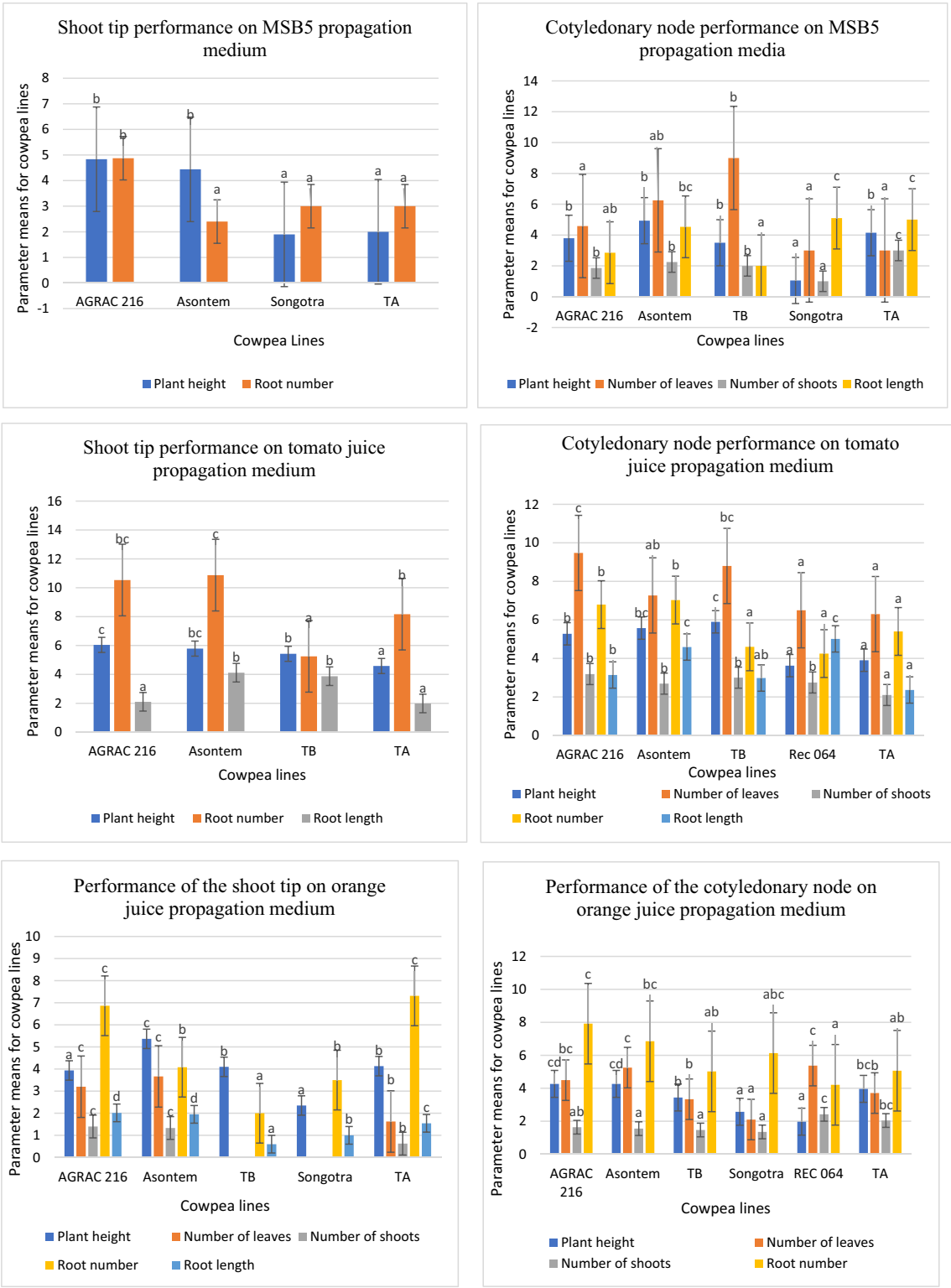
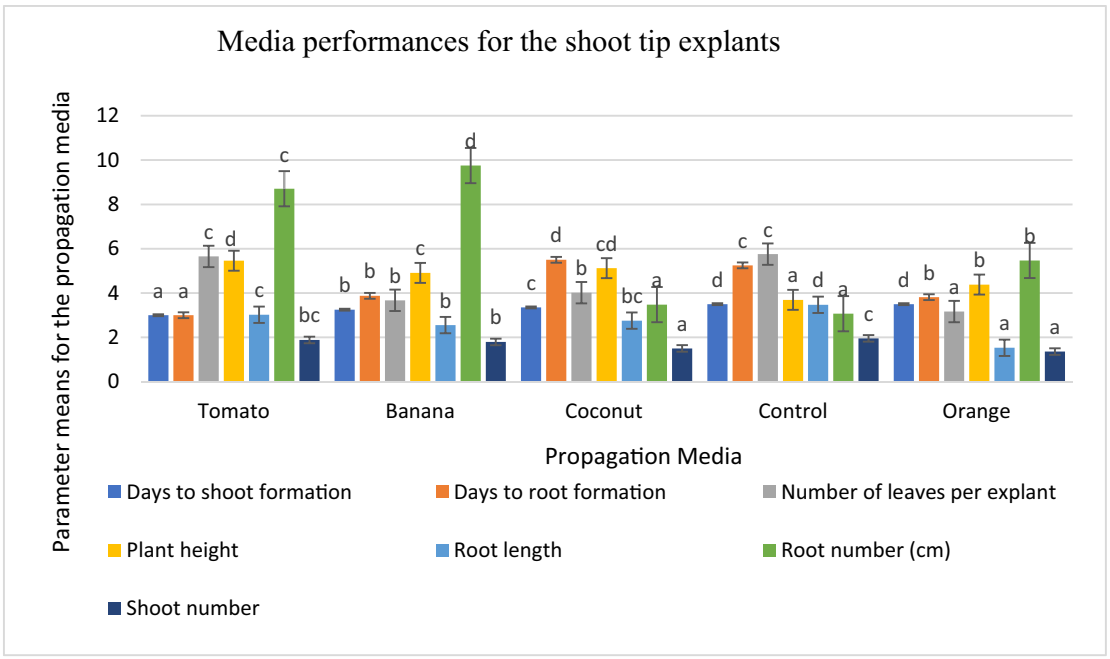


Fig. 4 (continued)

days to root formation and the highest significant difference for plant height for both cotyledonary node and shoot tip explants. Meanwhile, banana juice displayed the highest difference for shoot number and tomatoes the highest for number of leaves for cotyledonary node explants. MSB5 media displayed the highest significant difference



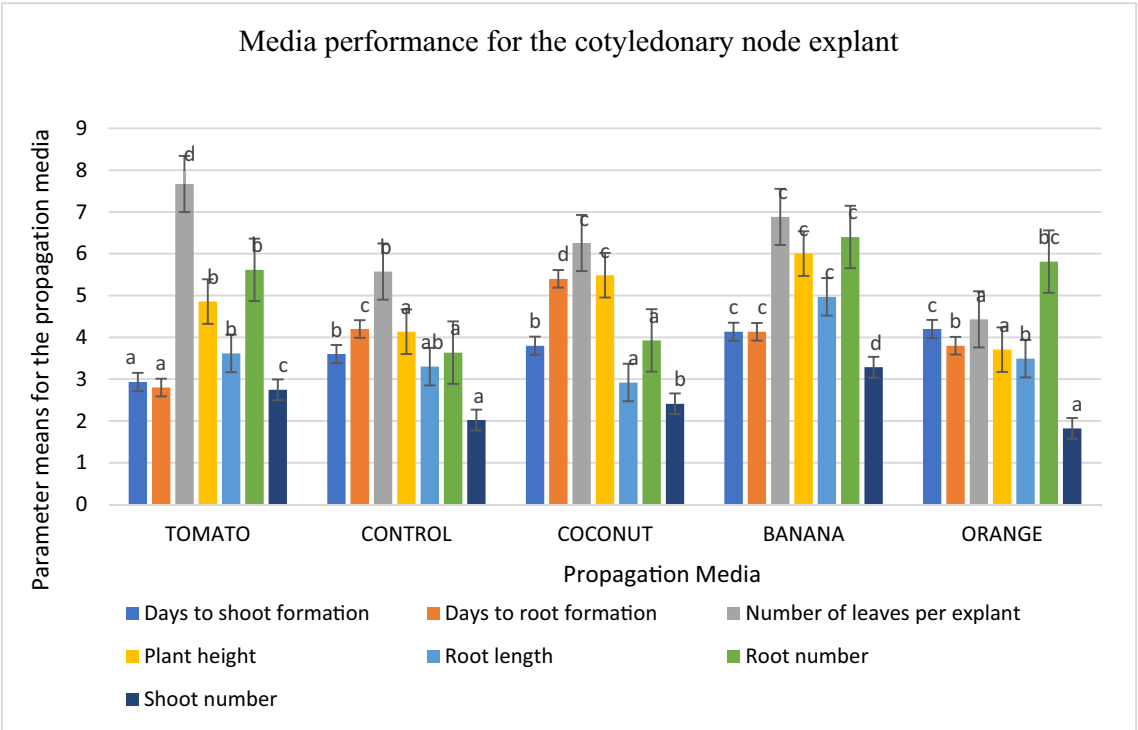


Fig. 5 Mean values of propagation media performances for the shoot tip and cotyledonary nodes based on the growth parameters **a** days to shoot formation, **b** days to root formation, **c** number of leaves, **d** plant height, **e** root number, **f** root length, **g** shoot number. All measurements in cm, error bars represent standard error of mean values

for root length of the shoot tip explants however the opposite is observed for the cotyledonary node explants. MS media supplemented with orange juice displayed the least significant difference for root length of shoot tips whiles MS media supplemented with Banana recorded the highest efficiency for cotyledonary nodes. The absent bars for the performance parameter indicates no significant difference. Furthermore, the variables on the error bars indicate significant differences in performance for both genotype and media.

Discussion

Cowpeas are essential leguminous crops that contribute immensely to the achievement of food security in developing and underdeveloped countries (UNDP 2020). To boost production and optimize the cost associated with the application of tissue culture tehnology for cowpea improvement via *in vitro* culture, low-cost tissue culture media options that allow the use of plant-derived extracts as replacements for synthetic plant growth regulators are employed (Akter et al. 2007; Datta et al. 2017).

The germination and *in vitro* propagation trends observed in the present study for the cowpea genotypes Rec 064, Songotra, and TB could presumably be due to the general recalcitrance of legumes such as cowpea to *in vitro* manipulation (Bakshi and Sahoo 2013; Somers et al. 2003).

The cotyledonary node explants were reported to have the highest survival rate than shoot tip explants. This observation agrees with the findings of (Adesoye et al. 2010; Chaudhury et al. 2007; Raji et al. 2008; Solleti et al. 2008) who opined that cotyledonary nodes were the most efficient and gave the best results for multiple shoot induction.

All the plant-derived extracts supported the micropropagation of cowpea and this response could be explained by the amounts of vitamins, minerals, and plant growth regulators present in the extracts. Though "undefined" because the exact amount of each constituent is unknown and variable for each extract, these supplements are reported to contain plant growth regulators coupled with some nutrients that support explant growth in plant tissue culture systems (Caplin and Steward 1948; Saad and Elshahed 2012). Majority of the time, the amount of auxin to cytokinin in plant culture media determines the type and extent of organogenesis present in a culture (Saad and Elshahed 2012; Skoog and Miller 1957). The performance of the control media could be explained by the very low concentrations of plant growth regulators present in the medium (Gamborg et al. 1968; Murashige and Skoog 1962).

MS basal media supplemented with tomato juice was determined as the best and most efficient plant-derived extract that supported cowpea *in vitro* propagation. This observation agrees with the findings of Ayanlola et al. (n.d.) and Norhayati et al. (2011) who reported on the successful regeneration of cowpea varieties Ife brown and TVU 943 and shoot regeneration of *Celosia* spp. respectively on MS media supplemented with tomato juice. Furthermore, MS media supplemented with banana extract was second in terms of shoot formation for the various cowpea genotypes. This is in line with the findings of Norhayati et al. (2011) who reported that MS basal media supplemented with banana extract came second to providing the best growth rate for *in vitro* shoot regeneration of *Celosia* spp. It was also deduced from the results of the study that MS media supplemented with orange juice induced both shoot and root formation for explants in culture. This observation is confirmed by the findings of Ubalua et al. (2015) who posited efficient shoot and root formation of cocoyam using orange juice. Finally, MS basal media supplemented with coconut water came forth as the best plant extract that supported cowpea micropropagation due to delayed root formation of the cowpea genotypes. This observation could be explained by the very high levels of cytokinin and low amounts of auxins present in coconut water compared to the other plant-derived extracts (Klerk et al. 2008; Kuraishi and Okumura 1961).

Significant growth rate differences were observed for plant height, root length, shoot number, shoot length and root number of the various genotypes on the different supplemented media. Explants' responses to the various *in vitro* propagation media differed one from the other presumably due to differences in genetic compositions (Brar et al. 1999) and varied absorption rates of nutrients and hormones contained in the media (Chee 1995; Drew et al. 1993). Gibberellins are responsible for the elongation of plants (Torres 1989) and all the plant-derived extracts used in this study contain gibberellins (Garmendia et al. 2019; Ge et al. 2007, 2008; Khalifah 1966; Radley and Dear 1958; Srivasta and Handa 2005). However, the significant increase in plant height of shoot tip and cotyledonary node explants in the growth media supplemented with tomato juice and coconut water respectively suggests an increased concentration of gibberellins in both juices. The number of roots formed can be attributed to the high amount of auxin present in the extracts. The works of Hu et al. (2015) and Klerk et al. (2008)imply a higher number of auxins in bananas, hence the most efficient for the highest root numbers and root length for the different explant types. From this study, MS basal media supplemented with tomato juice, banana extract, or orange juice could be utilized in further cowpea tissue culture works since they provided optimum growth conditions. Furthermore, protocols that involved the use of MS basal media supplemented with coconut water could be improved further to provide optimum growth conditions for the cowpea genotypes in culture. In addition, AGRAC 216, TA, and Asontem proved to have high amenability to growth in MS media supplemented with plant-derived extracts. They could be optimized for further tissue culture works on cowpea that involves the use of plant-derived extracts.

Conclusion

Cowpea can undergo successful *in vitro* propagation on MS media supplemented with varied plant-derived extracts by optimizing efficient tissue culture media and protocols. Based on regeneration efficiency in descending order, tomato juice, banana extract, orange juice, and coconut water best-supported cowpea growth *in vitro*. Based on organ development, tomato juice—promoted early shoot and root development. Banana extract and orange juice came second and third respectively followed by coconut water. Additionally, cowpea genotypes AGRAC 216, TA, and Asontem were most amenable to *in vitro* manipulation in MS basal media fortified with plant-derived extracts. Furthermore, the cotyledonary node explants were the most receptive to *in vitro* culture. While there may be high variations in the quality and quantity of growth-promoting factors in the various plant-derived extracts as a result of the non-standardization of juices and extracts, the findings from this study will encourage tissue culture laboratories with fewer resources to conduct similar research using efficient but less expensive approaches. Finally, the mass production of clean cowpea plants within the shortest possible time using this approach would allow farmers to meet high consumer demands all year-round whiles keeping the spread of infection amongst plants to the barest minimum. This would lead to an increased yield and higher profits from sales for local farmers and contribute towards the achievement of food security in developing and underdeveloped countries. Furthermore, these micropropagation media can be utilised in cowpea transformation works worldwide.

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Author contribution JESY and JNA contributed to the study conception and design. Material preparation, data collection, and laboratory analyses were performed by GM and SR. Data analyses was performed by GM. The first draft of the manuscript was written by GM and all authors commented on previous versions of the manuscript. All authors read and approved the final version of the manuscript.

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Data availability The data supporting the findings are available from the corresponding author upon request.

Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the contents of this article.

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