Trop J Nat Prod Res, December 2020; 4(12):1174-1177 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

In vitro Regeneration of *Solanecio biafrae* Through Direct Shoot Organogenesis

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ARTICLE INFO

Article history:
Received 08 September 2020
Revised 26 November 2020
Accepted 24 December 2020
Published online 02 January 2021

ABSTRACT

Solanecio biafrae (Olive & Hierne) C. Jeffrey (Asteraceae) is a tropical, underutilized African indigenous vegetable, highly nutritious and medicinal. It grows as undercover and is mostly collected in tree plantations such as cocoa and kola. Its propagation is seldom carried out by vines, but its climbing habit poses a problem for farmers, which leads to the application of herbicide to control their spread. In order to ascertain its potential for genetic improvement, the protocol for direct shoot organogenesis of *S. biafrae* was developed. Leaf lamina, petiole, and leaf with petiole explants were cultured on Murashige and Skoog (MS) medium supplemented with varying concentrations of 6-benzylaminopurine (BAP) alone and in combination with Indole butyric acid (IBA). Direct shoot organogenesis was obtained from the cut end of leaf lamina and petiole explants as well from the petiolar region of the leaf with petiole explant. The results obtained from this study show the potential of these explants of *S. biafrae* to serve as culture material for its micropropagation, genetic improvement via transgenesis. Hence, this regeneration protocol of *S. biafrae* can be coupled to its genetic transformation to improve species with shorter internode and more leaves to enhance the structure of tropical vegetation.

Keywords: *Solanecio biafrae*, *Woorowo*, *Bologi*, African Indigenous Vegetable, Micropropagation.

Introduction

Solanecio biafrae (Olive & Hierne) C. Jeffrey (Asteraceae) is a perennial climbing herb, which grows in a considerable amount as understory in tree crop plantation, e.g., cocoa[1] and kola.[2] It is ubiquitous in Osun, Ekiti, and Oyo States of southwest Nigeria (majorly in cocoa farms) as a result of intentional safeguarding of the growing stands and ignorable cultivation done with little or no coordination[1] because of its culinary importance as a potherb.[3] Popularly called "Woorowo" or "Bologi", it is an indigenous medicinal vegetable eaten and medicinally used in southwest Nigeria. However, its need for physical support and shade is hampering its cultivation.[3] Breeding to improve this vegetable and biotechnological research are urgently required.[4] Till now, the reports on the *in vitro* propagation of *S. biafrae* described protocols using nodal explants as the starting material[5,6] whereas there are no reports on the use of internode, leaf with petiole, leaf lamina, and petiole explants excised from *in vitro* grown plantlets.

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Citation: Bello OA, Fajimi O, Esan EB, Obembe OO. *In vitro Regeneration of Solanecio biafrae Through Direct Shoot Organogenesis.* Trop J Nat Prod Res. 2020; 4(12):1174-1177. doi.org/10.26538/tjnpr/v4i12.24

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Materials and Methods

Explant preparation

Young growing vines of *S. biafrae* were collected from potted plants. Nodal segments were thoroughly cleaned under running tap water. They were surface-disinfected according to Bello[7] and the disinfectants were washed off using sterile distilled water (SDW).

Culture initiation

The surface-sterilized explants were cultured with vertical orientation on semi-solid Murashige and Skoog (MS) basal medium[8] containing 30 g/L sucrose and gelled with 8 g/L agar. The pH of the medium was adjusted to 5.7 and the media autoclaved for 15 min at 121°C. They were then kept in a culture room under fluorescent light for a 16/8 h photoperiod at 25 ± 2°C.

Direct shoot organogenesis

After six (6) weeks, internode explants from the resulting *in vitro* grown plantlets were excised and cultured on MS basal medium supplemented with 6-benzylaminopurine (BAP) at varying concentrations (0, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 2.0, and 4.0 mg/L). Also, leaf lamina, leaf lamina with petiole and petiole explants were cultured on MS basal media supplemented with different concentrations (0.05 and 0.1 mg/L) of BAP alone and in combination with different concentrations (0, 0.0625, 0.125, 0.25 and 0.5 mg/L) of indole butyric acid (IBA).

Experimental design

All experiments were conducted under a completely randomized design. Each treatment or combination of treatments was replicated ten (10) times with an explant in a culture tube. Morphogenic response, callus formation, shoot number, and shoot length were monitored and data recorded within 4 ± 1 week in culture.

Statistical analysis

The data were analyzed with GraphPad Prism (version 5.0). The data were presented as mean ± standard error (SE) and means were compared using Tukey Multiple Comparison Test at the 5% probability level.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Results and Discussion

BAP influenced direct organogenesis in internode explants of in vitro grown plantlets of Solanecio biafrae

The ability of somatic cells aseptically cultured on plant growth medium to regenerate whole plants is a significant fundamental for manipulating plant genetic constituent *in vitro*.[9] Under the influence of plant growth regulators (PGRs), somatic cells are re-programmed to commence definite developmental events in morphogenesis *in vitro*.[10] Direct shoot organogenesis obtained in explants without pre-existing meristem of *Solanecio biafrae* was reported in this study. Four explants (internode, leaf lamina, leaf lamina with petiole, and petiole) from *in vitro* raised plantlets of S. *biafrae* were excised and cultured in culture media supplemented with varied PGR alone and in combination to study the regenerative potential of the vegetative parts. The response of internode explants of *in vitro* raised plantlets of *S. biafrae* depended mainly on the PGR concentration(s) in the culture medium. All the internode explants cultured on MS basal medium enhanced with varying concentrations of BAP (0-5.0 mg/L) induced callus except treatment control (Table 1). Seven (7) days after culture initiation, callus interspersed with the shoot was observed to be emerging straight from the epidermal cell layers of the internode explants cultured on MS medium enhanced with 0.4 mg/L BAP while shoot clumps were induced on 0.1 mg/L BAP. However, MS medium without PGRs induced no shoot. This is in agreement with Pandey[11] that intermodal explants cultured on MS only did not induce shoot regeneration. At four weeks after culture initiation, the initiated shoot(s) further elongated, and more new shoots developed from the explants (Figure 1). Among the treatments, only the treatment with 0.4 mg/L BAP resulted in 2 shoots with a mean shoot length of 3.00 cm, four weeks after culture initiation. The result may be due to abrupt modification in the cellular environment, which resulted from the exposure of injured tissues to low nutrient concentrations or PGRs, resulting in stress and subsequently inducing regeneration.[12] Similar reports of successful shoot induction from internode explants on media supplemented with BAP abound in *Solanum viarum*[11], *Solanum tuberosum*.[13] Similar report was obtained in *Monochasma savatieri*.[14] In *Digitalis lamarckii*, 0.5mg/L and 1.0 mg/L BAP was effective for direct shoot induction from leaf explants, while the lowest concentration (0.1 mg/L) and the highest concentration (3.0 mg/L) did not induce shoot.[15]

BAP alone or in combination with IBA influenced direct organogenesis in leaf lamina, leaf with petiole and petiole explants of in vitro grown plantlets of Solanecio biafrae

The synergy between PGRs (primarily auxins and cytokinins) modulates the initiation and expression of an explant's capacity for regeneration[16]. The effect of BAP and IBA on direct organogenesis from leaf lamina, leaf with petiole, and petiole explants obtained from *in vitro* grown plants of *Solanecio biafrae* is shown in Table 2. Multiple shoots induction has been reported in explants obtained from *in vitro* seedling of *Cucurbita pepo*.[17] The leaf lamina explants cultured on 0.1 mg/L BAP+0.25 mg/L IBA started to enlarge and produced direct multiple shoots (2 shoots of 1.00 cm length) within 7-8 weeks after culturing, at the cut ends (Figure 2). The leaf with petiole explant also enlarged, and treatment 0.1 mg/L BAP produced a single shoot (4.00 cm long) while 0.05 mg/L BAP+0.5 mg/L IBA gave multiple shoots (2.00) with mean shoot of 1.00 cm long after 7-8 weeks of culture, at the petiolar region (Figure 2). Previous reports revealed that shoots were successfully induced from petiole explants with BA in *Solanum viarum* Dunal.[11] However, shoots were formed directly in the leaf explants of *Chrysanthemum morifolium* on the media supplemented with BAP.[18] In contrast, leaf segments of *S. viarum* did not induce any shoot with BA.[11] Adventitious shoots were also obtained in *Titanotrichum oldhamii* using medium supplemented with 0.1mg/L BA.[19] Meanwhile, in *Bacopa monnieri*, BAP (1.5 mg/L) in combination with IAA (0.5 mg/L) induced 220 multiple shoots regenerated from leaf explants.[20] Petiole explants with a concentration of 0.05 mg/L BAP+0.125 mg/L IBA enlarged and induced an adventitious shoot of 1.50 cm (Figure 2). In this study, the synergy of lower cytokinins and higher auxin concentrations induced adventitious shoot induction. This result disagrees with cytokinin alone reported for the formation and differentiation of shoot from petiole explants in *Digitalis lanata*.[21]

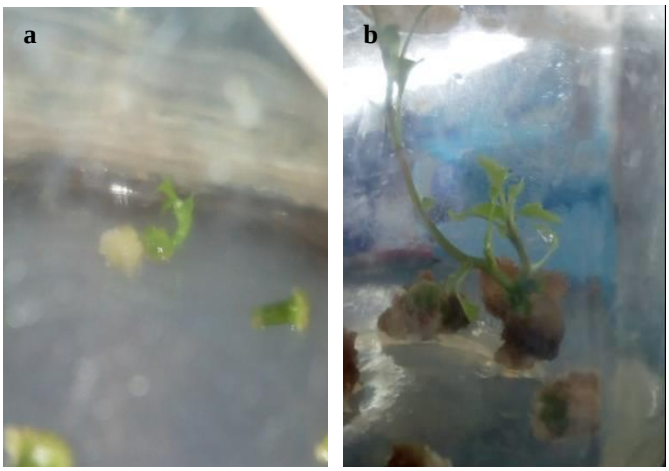

Figure 1: (a) Two-week-old direct shoot formed from internode explant on MS medium enhanced with BAP (0.4 mg/L) **(b)** Four-week-old direct shoot formed from internode explant on MS medium enhanced with BAP (0.4 mg/L).

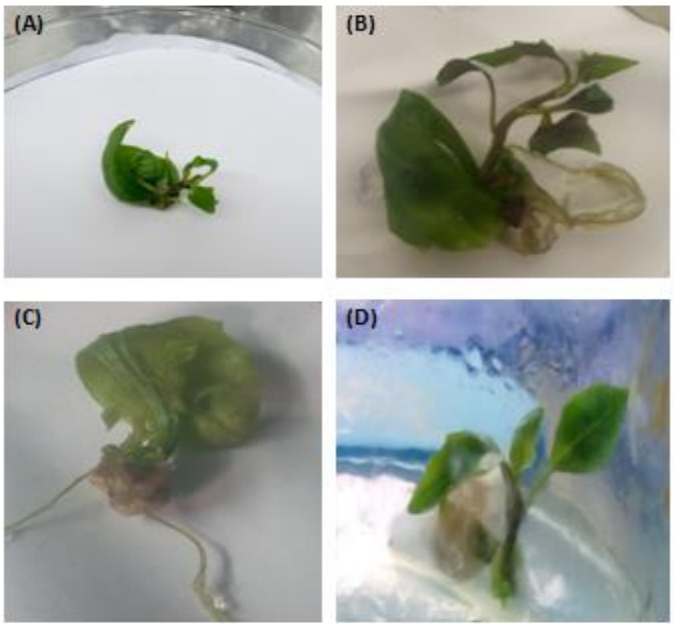

Figure 2: Direct shoot organogenesis in *Solanecio biafrae* obtained from various explants cultured on MS medium enhanced with varied concentrations of BAP alone and in combination with IBA:
(A) Leaf lamina explants using 0.1 mg/L BAP and 0.25 mg/L IBA; **(B)** Leaf lamina with petiole explants using 0.1 mg/L BAP; **(C)** Leaf with petiole explants using 0.05 mg/L BAP and 0.5 mg/L IBA; and **(D)** Petiole explants using 0.05 mg/L BAP and 0.125 mg/L IBA.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 1: Influence of BAP on direct organogenesis from internode explants of *Solanecio biafrae*

BAP (mg/L)	Callus	Number of shoots	Shoot length (cm)
0	-	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}
0.1	+	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}
0.2	+	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}
0.3	+	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}
0.4	+	2.00 ± 0.00 ^{a}	3.00 ± 0.50 ^{a}
0.5	+	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}
1.0	+	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}
2.0	+	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}
3.0	+	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}
4.0	+	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}
5.0	+	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}

BAP: 6 Benzylamino purine; present (+) and absent (-); treatment means followed by different letters in their superscript significantly differ from one another ($P < 0.05$) according to the Tukey's Multiple Comparison Test

Table 2: Influence of BAP alone or its combination with IBA on direct organogenesis from three explants of *Solanecio biafrae*

		Leaf lamina		Leaf with petiole		Petiole	
BAP (mg/L)	IBA (mg/L)	Number of shoots/explant	Shoot length (cm)	Number of shoots	Shoot length (cm)	Number of shoots/explant	Shoot length (cm)
-		0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}
0.1	0	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}	1.00 ± 0.00 ^{a}	4.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}
0.1	0.0625	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}
0.1	0.125	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}
0.1	0.25	2.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}
0.1	0.5	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}
0.05	0	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}
0.05	0.0625	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}
0.05	0.125	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}	1.00 ± 0.00 ^{a}	1.50 ± 0.00 ^{a}
0.05	0.25	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}
0.05	0.5	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}	2.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ab}

BAP: 6 Benzylamino purine, IBA: Indole butyric acid; present (+) and absent (-); treatment means followed by different letters in their superscript significantly differ from one another ($P < 0.05$) according to the Tukey's Multiple Comparison Test.

Conclusion

Solanecio biafrae has shown great potential for plant tissue culture techniques, especially direct shoot organogenesis. All the explants (internodes, leaf lamina, leaf lamina with petiole and petiole) obtained from the *in vitro* grown plantlets responded to the influence of PGRs. This, therefore, showed that *S. biafrae* explants have the potential for genetic transformation for its genetic improvement.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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