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IN VITRO PROPAGATION OF *SOLANECIO BIAFRAE* AND DETERMINATION OF GENETIC STABILITY OF PLANTLETS USING RAPD AND ISSR MARKERS

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Abstract

An efficient and reproducible micropropagation protocol of *Solanecio biafrae* (Oliv. & Hiern) C. Jeffrey has been developed from nodal stem segments. Shoot development was obtained on Murashige and Skoog (MS) medium supplemented with benzylaminopurine (BAP) alone and in combination with zeatin and 1-naphthaleneacetic acid (NAA). Elongated shoots were rooted in the presence of zeatin or 3-indolebutyric acid (IBA) alone or in combinations. The highest number of explants forming shoots (100%) as well as the highest number of shoots per explant (3.4) and the longest shoots (22 mm) were recorded on medium containing 4.0 mg·dm^{-3} BAP, 2.0 mg·dm^{-3} NAA, and 1.0 mg·dm^{-3} zeatin. About 76% of shoots formed roots on half-strength MS medium free of plant growth regulators. The best root formation (approximately 88%) was recorded on the medium containing 1.0-1.5 mg·dm^{-3} IBA. The micropropagated shoots with well-developed roots were efficiently acclimatized under greenhouse conditions. The random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) amplification products were monomorphic in micropropagated plants and similar to those of mother plant showing their genetic uniformity. This is the first report of micropropagation of *S. biafrae*, which will facilitate *in vitro* mass propagation, conservation, and germplasm exchange of this endangered African vegetable.

Key words: genetic analysis; leaf vegetable; micropropagation; worowo

INTRODUCTION

Solanecio biafrae (Oliv. & Hiern) C. Jeffrey called worowo is a traditional leaf vegetable in Africa, and it is a member of Asteraceae plant family (Adebooye 1996; Schippers 2000). The importance of *S. biafrae* as a leaf vegetable arises from its high nutritive values. Fresh succulent leaves of *S. biafrae* are used as a leaf vegetable in West Africa (Adebooye & Opabode 2004). Leaves of *S. biafrae* contain per 100 g dry matter: crude protein 12.3 g, crude fibre 11.8 g, Ca 342 mg, P 39 mg, and Fe 52 mg (Adebooye 1996). Furthermore, medicinal value of *S. biafrae* is being exploited as leaf extract to stop bleeding from fresh cuts and sore treatment (Adebooye 2004). *S. biafrae* is being used as a biological control agent for weed suppression in plantation crops. It has considerable potential as a cash income earner, enabling the poorest people in the rural communities to earn a living from its domestication. Agronomically, *S. biafrae* is well adapted to harsh climatic conditions and diseases. Moreover, it is easier to grow in comparison to its counterparts, such as cabbages and broccoli (Adebooye & Opabode 2004).

The existence of *S. biafrae* is being threatened despite its nutritional, medicinal, and agronomical importance because of it being considered a weed by researchers and thus the tendency to eradicate and not conserve it (Adebooye & Opabode 2004). *S. biafrae* is propagated usually by stem cuttings. Little is known about the distribution of the species as its genetic diversity is not investigated. The biology of *S. biafrae* and its nutrition and response to abiotic stresses such as water, temperature, and nutrients, as well as protection against diseases and pests has not been adequately investigated and described (Adebooye 2004; Opabode & Adebooye

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2005). Lack of quality seeds and seed dormancy are other constraints to sustainable production and utilization (Adebooye 2004). *S. biafrae* perpetuates itself untended and as a volunteer crop. To prevent *S. biafrae* from becoming extinct, there is an urgent need to use micropropagation to solve problems associated with its production (Adebooye 2004; Opabode & Adebooye 2005). Establishment of a micropropagation protocol will ensure mass propagation of the vegetable and reverse its status of being a threatened species within a short time. The objective of this study was to establish a micropropagation protocol from the nodal segments and confirm the genetic fidelity of plants raised *in vitro* by random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) markers.

MATERIALS AND METHODS

Plant materials and surface sterilization

The morphotype of *S. biafrae* with green stem was used for the study. Seventy donor plants were raised from stem cuttings at the screen house of the Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife, Nigeria. Young plants were treated with 0.5% (w/v) Bavistin 50 DF (carbendazim), a broad-spectrum fungicide. Nodal segments (1-2 cm) from actively growing plants were excised and the surfaces sterilized with 0.1% mercuric chloride (w/v) for 3 min followed by four to five rinses with sterile distilled water.

Culture media and conditions

Basal medium (BM), which consisted of full-strength Murashige and Skoog (MS) (Murashige & Skoog 1962) mineral salts (Sigma-Aldrich, USA), 0.8% agar, and 3% sucrose, was used for shoot induction and elongation experiments. Rooting medium (RM), which consisted of half-strength MS salts, 0.8% agar, and 3% sucrose, was used in rooting experiments. The pH of the medium was adjusted to 5.8 before autoclaving at 121 °C and 1.05 $kg \cdot cm^{-2}$ pressure for 20 min. All cultures were kept at 26 ± 1 °C and a 16-h photoperiod with 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$ irradiation provided by Philips 32-W cool white fluorescent lamps (Philips Electric Company, Hyderabad, India). In all the experiments, the explants were cultured singly and vertically on 20 cm^3 medium in glass test tube (25 × 150 mm).

Shoot development and elongation

Initially, **s**ingle-node segments of about 1 cm in length were used for the study. They were cultured for 3 weeks on BM media containing plant growth regulators (PGRs) at various concentrations and combinations. Then, for shoot elongation, shoot clumps (1-2 mm) developing on initial explants were excised and divided to have single shoot explants and cultured for elongation on BM without PGRs for further 3 weeks. In the first experiment, the elongated shoots were cultured on BM supplemented with different concentrations of benzyl aminopurine (BAP: 0.0, 2.0, 4.0, 6.0, 8.0 10.0, 12.0, and 16.0 $mg \cdot dm^{-3}$) for shoot proliferation. At the second experiment, the shoot induction was stimulated by the combination of BAP (4.0 $mg \cdot dm^{-3}$) and naphthalene acetic acid (NAA: 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 $mg \cdot dm^{-3}$). In the third experiment, nodal segments were cultured on BM supplemented with 4.0 $mg \cdot dm^{-3}$ BAP, 2.0 $mg \cdot dm^{-3}$ NAA and different concentrations of zeatin (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 $mg \cdot dm^{-3}$). In each experiment, observations were made on the survival of explants, frequency of explants forming shoots, number of shoots per explant, and average shoot length after 3 weeks of shoot elongation.

Root induction

Shoots (3-4 mm) for rooting experiments were derived from the medium containing 4.0 $mg \cdot dm^{-3}$ BAP followed by shoot elongation on BM. In the first experiment, the shoots were transferred on RM containing six concentrations (0.0, 2.0, 4.0, 6.0, 8.0, and 10.0 $mg \cdot dm^{-3}$) of zeatin. To determine the influence of IBA on root formation, the second experiment was conducted by transfer of shoots on RM medium supplemented with five concentrations (0.5, 1.0, 1.5, 2.0, and 2.5 $mg \cdot dm^{-3}$) of 3-indolebutyric acid (IBA). In the third experiment, shoots were cultured on RM supplemented with 2.0 $mg \cdot dm^{-3}$ zeatin and 1.0, 1.5, and 2.0 $mg \cdot dm^{-3}$ IBA. In all experiments, frequency of root formation and number of root per shoot were recorded after 3 weeks.

Hardening of plantlets and establishment in a greenhouse

Plantlets (4-5 cm) with well-developed roots were rinsed with water to wash off the agar medium and transplanted to peat pellets (AS Jiffy Products Ltd, Norway) in plastic pots that were covered to maintain high humidity. Fifteen plantlets from each of rooting treatment were subjected to the hardening process. They were grown at 22-26 °C for 3 weeks and after that were transferred to the greenhouse.

Molecular identity of microplants

DNA was extracted from fresh leaves (1.0-1.5 g) of two plants per rooting treatment 7 weeks after soil establishment using the cetyl-trimethyl-ammonium bromide (CTAB) method of Doyle and Doyle (1990). Quantification of DNA was done by a Nanodrop spectrophotometer (Nanodrop 1000, Thermo Fischer Scientific, Wilmington, USA). Four random primers (Table 1) were used based on RAPD results from other members of *Asteraceae* (Salvi et al. 2001). All reactions were repeated at least twice. RAPD amplification was carried out in 20-mm^3 reaction volume containing 25 ng DNA (2 mm^3), 2.0 mm^3 of 10× polymerase chain reaction (PCR) buffer (Taq buffer A containing $MgCl_2$), 0.5 mm^3 of 100 µM dNTP, 2.0 mm^3 of RAPD primer, 0.3 mm^3 of Taq DNA polymerase (Bioline Inc., Taunton, MA, USA), and water to make up the volume. The PCR program used was as described by Patamsytė et al. (2011). The PCR products obtained were separated on 1.5% agarose gel through electrophoresis using size standards GeneRuler 100 bp DNA Ladder Plus and photographed using Gel Documentation System (Bio-Rad, Munchen, Germany).

Five ISSR primers were finally used in the study after an initial screening of 15 primers for the production of distinct and scorable bands (Table 1). Amplification was carried out in 25 mm^3 reaction volume containing 1.5 mm^3 $MgCl_2$, 0.5 mm^3 of 100 µM dNTP, 2.0 mm^3 of 10 × PCR buffer, 2.0 mm^3 of ISSR primers (10 pM), 0.3 mm^3 of Taq DNA polymerase (Bioline, USA), and 20 ng genomic DNA (2 mm^3) as template and water to make up the volume. The PCR program used was as described by Patamsytė et al. (2011). The PCR products obtained were separated on 2% agarose gel through electrophoresis using size standards GeneRuler 100 bp DNA Ladder Plus and photographed using Gel Documentation System (Bio-Rad, Munchen, Germany).

Table 1. Primers and amplification products of RAPD- and ISSR-PCR used for the checking identity of micropropagated plants of *Solanecio biafrae*

s/n	Primer	Sequence	Number of bands	Range of applicon (pb)
1.	OPB-01	TTCGAGCCAG	03	200-1000
2.	OPB-06	TGCTCTGCCC	03	250-1050
3.	OPK-01	TGCCGAGCTG	04	250-2000
4.	OPK-02	GTGAGGCGTC	05	450-3000
5.	UBC-836	$(AG)_8$YA	04	325-1990
6.	UBC-843	$(CT)_8$RA	06	350-1965
7.	UBC-857	$(AC)_8$YG	08	200-2000
8.	UBC-859	$TG)_8$RC	04	1340-3000
9.	UBC-860	$(TG)_8$RA	05	330-400

R = purines: G or A; Y = pyrimidines: C or T pb – base pair

Experimental design and statistical analysis

In all experiments, treatments were arranged in a completely randomized design with 15 replicates (explants). Each experiment was repeated twice. Data were further subjected to analysis of variance to detect differences among treatments using PROC GLM procedure of the Statistical Analysis Systems (SAS 2002). Means were separated by Tukey's test at 5% level of probability.

RESULTS AND DISCUSSION

This work is the first report on *in vitro* propagation of *S. biafrae*; therefore, information at every stage is important for a successful application of tissue culture techniques for improvement of the crop. The disinfection procedure described earlier yielded nearly 98% aseptic bud cultures, while explant discoloration was 0.5%.

After 2 weeks of culture, surviving nodal segments retained their green appearance, while explants that could not survive turned brown or yellow. Survival of nodal segments, frequency of explants forming shoots, number of shoot per explant, and shoot length as influenced by BAP alone is presented in Table 2. Shoot formation was not observed on medium free of BAP. The highest survival of explants

was in the presence of BAP at a concentration of 4.0 and 6.0 mg·dm^{-3}. However, the highest number of shoots and the longest shoots were obtained using 4 mg·dm^{-3} BAP. Compared to the treatments with BAP alone, an improvement in explant survival and shoot length was observed after the addition of NAA to BAP medium (Table 3). However, there was no significant difference among NAA treatments in the number of shoots per explant (Table 3). The highest survival rate and the growth of shoots were observed on medium containing 4.0 mg·dm^{-3} BAP and 2.0 mg·dm^{-3} NAA.

Further improvement of shoot development from nodal explants was obtained when BM containing 4.0 mg·dm^{-3} BAP and 2.0 mg·dm^{-3} NAA was supplemented with zeatin. Addition of this cytokinin resulted in markedly higher number of shoots per explant (3.0-3.4) compared to those recorded in the presence of BAP alone or BAP combined with NAA (Table 3). In all zeatin treatments, 100% survival of explants was observed, with formation of shoots on more than 70% of explants. All explants grown on the medium containing 1 mg·dm^{-3} zeatin formed shoots (Table 4).

Table 2. Effect of BAP on shoot development from initial explants (nodal segments) of *Solanecio biafrae*

BAP (mg·dm^{-3})	Survival (%)	Explants forming shoots (%)	Number of shoots per explant	Shoot length (mm)
0.0	85.5 ± 6.7b	0.0 ± 0.0d	-	-
2.0	87.5 ± 7.2b	43.8 ± 4.8c	1.3 ± 0.6c	3.7 ± 1.2b
4.0	98.6 ± 7.4a	75.4 ± 7.1a	2.4 ± 0.8b	8.5 ± 2.4a
6.0	98.2 ± 6.2a	73.6 ± 7.6a	1.2 ± 0.5c	4.6 ± 0.8b
8.0	83.2 ± 6.8b	65.3 ± 5.5b	1.2 ± 0.3c	3.8 ± 0.8b
10.0	80.1 ± 5.6b	64.5 ± 5.3b	1.0 ± 0.6c	4.5 ± 1.3b
12.0	81.5 ± 6.5b	60.7 ± 5.4b	1.0 ± 0.5c	4.3 ± 1.2b
14.0	85.7 ± 5.8b	57.9 ± 4.7b	1.0 ± 0.4c	4.6 ± 0.7b
16.0	84.6 ± 5.4b	52.8 ± 4.6b	1.0 ± 0.5c	3.8 ± 0.6b

Values are means (±standard error). Means followed by different letters in same column are significantly different at 5% level of probability according to Tukey's Test.

Table 3. Effect of NAA combined with 4 mg·dm^{-3} BAP on shoot development from initial explants of *Solanecio biafrae*

NAA (mg·dm^{-3})	Survival (%)	Explants forming shoots (%)	Number of shoots per explant	Shoot length (mm)
0.5	95.6 ± 5.6b	54.4 ± 4.8d	2.4 ± 0.8a	3.8 ± 1.2d
1.0	95.8 ± 6.8b	75.2 ± 4.6b	2.0 ± 0.6a	15.2 ± 2.6b
1.5	95.8 ± 6.3b	78.0 ± 6.5b	2.0 ± 0.7a	10.2 ± 3.3c
2.0	100.0 ± 0.0a	84.0 ± 6.2a	2.2 ± 0.9a	10.2 ± 3.2c
2.5	100.0 ± 0.0a	68.8 ± 6.2c	2.2 ± 0.5a	18.6 ± 3.8a
3.0	100.0 ± 0.0a	63.7 ± 5.7c	2.2 ± 0.4a	12.8 ± 2.7b

Explanation: see Table 2.

Table 4: Effect of zeatin combined with 4 mg·dm^{-3} BAP and 2 mg·dm^{-3} NAA on shoot development of *Solanecio biafrae*

Zeatin (mg·dm^{-3})	Survival (%)	Explants forming shoots (%)	Number of shoots per explant	Shoot length (mm)
0.5	100.0 ± 0.0a	98.2 ± 7.8b	3.2 ± 1.1a	10.2 ± 3.5c
1.0	100.0 ± 0.0a	100.0 ± 0.0a	3.4 ± 0.8a	22.0 ± 6.8a
1.5	100.0 ± 0.0a	88.7 ± 5.8c	3.0 ± 1.0a	20.0 ± 4.1a
2.0	100.0 ± 0.0a	81.3 ± 5.2c	3.2 ± 0.7a	19.8 ± 3.8b
2.5	100.0 ± 0.0a	75.5 ± 6.4d	3.0 ± 0.9a	15.4 ± 3.7c
3.0	100.0 ± 0.0a	74.8 ± 6.3d	3.0 ± 0.8a	15.8 ± 3.6c

Explanation: see Table 2.
BAP – benzyl amino purine NAA – naphthalene acetic acid

Micropropagation of members of *Asteraceae* family has been reported from various explants such as flower stalk, nodal segment, cotyledon, shoot, and leaf explants (Rossato et al. 2015; Lucchesini et al. 2009; Hristova et al. 2013; Subhan & Agrawal 2011). Our observation that the best survival of explants occurred at 4.0 and 6.0 mg·dm^{-3} BAP confirmed the reports of Lucchesini et al. (2009) on *Echinacea angustifolia*. On the other hand, Hristova et al. (2013) reported that the presence of PGRs in medium had no effect on explant survival of *Artemisia chamaemelifolia*. Furthermore, shoot induction in the presence of BAP observed in current work agrees with previous works. For example, MS medium supplemented with BAP alone produced high shoot induction and multiplication rate in some Asteraceae members such as *Wedelia calendulacea* (Emmanuel et al. 2000), *Echinacea purpurea* (Korochi et al. 2002), and *Carlina acaulis* (Grubisić et al. 2004). Marked improvement of shoot development from initial explants was recorded when 4.0 mg·dm^{-3} BAP was combined with different concentrations of NAA. Such synergetic effect of BAP and NAA was also reported in other micropropagated plant species belonging to *Asteraceae* family (Jain et al. 2008; Trejgell et al. 2010; Joshi et al. 2015).

Before rooting, the induced shoots were separated and transferred to basal medium for elongation. Table 5 presents the effect of zeatin alone on shoot rooting. Most root formation (75.6%) was observed on the medium free of zeatin, while its addition decreased rooting by 12-33%. However, plantlet acclimatization was the poorest when they were rooted on this medium (52.8%). Most plantlets that successfully acclimatized were from media containing 2 and 4 mg·dm^{-3} zeatin (94.3% and 97.6%, respectively). Zeatin had no effect on the number of roots. Root formation was promoted by IBA (Table 6).

Table 5: Effect of zeatin alone on root formation, number of roots per plantlet and plantlet acclimatization in *Solanecio biafrae*

Zeatin (mg·dm^{-3})	Root formation (%)	Number of roots per shoot	Plantlet acclimatization (%)
0.0	75.6 ± 6.8a	4.2 ± 0.8a	52.8 ± 4.4c
2.0	63.3 ± 5.6b	4.3 ± 1.0a	87.3 ± 6.3a
4.0	51.8 ± 6.5ab	3.6 ± 0.7c	88.2 ± 5.6a
6.0	45.3 ± 4.8c	4.0 ± 0.7ab	73.5 ± 6.0b
8.0	42.3 ± 3.6c	4.3 ± 0.8a	74.8 ± 5.8b
10.0	42.8 ± 3.5c	4.3 ± 2.3a	73.4 ± 5.9b

Explanation: see Table 2.

Table 6: Effect of IBA alone on root formation, number of roots per plantlet and plantlet acclimatization in *Solanecio biafrae*

IBA (mg·dm^{-3})	Root formation (%)	Number of roots per shoot	Plantlet acclimatization (%)
0.0	75.3 ± 5.4b	4.3 ± 0.9ab	54.5 ± 0.8b
0.5	71.3 ± 6.8ab	5.4 ± 1.8a	94.3 ± 8.5a
1.0	87.8 ± 7.1a	6.5 ± 1.4a	97.6 ± 10.2a
1.5	88.3 ± 6.8a	5.0 ± 1.1a	93.7 ± 9.4a
2.0	87.5 ± 7.2a	4.3 ± 0.8ab	97.8 ± 9.2a
2.5	86.1 ± 7.4a	4.2 ± 0.7ab	93.7 ± 9.8a

Explanation: see Table 2.

Table 7: Effect of IBA combined with 2 mg·dm^{-3} zeatin on root formation, number of roots per plantlet and plantlet acclimatization in *Solanecio biafrae*

IBA (mg·dm^{-3})	Root formation (%)	Number of roots per explant	Plantlet acclimatization (%)
1.0	43.8 ± 3.2a	3.8 ± 0.7a	100.0 ± 0.0a
1.5	40.7 ± 3.4a	3.8 ± 0.6a	100.0 ± 0.0a
2.0	32.5 ± 3.2ab	3.6 ± 0.6a	100.0 ± 0.0a

Values are means (±standard error). Means followed by different letters in same column are significantly different at 5% level of probability according to Tukey's Test.

The addition 1.0 to 2.5 mg·dm^{-3} IBA increased significantly root formation by 13-15% and acclimatization by 42-44% in comparison with control (no IBA). IBA did not affect root number. Zeatin and IBA combinations did not have a positive effect on rooting. Less than 44% of shoots formed roots and their number per shoot was lower than that without zeatin at the same IBA concentration (Table 7).

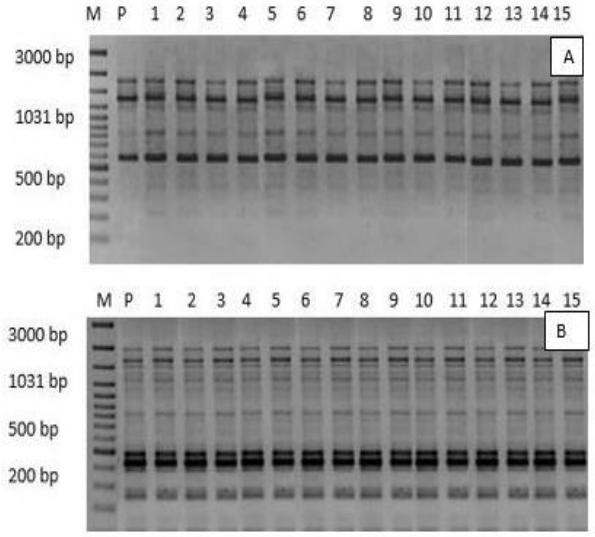

Fig. 1. RAPD analysis with the primer OPK-02 (A) and ISSR analysis with the primer UBC 857 (B) M – GeneRuler ladder, P – Donor plant, 1 – 0.0 mg·dm^{-3} zeatin, 2 – 2.0 mg·dm^{-3} zeatin, 3 – 4.0 mg·dm^{-3} zeatin, 4 – 6.0 mg·dm^{-3} zeatin, 5 – 8.0 mg·dm^{-3} zeatin, 6 – 10.0 mg·dm^{-3} zeatin, 7 – 0.0 mg·dm^{-3} IBA, 8 – 0.5 mg·dm^{-3} IBA, 9 – 1.0 mg·dm^{-3} IBA, 10 – 1.5 mg·dm^{-3} IBA, 11 – 2.0 mg·dm^{-3} IBA, 12 – 2.5 mg·dm^{-3} IBA, 13 – 2.0 mg·dm^{-3} zeatin + 1.0 mg·dm^{-3} IBA, 14 – 2.0 mg·dm^{-3} zeatin + 1.5 mg·dm^{-3} IBA, 15 – 2.0 mg·dm^{-3} zeatin + 2.0 mg·dm^{-3} IBA.

Some members of *Asteraceae* developed *in vitro* roots on medium free of PGRs, while others required the presence of PGRs in medium to form roots. In this study, the presence or absence of PGRs in the rooting medium did not always produce the expected results. For instance, av. 76.9% of shoots produced roots in the absence of PGRs with moderate (av. 53.2%) plantlet acclimatization. In the presence of zeatin, root formation decreases, while acclimatization reached 100%. In addition, the rate of acclimatized microplants increased to 100% when rooted on IBA containing media. Clearly, our data suggested that IBA is the most suitable PGR for rooting of *S. biafrae*. The presence of IBA has been reported to increase the number of rooted shoots as well as the number of roots per shoot in *Saussurea obvallata* (Joshi & Dhar 2003). The incidence of root formation on auxin-free medium may be due to the presence of endogenous auxin in regenerated shoots. In agreement with our observation on plantlet acclimatization, microplants of members of *Asteraceae* (*Senecio macrophyllus* and *Spilanthes acmella*) have been reported to exhibit 100% plantlet acclimatization (Trejgell et al. 2010; Joshi et al. 2015). Genetic stability of the micropropagated plants was confirmed by RAPD and ISSR analyses. Twelve random RAPD primers were screened, of which four (OPB-01, OPB-06, OPK-01, OPK-02) produced clear, distinct, and scorable bands. A total of 240 bands were obtained and primer OPK-02 generated the highest number of bands (5) (Fig. 1A). From 15 ISSR primers, 5 (UBC-836, UBC-843, UBC857, UBC-859, and UBC-860) produced distinct and scorable bands. A total of 405 bands were obtained, and primer UBC 857 produced the highest (8) number of bands (Fig. 1B). DNA amplification profiles of both RAPD and ISSR primers revealed similarity in banding patterns among micropropagated plants. Similarly, no variation in banding pattern was observed between the mother plant and the micropropagated plants. This indicates identity at DNA level among micropropagated plants and between the micropropagated plants and their donors. Both RAPD and ISSR markers have been successfully applied to check the genomic identity of micropropagated plants of *Asteraceae*. Martins et al. (2004) suggested that a better analysis of genetic

stability of plantlets can be made by using a combination of two types of markers that amplify different regions of the genome (Salvi et al. 2001; Patamsyte et al. 2011). True-to-type clonal fidelity is one of the most important prerequisites in the micropropagation of any crop species. Sometimes, the presence of somaclonal variation among subclones of one parental line, arising as a direct consequence of *in vitro* culture of plant cells, tissues, or organs, was reported. Using shoot propagation and rooting described here, no variation in the banding patterns was detected what clearly indicates that *in vitro* conditions applied in this study do not induce any genetic variability in *S. biafrae*.

CONCLUSION

In conclusion, this is the first report of *in vitro* propagation of *S. biafrae* (Oliv. & Hiern) C. Jeffrey. Our data suggested that shoot development was best on MS medium fortified with 4.0 $mg \cdot dm^{-3}$ BAP, 2.0 $mg \cdot dm^{-3}$ NAA, and 1.0 $mg \cdot dm^{-3}$ zeatin and rooting preferable on half-strength MS supplemented with 1 $mg \cdot dm^{-3}$ IBA. The protocol developed here is simple and can be used for the sustainable supply of genetically stable *in vitro* plant materials with the prospect to apply in plant propagation and biotechnology. Furthermore, the protocol described here opens a pathway for *in vitro* conservation of *S. biafrae* by apical and nodal segments. In addition, the protocol may promote germplasm exchange for production, improvement, and conservation of the vegetable. More importantly, our work may facilitate *in vitro* production of phytochemicals with medicinal properties isolated from *S. biafrae* by pharmaceutical industry.

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