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EFFECT OF NAPHTALENE ACETIC ACID AND BENZYLAMINOPURINE ON *IN VITRO* CLONAL PROPAGATION OF *SOLANECIO BIAFRAE* – A THREATENED INDIGENOUS LEAFY VEGETABLE

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ABSTRACT

Clonal propagation study was carried out on *Solanecio biafrae*; a threatened indigenous leafy vegetable, using nodal and leaf explants. The aim of this study was to identify a suitable explant and determine an optimal hormonal concentration for callus induction and shoot proliferation. Nodal and leaf explants of this species were cultured on Murashige and Skoog (MS) medium, supplemented with various concentrations of Benzylaminopurine (BA) or Naphtalene acetic acid (NAA) alone and in combination. Nodal explants were found to be suitable for shoot and root proliferation and callus formation. The highest shoot regeneration frequency (100%) was obtained from nodal explants cultured on MS medium supplemented with a combination of 1.25 mg/L BA and 0.25 mg/L NAA. The highest root induction frequency (80%) was obtained from leaf explants cultured on MS supplemented with 0.50 mg/L BA. Multiple shoots and plantlets were produced from nodal explants cultured on MS medium supplemented with 1.25 mg/L BA and 0.25 mg/L NAA. The highest callus induction frequency (100%) was obtained from nodal explants cultured on MS medium supplemented with a combination of 0.25 mg/L BA and 0.50 mg/L NAA. A suitable explant for callus formation and organogenesis has been identified. This report is the first on *in vitro* propagation of this threatened indigenous leafy vegetable using leaf explants.

Keywords - *Solanecio biafrae*, *in vitro*, shoots, roots, propagation

INTRODUCTION

Solanecio biafrae (Oliv. & Hiern) C. Jeffrey is an indigenous leafy vegetable consumed in southwest Nigeria. The genus Solanecio is comprised of *S. biafrae* and 15 other species, all belonging to the family Asteraceae. Naturally, *S. biafrae* is found in the rainforest zones of Central and West Africa (Adebooye, 2004). It is a perennial climbing herb with stems which can grow as high as 3m. Its leaves are alternate and succulent with triangular blade and glossy surface. *Solanecio biafrae* is an over exploited vegetable because it is usually collected from the wild without cultivation. In addition, seeds of *Solanecio biafrae* have low viability.In south-western Nigeria, *Solanecio biafrae* occurs in two distinct types: plants with purple stems and plants with green stems.

Indigenous leafy vegetables are an integral part of agricultural systems in Africa, but most African governments have not given them priority in crop development (Adebooye and Opabode, 2004). Adebooye *et al*. (2003) reported about the status of food in Nigeria, that Indigenous leaf vegetables often disappear and Research Institutes and Universities have focused attention on routinely cultivated species of indigenous leaf

vegetables only. However, presently the importance of African indigenous leaf vegetable to human nutrition is being emphasized.

Indigenous leaf vegetables play a vital role in income generation and subsistence (Adebooye and Opabode, 2004). In southwest Nigeria, *Solanecio biafrae* is several times more expensive than routinely cultivated species especially during the dry season (Adebooye, 2004). In Kenya, a survey carried out by Abukutsa-Onyago (2003) showed that indigenous leaf vegetables offer a significant opportunity for the people in western Kenya to earn a living because their cultivation requires a little capital. Indigenous leafy vegetables contain appreciable amounts of crude protein, fat and oil, energy, vitamins and minerals (Adebooye, 1996; 2001; 2002; 2004; Adebooye and Bello, 1998; Chweya, 1997). They can also serve as a source of traditional medicine in southwest Nigeria. The indigenous species are adapted to many tropical conditions, pests and diseases, as such they can be very good sources of genes for genetic improvement of cultivated species especially in the area of pests and disease resistance (Adebooye and Opabode, 2004). Vegetables on consumption have a protective action attributed to the presence of anti - oxidant and anti – oxidant vitamins such as ascorbic acid, α-tocopherol and β-carotene (Grivetti and Ogle, 2000). Fresh succulent leaves of *S. biafrae* are eaten in Sierra Leone, Ghana, Benin, Nigeria, Cameroon and Gabon (Ajala, 2009). In addition the leaf extract of *S. biafrae* is used to stop bleeding from cuts and injury in southwest Nigeria; in Congo it is used to treat oedema, to relieve rheumatic pain and also as a tonic (Stevels 1990). An infusion of the leaves is taken as a drink (Adebooye, 2004).

Propagation of *S.biafrae* is by means of seeds and semi - hard stem cuttings. The seeds however show very low viability – less than 2%. Culturally in most African countries, it is only occasionally cultivated and collection is done from the wild (Adebooye, 2004). As a result *S. biafrae* has become rare, expensive and endangered (Adebooye, 2004)

The objective of this study, therefore, was to investigate the effect of Naphthalene acetic acid and Benzylaminopurine on nodal and leaf explants and to identify the most suitable explants for *in vitro* propagation of the species.

MATERIALS AND METHOD

Leaf and nodal explants of *Solanecio biafrae* were collected from young and actively growing plants grown at the Reforestation unit, Obafemi Awolowo University, Ile Ife, Nigeria (Lat 7^0 32'N, Long 4^0 31'E). The explants collected were presterilized with 70% ethanol for 5 minutes and then sterilized with 80% (v/v) sodium hypochlorite solution. Two drops of Tween 20 were added to the sterilizing solution and decanted after 10 minutes; explants were then rinsed three times in sterile distilled water. Nodal explants of 1cm length and leaf explants of $1cm^2$ area were excised and cultured on full strength Murashige and Skoog, (1962) (MS) medium (composed from MS salts) which served as the control and MS medium supplemented with different concentrations and combinations of plant growth regulators. The MS medium was supplemented with 3% sucrose and the pH was adjusted to 5.7 ± 0.2 after which it was gelled with 0.8% agar. The following concentrations were tested for their effects on callus induction and plant regeneration; 0.25 mg /L benzylaminopurine (BA), 0.50 mg /L BA, 1.25 mg /L BA, 0.25 mg /L NAA, 0.50 mg /L NAA, 0.25 mg /L BA + 0.25 mg /L NAA, 0.25 mg /L BA + 0.50 mg /L NAA, 0.50 mg /L BA + 0.25 mg /L NAA, 1.25 mg /L BA + 0.10 mg /L NAA, 1.25 mg /L BA + 0.25 mg /L NAA. The

Five explants were used per treatment and were observed within the period of 4 to 12

The cultured explants were then incubated at 25 ^{o} C.

weeks. The results were subjected to one way analysis of variance (where concentration of plant growth regulator was the varying factor) and means were separated using least significant difference (LSD) using SPSS version 16.0 for windows.

Results and Discussion

The *in vitro* regeneration of shoots was low when nodal explants were cultured on MS alone (Table 1, Plate 1) and MS supplemented with low concentrations of BA. MS medium supplemented with 1.25 mg/L BA and 0.25 mg/L NAA showed the best efficiency of shoot regeneration producing a 100% shoot regeneration frequency and 1.8 mean numbers of shoots per nodal explants (Table 1, Plate 2). Relatively low concentration of NAA in combination with a high cytokinin concentration has been reported to promote shoot proliferation (Arya *et al.,* 2012). Interaction effect (synergism) observed with BA (1.25 mg/L) and NAA (0.25 mg/L) was significant. No significant difference was observed in the mean number of shoots formed from nodal explants cultured on media supplemented with 1.25 mg/L BA and 0.25 mg/L NAA separately. There was however significant difference in the mean number of shoots obtained on media supplemented with 1.25 mg/L BA + 0.25 mg/L NAA and that obtained on media supplemented with either 1.25 mg/L BA or 0.25 mg/L NAA. Similar synergistic effect of BA and NAA has been observed in many medicinal plants such as *Hemidesmus indicus* (Sreekumar *et al.,* 2000), *Holostemma ada*-kodien (Martin, 2002), *Leptadenia reticulata* (Arya *et al.,* 2003), *Tylophora indica* (Thomas and Philip, 2005; Faisal *et al*., 2007), *Huernia hystrix* (Amoo *et al*., 2009) and *Sarcostemma brevistigma* (Thomas and Shankar, 2009) and among other species in the family Asteraceae such as *Launea taraxacifolia* (Sakpere *et al*., 2011).

Plate 1: Nodal explant cultured on MS alone after 28 days.

Plate 2: Nodal explant cultured on MS medium Supplemented with 1.25 mg/L BA and 0.25mg/L NAA after 150 days.

Plantlets from nodal explants cultured on MS medium without Plant Growth Regulators (PGR) were observed to be initiated faster (28 days) than other plantlets initiated on MS media supplemented with PGR (0.5 mg /L BA, 0.25 mg/L BA + 0.5 mg /L NAA, 0.25 mg /L NAA and 1.25 mg /L BA + 0.25 mg /L NAA) for 32, 43, 72 and 150 days respectively.

Table 1 - Response of nodal and leaf explants on MS media supplemented with BA and NAA alone and in combination.

	NODAL EXPLANT			LEAF EXPLANT		
Concentration of media (mg/L)	MNS/SP	MNR/RP	MSC/CP	MNS/SP	MNR/RP	MSC/CP
MS	0.4^{bc} /40	0.8^{b} /40	0.00	0.00	0.00	0.00
0.25 BA	0.8^{b} /80	0.00	0.00	0.00	0.00	0.00
0.50 BA	0.2^{bc} /20	0.00	0.00	0.00	2.4^{a} /80	0.8^{a} /80
1.25 BA	0.6^{bc} /40	0.00	0.4^{cde} /40	0.00	0.00	0.00
0.25 NAA	0.2^{bc} /20	0.6^{b} /20	0.4^{cde} /20	0.00	0.00	0.4^{ab} /40
0.50 NAA	0.00	1.4^{b} /40	1^{bcd} /60	0.00	0.00	0.4^{ab} /40
0.25BA+0.25NAA	0.6^{bc} /60	0.00	0.2^{de} /20	0.00	0.00	0.00
0.25BA+0.50NAA	0.8^{b} /80	4.8^{a} /60	2.8^{a} /100	0.00	0.00	0.6^{a} /60
0.50BA+0.25NAA	0.8^{b} /80	0.4^{b} /40	1.4^{b} /60	0.00	0.00	0.8^{a} /80
1.25BA+0.10NAA	0.6^{bc} /60	0.00	1.2^{bc} /60	0.00	0.00	0.4^{ab} /40
1.25BA+0.25NAA	1.8^{a} /100	0.8^{b} /40	0.6^{bcde} /60	0.00	0.00	0.00

KEY: MS – Murashige and Skoog medium, BA – Benzyladenine, NAA – Naphthalene acetic acid, MNS – mean number of shoot, MNR – mean number of root, MSC – mean size of callus, SP – shooting percentage, RP – rooting percentage, CP – callusing percentage. Note that means with the same letter along the column are not significantly different.

For *in vitro* root regeneration, root formation was inhibited when nodal explants were cultured on MS supplemented with BA alone. It has been reported that root formation is often inhibited by cytokinins, especially BA (Poonawala *et al*., 1999; Chaudhuri *et al*., 2004). In *Senecio macrophyllus*; an Asteraceae plant, inhibitory effects of BA supplemented media on root induction was also reported (Trejgell *et al*., 2009). The addition of BA alone to basal medium inhibited root induction, while the addition of NAA alone favored root induction in nodal explants of *S. biafrae*. NAA has been an auxin known to be suitable for root formation and increasing concentrations of NAA increased rhizogenesis (Table 1, Plate 3). Mahesh *et al*. (2012) reported that increasing concentration of NAA favored root induction in nodal explants of *Commelina ensifolia*. There is however, a synergistic effect of BA and NAA with respect to rhizogenesis. Low concentrations of NAA when combined with very high concentrations of BA improved rhizogenesis. Higher concentration of NAA in combination with even a low concentration of BA significantly increased rhizogenesis. The highest root regeneration frequency (60%) and the maximum number of roots (4.8) was observed on MS supplemented with 0.25 mg/L BA and 0.50 mg/L NAA (Table 1, Plate 4).

Plate 3: Nodal explant cultured on MS medium supplemented with 0.25 mg/L after 72 days

Plate 4: Nodal explant cultured on MS medium supplemented with 0.25 g/L. BA and 0.50 mg/L NAA after 43 days

Though shoots alone were formed on MS supplemented with 0.25 mg/L BA and roots and callus on MS supplemented with 0.50 mg/L NAA, the interaction of both PGR had significant effect on root formation. Interaction effect of BA and NAA has also been reported to be significant for root number in potato (Moeinil *et al*., 2011).

Callus induction from nodal explants was observed on all concentrations tested except on basal MS, MS supplemented with 0.25 mg/L BA and MS supplemented with 0.50 mg/L BA. The concentration of endogenous auxin present in this species is not optimum for callus induction and at lower concentrations of BA, callus induction is inhibited. Callus induction was however recorded on media supplemented with a higher concentration of BA (1.25 mg/L) alone and on media supplemented with a combination of BA and NAA. Steephen *et al.* (2010) reported formation of callus from nodal explants of *Vitex negundo* on MS supplemented with a high concentration of BA (5 – 10 mg/L), also cotyledonary explants of *Lycopersicum esculentum* inoculated on MS supplemented with BA at high concentration (5 – 15 µM) produced significantly high callus size (Bhatia *et al*., 2004). Media supplemented with a combination of BA and NAA favoured callus induction significantly than media supplemented with NAA alone. The presence of BA might have enhanced the effectiveness of NAA. Balogun *et al*., (2002) reported that for good callusing, BA combined with NAA is optimum for stem sections of *Telfairia occidentalis*.

Shoot regeneration was not observed from leaf explants cultured on any of the media tested while root regeneration was observed on only MS supplemented with 0.50 mg/L BA (Plate 5) and callus induction observed on some other media combinations.

Plate 5: - Leaf explant cultured on MS medium supplemented with 0.50 mg/L BA showing callus after 32 days

Different explants respond differently to media combinations. Li *et al*. (2012) reported formation of shoots from nodal explants of *Solidago Canadensis* L. while leaf explants showed no shooting response on basal medium.

Conclusion

Nodal explants are more suitable for *in vitro* propagation of *S. biafrae* than leaf explants. High concentration of BA in combination with a low concentration of NAA is adequate for shoot proliferation. A combination of NAA and BA has also been determined to be better for rhizogenesis and callogenesis than NAA alone. Further investigation is required to optimize the regeneration potential of *Solanecio biafrae*. This report is the first on *in vitro* propagation of this threatened indigenous leafy vegetable using leaf explants.

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