

Influence of Exogenous 6-Benzylaminopurine on Growth, Physiological Parameters, Proximate Content and Mineral Element Composition of Pot-Grown *Solanecio bialfrae*

Jelili T Opabode* and Iqmot B Raji

Department of Crop Production and Protection, Obafemi Awolowo University, Ile-Ife, Nigeria

*Corresponding author: Jelili T Opabode, Department of Crop Production and Protection, Obafemi Awolowo University, Ile-Ife, Nigeria, Tel: +2348034172865; E-mail: jopabode@gmail.com

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Abstract

The influences of exogenous 6-benzylaminopurine (BAP) on shoot emergence, growth, fresh shoot weight, physiological parameters, proximate and mineral element contents of green-stemmed morphotype of *Solanecio bialfrae* at early stage were investigated. Stem-cuttings were sprayed with solution containing concentrations (0, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 mg L⁻¹) of BAP a day after planting in plastic pots. Medium (60-70 mg L⁻¹) BAP promoted highest number of shoot per stem-cutting, total number of leaves, total leaf area, shoot to root ratio, total soluble sugars, ash, carbohydrate, leaf Mg and Ca. Intermodal length, phenolic, crude fibre, leaf Na, P and Zn increased as BAP concentration increased. Shoot height, dry weight, chlorophylls a and b, total soluble protein, moisture, crude protein, fresh shoot weight and leaf Fe did not differ significantly ($P>0.05$) at medium to high (50-100 mg L⁻¹) concentrations of BAP. The study concluded that 60 mg L⁻¹ BAP is the best concentration as it promoted highest number of shoot per stem-cutting, total number of leaves, total leaf area, shoot height, dry weight, fresh shoot weight, shoot to root ratio, chlorophyll a and b, phenolics, total soluble sugars and proteins, crude protein, fat, ash and carbohydrate, leaf K, Mg and Ca.

Keywords: Cytokinin; External application; Mineral nutrients; Photosynthetic pigments; Regeneration; Shoot yield

Introduction

Solanecio bialfrae (Oliv. & Hiern) C. Jeffrey is a important traditional leaf vegetable in Africa [1]. The vegetable mostly exists as an understory climber that occurs naturally in the forest zone of West and Central African countries. Occasionally, *S. bialfrae* is cultivated on a small scale in Nigeria and Cameroon, where average annual rainfall is about 1500 mm, from sea-level to 1300 m altitude. The vegetable is a perennial climber that produces stems of about three meters long. The long stems of the vegetable twine around other plants for support and branch profusely at 50 cm above ground level. *S. bialfrae* cannot survive under dry conditions, grows best in a well-drained, fertile soil rich in organic matter and prefers a position in light shade [2]. The vegetable strongly responds to water-deficit stress by developing shriveled stems and yellowing of the leaves.

The importance of *S. bialfrae* as leaf vegetable is derived from its high nutritive and food values. Fresh leaves and young stems of *S. bialfrae* are widely consumed by cacao farmers in West Africa and Central Africa [3]. Leaves of *S. bialfrae* have been reported to contain 12.3 g, 11.8 g, 342 mg, 39 mg and 52 mg of crude protein, crude fibre, Calcium, Phosphorus and Iron respectively, per 100 g of dry matter [4]. Also, proximate composition of leaves of the vegetable were reported to be 6.4, 15.3, 17.8, 15.1 and 46.9% moisture, crude protein, crude fibre, crude fat, ash and carbohydrate, respectively by Ajiboye et al. [5]. Furthermore, medicinal value of *S. bialfrae* is being exploited by herbalists in many parts of Africa. For example, leaf extracts are used to stop bleeding from fresh cuts and sore treatments [2]. The spread of *S. bialfrae* makes it a suitable biological control agent for weed

suppression in plantation crop in West Africa. Most importantly, the vegetable has a considerable potential as a cash income earner, enabling the poorest people in the rural communities to earn a living from its domestication and marketing. In addition, *S. bialfrae* is well adapted to some extreme weather conditions, pests and diseases, which are traits that are agronomically important. Moreover, vegetative propagation by stem cuttings makes cultivation of the vegetable easier than its exotic counterparts, such as cabbages and broccoli [3].

Despite its nutritional, medicinal, and agronomical importance, production of the vegetable falls short of demand throughout the year [3]. In open markets in southern West African cities, *S. bialfrae* supply is inadequate when compare with other indigenous leaf vegetables like *Telfairia occidentalis*, *Celosia argentea* and *Solanum macrocarpon*. This is attributed to low multiplication rate, slow regeneration and limited bud initiation arising from vegetative propagation of the vegetable by stem cuttings [6]. The problem is exacerbated by the fact that the vegetable produces seeds only after an uninterrupted growth of 3-4 years. In addition, the situation is compounded by fruit abortion and seed dormancy that the plant experience [2]. Thus, alternative method of boosting regeneration and growth of *S. bialfrae* is needed to meet market demands [7].

External application of plant growth regulators is a viable method of increasing the production of *S. bialfrae*. Studies have established that cytokinin and gibberellins-driven diversion of assimilates and mineral nutrients towards shoot meristems, rather than to roots, resulted in an increase in aerial biomass in a wide number of species [8,9]. As a result, 6-benzylaminopurine (a cytokinnin) has been externally applied to promote the growth and development of crop species, including vegetables. For example, exogenous benzyl aminopurine supplied to pot-grown rooted-cuttings of *Epipremnum aureum* (an ornamental

plant) resulted in promotion of shoot development, leaf area growth and fresh and dry weights accumulation [10]. Exogenous applications of 6-benzyl aminopurine to the ornamental plant, *Monstera deliciosa* (Liebm.), grown in pots overcomes the limitations on shoot growth caused by root restriction, as well as increasing biomass accumulation and the partitioning of photosynthate to shoots [11]. Also, Hosseini et al. [12] observed an increase in the photosynthetic rate and chlorophyll content of *Hordeum vulgare* of BAP-sprayed plants at the late period of grain filling. Previous reports on lettuce, celery and spinach have shown that a single BAP spray during the first days after emergence increases total leaf area, fresh weight and dry weight [13,14]. Exogenous BAP spray on pumpkin grown in commercial facilities by Gaspera et al. [15] resulted in 20% increase in biomass accumulation and yield by increasing population density and manipulating crop architecture. Application of 6 mg/L BAP at grain filling stage of wheat increases flag leaf area duration, chlorophyll index and seed filling duration and grain yield [16]. The response of *S. bialfrae* to external application of 6-benzylaminopurine is not known. The objective of this study was to determine the influence of a single external application of 6-benzylaminopurine on stem-cuttings on growth, physiological parameters, proximate content and mineral element composition of pot-grown *Solanecio bialfrae*.

Materials and Methods

Location of the study, plant materials and growth conditions

The study was conducted at the greenhouse facilities of the Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife, Nigeria. The study was conducted between June 10 to December 23 2016 and was repeated between March 17 to October 23 2017. The morphotype of *S. bialfrae* that produces green stems was used for the study. Healthy and uniform stem-cuttings were obtained from a two-year old actively growing culture of *S. bialfrae* at cacao plantation of the University's Teaching and Research Farm. Stem-cuttings (5 cm long with five nodes) were planted vertically and singly on polythene pot containing 2 kg of a rich loamy soil. The soil has the following properties: pH=7.2, organic carbon=4.3%, total nitrogen=5.1%, and cation exchange capacity=15.3 c mol.kg⁻¹. Each plant was irrigated manually and daily with 800 ml tap water of pH 6.8.

Experimental treatments and design

Eleven concentrations of 6-benzylaminopurine (BAP; Sigma-Aldrich Co., St. Louis, MO, USA; 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 mg L⁻¹) were applied 24 hours after planting by spraying the stem-cuttings to run-off with the aid of an atomizer at sunset. Addition of two drops of 0.5% Tween-20 to BAP solution was done to ensure adequate wetting of leaf surfaces. Treatments were arranged in randomized complete block design with three replicates. Fifteen stem-cuttings were used per treatment.

Growth measurements

Data were recorded at six weeks after planting the stem-cuttings on the following parameters: survival of stem-cuttings, number of days to emergence of first shoot, number of shoots emerged per stem-cutting, total number of leaves of shoots per stem-cutting, average shoot height, total leaf area and dry weights. For dry weights, the plants were removed along with soil and dipped in water to dislodge the adhering soil particles without injuring the roots. The roots were then separated

from the shoot and blotted. The roots and shoot were weighed separately to record their fresh mass and placed in an oven (80°C for 72 h). The samples were weighed again to record the respective dry mass. Leaf area was ascertained by gravimetric method by tracing the outline of the leaf on graph sheet and counting the squares covered by it on graph paper.

Determination of photosynthetic pigments

Pigments were extracted from fresh leaves (1 g) with 80% acetone following homogenization. The homogenized mixture was separated by centrifugation at 5000 × g for 10 min. The absorbances of the supernatant were read at the following wavelengths with a spectrophotometer: 645, 653, 662 and 664 nm, for chlorophyll a and b and 470 nm for carotenoids, as outlined by Lichtenthaler and Wellburn [17]. Measurements were performed in triplicates. Equations used for calculations are presented below:

$$\text{Chlorophyll a} = 11.75 A_{662} - 2.350 A_{645}$$

$$\text{Chlorophyll b} = 18.61 A_{645} - 3.960 A_{662}$$

$$\text{Carotenoids} = 1000 A_{470} - 2.270 \text{Chl a} - 81.4 \text{Chl b} / 227$$

Measurement of total phenolics content

Fresh leaves (0.5 g) were ground in 80% acetone (10 mL) following Julkunen-Titto [18]. Thereafter homogenate mixture was collected. After that, Folin-Ciocalteu reagent (1 mL)+H₂O (2 mL) and the supernatant (0.1 mL) were homogenized, followed by vigorous shaking. To it was added 5 mL of Na₂CO₃ (20%) and the volume was raised to 10 mL using distilled H₂O. The absorbance was noted at 750 nm and the results expressed as mg g⁻¹ fresh weight of sample.

Determination of total soluble proteins

Fresh leaves (0.25 g) were ground in 10 mL of 50 mM potassium phosphate buffer (pH 7.8) in a cool environment. The samples were centrifuged at 10,000 × g at 4°C for 15 min as described by Bradford [19]. The aliquot (0.1 mL) was mixed with 2 mL of Bradford reagent and the absorbance read by spectrophotometer at 590 nm using bovine serum albumin as standard.

Total soluble sugars measurement

For measuring total soluble sugars, fresh green leaves (0.1 g) were ground in ethanol solution (10 mL; 80%) and then shaken for 6 h at 60°C. The supernatant (0.1 mL) was taken and mixed in anthrone reagent (3 mL). Then the mixture was heated for 10 min, ice cooled, and incubated for 20 min. The optical density of the aliquot was recorded at 625 nm using a spectrophotometer. Total soluble sugars were determined from a standard curve of glucose following Yemm and Willis [20].

Determination of proximate contents

Moisture content: Five grams of leave sample (in duplicate) were accurately weighed in crucibles of known weights. The crucibles and the samples were heated at 100°C in a Gallen Kamp oven until constant weights were obtained. The dishes and their contents were cooled in a desiccator containing fused calcium chloride as drying agents and then weighed. The loss in weight was expressed in percentage [21].

Ash: Ash content was determined using the method of AOAC [22] which involved igniting the samples in muffled furnace at 550°C (dull red) until grayish white ash were obtained. The crucible and their contents were cooled in desiccator and weighed soon after reaching room temperature.

Crude protein: The method of AOAC [22] was used. Two grams of each sample was accurately weighed and put into a 300 ml standard Kjeldahl digestion flask containing 8 g of the sodium sulphate catalyst, some anti-bumping chips and 30 ml of concentrated sulphuric acid followed by addition of 20 ml of concentrated sulphuric acid at 200°C for 45 minutes and allowed to cool at room temperature. After which the contents were transferred into Kjeldahl distillation apparatus and 10 ml of distilled water with 15 ml of 45% NaOH was added until the volume in the recording flask reach 20 ml thus, producing ammonium borate complex. The ammonium borate complex was diluted to 50 ml and titrated with 2% HCl to a pink end point. The crude protein content was determined by multiplying the percentage nitrogen content by the conversion factor of 5.3 recommended for vegetable analysis.

Crude fat content: Petroleum ether extraction method as described by AOAC [22] was used. Two grams of the powdered samples was mixed with petroleum extract in Soxhlet apparatus at 50°C for 5 hours (AOAC). The fat content was calculated using the formula below:

$$\% \text{-crude fat content} = \frac{E-F}{G} \times 100$$

Where F=weight of empty conical flask, E=weight of flask+content after evaporation, G=weight of sample extract.

Crude fibre content: The method of AOAC [22] was used. Two grams of each sample was distributed into conical flasks followed by addition of 1.5% H₂SO₄ solution and heated for minimum of 30 minutes. Vacuum filter was used to filter, and filtrate collected and washed with distilled water and using a pH paper to ensure that no trace of acid is detected. The extracts were then put into another set of conical flasks as 1.25% NaOH was added and heated for 30 minutes. The filtrate was also collected and washed until no trace of base was detected using pH papers. The samples were then transferred into crucibles after which the crucibles were dried at 105°C for 24 hours. After which the crucible was placed in muffle furnace at 400°C for 6 hours and the weight of the crucible was taken. The ash was weighed and the differences in weight gave the amount of crude fibre in the sample.

Carbohydrate content: Carbohydrate content of samples was determined by adding the values obtained for crude protein, fat, total ash and fiber and subtracting from 100 g.

Analysis of leaf mineral composition: Five grams of dry milled samples were ashed in a furnace at 550°C for 12 hours. The ash was allowed to cool in desiccators and then weighed. 2 mls of concentrated hydrochloric acid (HCl) were added to dissolve the ash, alongside few drops of nitric acid [23]. The solution was evaporated to almost dryness in a boiling water bath. The content was transferred to 100 ml volumetric flask and diluted to mark with deionized water. A PU9000 atomic adsorption spectrophotometer (Pye Unicam Ltd, Cambridge, UK) with acetylene flame was used to analyses for phosphorus, calcium, magnesium, zinc and iron as described in AOAC [23]. Sodium and potassium were determined with AIM 049000 flame photometer (Aimil Ltd, New Delhi, India).

Statistical analysis

Data obtained from tagged randomly selected five plants per replicate were averaged and used for analysis of variance. Since there were no significant differences between the two experiments, the data from each year were pooled and considered together. Count and percentage data, being not normally distributed were transformed as follows: a square root transformation of count data and arc-sine \sqrt{x} transformation of percentage data, which were then subjected to one-way analysis of variance using PROC GLM of the Statistical Analysis Systems [24]. Means were separated by Duncan's New Multiple Range Test at 5% level of probability.

Results

Growth measurements

Survival of both treated and untreated stem-cuttings was 100% (data not shown). External application of BAP on stem-cuttings significantly ($P < 0.05$) influenced number of days to first emergence of shoot buds, number of shoot emerged per cuttings, number of leaves, shoot height and leaf area (Table 1). On the average, the first shoot bud emerged from BAP-applied stem-cuttings four days earlier than the control treatment.

BAP (mg L ⁻¹)	NDST	NSEP	TNL	SHT	LA	DW g/plant	Inter Nodal Length
0	12.0 ± 2.0 ^a	1.5 ± 0.5 ^d	7.5 ± 1.3 ^d	7.5 ± 1.5 ^b	14.5 ± 2.8 ^e	8.2 ± 0.2 ^d	1.2 ± 0.8 ^b
10	8.0 ± 2.2 ^b	2.5 ± 0.4 ^c	10.5 ± 1.4 ^c	9.1 ± 2.3 ^{ab}	20.0 ± 3.2 ^d	11.8 ± 0.4 ^c	1.3 ± 0.2 ^b
20	8.0 ± 2.1 ^b	3.0 ± 0.7 ^b	12.7 ± 2.3 ^c	9.5 ± 2.0 ^{ab}	24.0 ± 3.5 ^c	15.3 ± 0.3 ^b	1.3 ± 0.2 ^b
30	7.0 ± 1.2 ^b	3.2 ± 1.4 ^b	13.7 ± 1.8 ^b	17.4 ± 2.3 ^a	30.0 ± 3.2 ^b	18.3 ± 0.2 ^b	1.3 ± 0.2 ^b
40	7.0 ± 0.9 ^b	3.5 ± 1.1 ^b	13.0 ± 1.6 ^b	15.2 ± 2.3 ^a	30.0 ± 2.8 ^b	18.2 ± 0.1 ^b	1.2 ± 0.1 ^b
50	7.0 ± 1.2 ^b	3.8 ± 1.2 ^b	14.0 ± 1.6 ^b	16.5 ± 1.8 ^a	35.0 ± 2.5 ^a	23.5 ± 0.3 ^a	1.3 ± 0.1 ^b
60	7.0 ± 1.1 ^b	4.7 ± 0.7 ^a	18.7 ± 1.8 ^a	16.5 ± 1.7 ^a	36.0 ± 2.1 ^a	24.0 ± 0.3 ^a	1.3 ± 0.1 ^b
70	7.0 ± 2.2 ^b	4.8 ± 0.5 ^a	19.0 ± 1.8 ^a	17.6 ± 2.4 ^a	39.0 ± 2.2 ^a	25.2 ± 0.3 ^a	2.2 ± 0.4 ^a
80	7.0 ± 1.3 ^b	4.2 ± 0.6 ^a	12.0 ± 1.9 ^b	16.2 ± 1.8 ^a	23.0 ± 2.7 ^c	25.0 ± 0.1 ^a	2.3 ± 0.5 ^a

90	7.0 ± 1.2 ^b	3.7 ± 0.5 ^b	11.7 ± 1.8 ^b	17.5 ± 1.9 ^a	24.0 ± 2.5 ^c	25.5 ± 0.2 ^a	2.5 ± 0.5 ^a
100	7.0 ± 1.3 ^b	3.7 ± 0.2 ^b	11.5 ± 2.1 ^b	17.9 ± 2.5 ^a	26.0 ± 2.8 ^c	25.2 ± 0.4 ^a	2.5 ± 0.5 ^a

Table 1: Influence of external application of BAP on growth parameters of *Solanecio bialfrae*. Means followed by different superscripts in the same column are significantly different at 5% level of probability using Duncan multiple range test. NDST-number of days to emergence of first shoot, NSEP-Number of shoot emerged per stem-cutting, TNL-Total number of leaves per cutting, SHT-average height of shoots, LA-total leaf area of shoots per cutting.

The number of days to emergence of shoot buds did not differ significantly ($P > 0.05$) among BAP treatments. The number of shoot emerged per stem-cutting treated with BAP was greater than those of control cuttings by an average of 60%. The stem-cuttings that were treated with 60-80 mg L⁻¹ BAP produced the highest number of shoots per cuttings (Table 1). Similarly, total number of leaves produced by shoots that emerged from each cutting treated with BAP nearly doubled those that served as control. Stem-cuttings on which external applications of 60 and 70 BAP mg L⁻¹ were treated produced the largest total number of leaves. Shoot height increased with an increase in the concentration of externally applied BAP from 0-30 mg L⁻¹. Thereafter, there was no increase in shoot height as the BAP concentration increased above 30 mg/l BAP, thus producing 73% shoots of uniform height. Similarly, total leaf area of shoots produced from each cutting increased as the concentration of BAP increased with a peak between 50-70 mg L⁻¹ BAP before declined. As the concentration of BAP was

increasing, dry weight of plants increased up to 60 mg/l BAP and thereafter no increment in dry weight was observed. High (70-100 mg L⁻¹) concentrations of external BAP promoted longer distance between nodes than low (10-60 mg L⁻¹) BAP concentration and control (0 mg L⁻¹) treatment.

Shoot/root ratio and physiological parameters

All treatments favored partitioned of more dry matter into above ground tissues except 0, 90 and 100 mg L⁻¹ BAP (Table 2). The plants partitioned tripled dry matter into the shoot when 50 and 60 mg L⁻¹ BAP were applied. Chlorophylls a and b of leaves of plants raised from BAP-applied cuttings were greater than the control plants (Table 2). Low and medium concentrations of BAP promoted chlorophyll a and b synthesis. Chlorophylls a and b of leaves of plants raised from 60-100 mg L⁻¹ BAP-applied cuttings did not differ significantly ($P > 0.05$).

BAP (mgL ⁻¹)	S/R Ratio	Chl.a	Chl.b	Carotenoids	Total Phenolics	Total Sugars	Soluble	Totals Protein	Soluble
0	1.2 ± 0.3 ^d	30.0 ± 3.3 ^d	14.5 ± 2.7 ^e	12.7 ± 3.8 ^d	4.6 ± 1.8 ^d	43.7 ± 4.9 ^c		25.0 ± 4.6 ^d	
10	2.0 ± 0.2 ^c	38.7 ± 3.6 ^c	17.6 ± 3.2 ^d	12.8 ± 3.2 ^d	5.6 ± 1.5 ^c	52.8 ± 3.8 ^c		28.5 ± 5.6 ^d	
20	2.0 ± 0.5 ^c	38.4 ± 3.7 ^c	17.8 ± 2.8 ^d	17.6 ± 2.8 ^c	5.8 ± 1.8 ^c	58.5 ± 3.2 ^b		30.4 ± 4.5 ^d	
30	2.3 ± 0.3 ^c	40.5 ± 4.2 ^b	21.8 ± 2.5 ^c	18.4 ± 3.7 ^c	13.5 ± 1.3 ^a	61.7 ± 3.7 ^b		43.3 ± 4.5 ^c	
40	2.7 ± 0.3 ^b	42.4 ± 4.0 ^b	21.9 ± 2.4 ^c	18.6 ± 3.4 ^c	12.0 ± 1.4 ^a	67.8 ± 3.5 ^b		60.2 ± 5.6 ^b	
50	3.0 ± 0.4 ^a	45.3 ± 3.0 ^b	22.8 ± 2.2 ^c	19.8 ± 3.2 ^c	10.3 ± 1.6 ^a	85.0 ± 3.8 ^a		65.5 ± 5.2 ^b	
60	3.0 ± 0.4 ^a	50.8 ± 4.8 ^a	25.4 ± 2.3 ^a	21.8 ± 3.2 ^b	12.6 ± 2.8 ^a	85.8 ± 4.6 ^a		70.6 ± 6.6 ^a	
70	2.5 ± 0.1 ^b	50.6 ± 4.7 ^a	27.6 ± 2.6 ^a	22.6 ± 4.2 ^b	5.9 ± 1.1 ^c	85.4 ± 4.8 ^a		72.8 ± 6.8 ^a	
80	2.0 ± 0.4 ^c	50.7 ± 5.2 ^a	27.8 ± 2.6 ^a	22.4 ± 4.3 ^b	5.4 ± 1.7 ^c	63.7 ± 4.2 ^b		70.7 ± 6.3 ^a	
90	1.5 ± 0.3 ^d	50.6 ± 4.9 ^a	28.8 ± 2.4 ^a	26.3 ± 3.8 ^a	5.4 ± 1.3 ^c	64.9 ± 4.3 ^b		73.5 ± 6.2 ^a	
100	1.9 ± 0.6 ^d	50.7 ± 4.9 ^a	28.3 ± 2.8 ^a	26.3 ± 3.8 ^a	5.6 ± 1.2 ^c	59.7 ± 4.6 ^b		72.5 ± 6.4 ^a	

Table 2: Influence of external application of BAP on shoot-root ratio and physiological parameters of *Solanecio bialfrae*. Means followed by different superscripts in the same column are significantly different at 5% level of probability using Duncan multiple range test.

However, carotenoids synthesis increased as BAP concentration was increasing. Medium concentrations (30-60 mg L⁻¹) of BAP facilitated synthesis of phenolics than low and high BAP concentrations. Phenolics contents of plants raised from 30-60 mg L⁻¹ BAP-applied stem-cuttings more than doubled those of other treatments (Table 2). Total soluble sugars increased from 0 to 40 mg L⁻¹ BAP, remained unchanged between 50-70 mg L⁻¹ BAP and declined thereafter. Total soluble proteins increased between 20-60 mg L⁻¹ BAP and remained unchanged thereafter as the BAP concentration increased (Table 2).

Fresh shoot weight and proximate contents

External application of BAP on stem-cuttings significantly ($P < 0.05$) influenced proximate contents and fresh shoot weight of the vegetable (Table 3). Exogenous BAP decreased the moisture content of the vegetable by an average of 23% and increase the crude protein content of the vegetable by an average of 40%. Irrespective of BAP concentration, the moisture and crude protein contents of the vegetable remained the same (Table 3).

BAP (mgL ⁻¹)	Moisture	Crude Protein	Crude Fibre	Crude Fat	Ash g/100 g	Carbohydrate	FSW g/Plant
0	12.3 ± 3.4 ^a	9.6 ± 3.6 ^b	11.2 ± 2.2 ^e	1.4 ± 0.8 ^d	9.5 ± 2.4 ^d	50.0 ± 5.8 ^c	9.3 ± 3.4 ^d
10	9.7 ± 2.8 ^b	13.4 ± 3.8 ^a	14.7 ± 2.2 ^d	3.5 ± 0.6 ^a	12.6 ± 2.7 ^c	46.1 ± 4.8 ^c	12.8 ± 3.7 ^c
20	9.7 ± 2.6 ^b	13.4 ± 3.2 ^a	16.7 ± 1.8 ^c	3.5 ± 0.6 ^a	15.7 ± 2.6 ^b	41.1 ± 4.9 ^c	16.6 ± 3.8 ^b
30	9.6 ± 2.6 ^b	13.4 ± 2.9 ^a	18.8 ± 1.6 ^c	3.5 ± 0.8 ^a	15.8 ± 2.6 ^b	38.9 ± 4.9 ^b	19.9 ± 4.3 ^b
40	9.2 ± 2.7 ^b	13.4 ± 2.9 ^a	18.6 ± 1.8 ^c	3.6 ± 0.4 ^a	18.5 ± 3.7 ^a	36.7 ± 4.5 ^b	19.8 ± 4.2 ^b
50	9.2 ± 2.6 ^b	13.4 ± 2.6 ^a	18.4 ± 2.4 ^c	3.6 ± 0.4 ^a	18.9 ± 3.8 ^a	56.5 ± 4.3 ^a	25.5 ± 4.6 ^a
60	9.4 ± 2.8 ^b	13.6 ± 2.7 ^a	17.5 ± 2.4 ^c	2.4 ± 0.7 ^b	18.7 ± 3.2 ^a	56.4 ± 4.4 ^a	26.0 ± 3.2 ^a
70	9.4 ± 2.8 ^b	13.5 ± 2.9 ^a	20.6 ± 2.6 ^b	2.2 ± 0.8 ^b	11.8 ± 3.7 ^c	52.5 ± 4.8 ^a	27.6 ± 3.5 ^a
80	9.5 ± 3.0 ^b	13.5 ± 3.3 ^a	23.8 ± 2.6 ^b	1.9 ± 0.0 ^c	11.6 ± 2.8 ^c	39.7 ± 3.8 ^b	27.8 ± 4.3 ^a
90	9.5 ± 3.1 ^b	13.5 ± 3.5 ^a	26.8 ± 2.7 ^a	1.9 ± 0.1 ^c	11.6 ± 2.6 ^c	36.7 ± 3.2 ^b	27.8 ± 4.6 ^a
100	9.5 ± 3.1 ^b	13.5 ± 3.6 ^a	26.6 ± 2.7 ^a	1.8 ± 0.1 ^c	11.4 ± 2.6 ^c	37.2 ± 3.6 ^b	27.4 ± 3.5 ^a

Table 3: Influence of exogenous BAP on proximate content and fresh shoot weight of *Solanecio bialfrae*. Means followed by different superscripts in the same column are significantly different at 5% level of probability using Duncan multiple range test.

Crude fibre content of the vegetable increased as the BAP concentration increases. The highest BAP concentrations (9 and 100 mg L⁻¹) had the largest fibre content. Crude fat accumulation was promoted by low and medium concentrations of exogenous BAP compare with high concentrations of BAP. The highest ash contents were observed on cuttings that received medium concentrations (40-60 mg L⁻¹) of BAP. Low and high concentrations of BAP decreased carbohydrate content of the vegetable tissues when compare with control plants. However, the medium concentrations (50-70 mg L⁻¹) of BAP improved the carbohydrate content of the vegetable tissue. The external application of BAP on stem-cuttings before planting enhanced fresh shoot weight of the vegetable by an average of 149%. The fresh shoot weight of the vegetable increased as BAP concentration increased up to 50 mg L⁻¹ BAP and thereafter remained unchanged as BAP concentration increased.

Leaf mineral composition

Sodium content in leaf increased with an increase in external BAP (Table 4). However, Potassium content of BAP-applied vegetable did not differ irrespective of the concentration of BAP but greater than that of the control by an average of 43%. Phosphorus content of the vegetable leaf tissues increased as the BAP concentration increased until a peak was attained at 80-100 mg L⁻¹ BAP. External application of 50 and 60 mg L⁻¹ BAP had highest Magnesium contents. Similarly, Calcium content of leaf tissues increased as BAP concentration increased with a peak at 50 and 60 mg L⁻¹ before declined. Exogenous BAP increased Zinc content by 17-fold on the average. Also, on an average, exogenous BAP increased Iron content of the vegetable by 33 folds.

BAP (mgL ⁻¹)	Sodium (g/100 g)	Potassium	Phosphorus	Magnesium	Calcium	Zinc	Iron
0	0.3 ± 0.0 ^d	0.5 ± 0.1 ^b	22.0 ± 0.0 ^e	24.5 ± 4.7 ^e	35.8 ± 2.4 ^e	0.8 ± 0.2 ^e	0.6 ± 0.1 ^g
10	0.5 ± 0.1 ^c	0.7 ± 0.1 ^a	25.4 ± 0.1 ^d	36.7 ± 3.6 ^d	47.9 ± 2.8 ^d	8.4 ± 0.3 ^d	9.0 ± 0.2 ^f
20	0.5 ± 0.1 ^c	0.7 ± 0.2 ^a	27.4 ± 0.0 ^d	36.1 ± 3.4 ^d	50.5 ± 3.4 ^c	8.5 ± 0.1 ^d	13.5 ± 2.3 ^e
30	0.5 ± 0.0 ^c	0.7 ± 0.0 ^a	27.8 ± 0.0 ^d	45.6 ± 4.2 ^c	57.5 ± 2.8 ^b	8.7 ± 0.1 ^d	13.4 ± 3.4 ^e
40	0.7 ± 0.2 ^b	0.8 ± 0.1 ^a	37.5 ± 0.2 ^c	47.2 ± 4.1 ^c	56.8 ± 2.8 ^b	8.6 ± 0.0 ^d	17.6 ± 3.6 ^d
50	0.7 ± 0.2 ^b	0.8 ± 0.0 ^a	41.0 ± 1.3 ^b	53.9 ± 5.8 ^a	58.3 ± 2.9 ^a	11.5 ± 2.3 ^c	20.6 ± 4.5 ^c
60	0.7 ± 0.2 ^b	0.9 ± 0.0 ^a	42.4 ± 1.3 ^b	54.9 ± 5.2 ^a	59.4 ± 3.8 ^a	15.4 ± 2.9 ^b	24.3 ± 4.5 ^b
70	0.7 ± 0.1 ^b	0.9 ± 0.0 ^a	42.6 ± 1.5 ^b	49.1 ± 4.3 ^b	44.2 ± 3.7 ^d	17.3 ± 3.8 ^b	27.2 ± 4.8 ^a
80	0.9 ± 0.1 ^a	0.9 ± 0.0 ^a	46.4 ± 2.4 ^a	49.4 ± 4.5 ^b	44.8 ± 3.8 ^d	19.6 ± 3.2 ^a	27.2 ± 4.8 ^a
90	0.9 ± 0.0 ^a	0.9 ± 0.0 ^a	46.3 ± 2.5 ^a	45.4 ± 4.4 ^c	44.8 ± 2.6 ^d	20.0 ± 2.7 ^a	27.8 ± 3.7 ^a

100	0.9 ± 0.0 ^a	0.9 ± 0.0 ^a	46.5 ± 2.1 ^a	40.4 ± 4.1 ^c	45.3 ± 2.8 ^d	25.0 ± 2.8 ^a	27.9 ± 2.9 ^a
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Table 4: Influence of plant growth regulators on mineral composition of *Solanecio bialfrae*. Means followed by different superscripts in the same column are significantly different at 5% level of probability using Duncan multiple range test.

Discussion

Solanecio bialfrae is an important indigenous leaf vegetable in tropical rainforest regions of Africa, whose supplies fall short of market demands as a result of its slow growth and regeneration from vegetative propagule. Productivity of *S. bialfrae* is measured by rapid formation of high quality and succulent shoots capable of providing nutritional and health benefits over a period of time. 6-benzylaminopurine is a cytokinin that promotes bud initiation, leafy shoot growth and development in many crop species, including vegetables by cell division and enlargement and tissue differentiation [25].

In this study, a single external application of BAP solution on stem-cuttings immediately after planting promoted rapid formation of shoot buds than the control treatment. It is possible that exogenous BAP stimulated rapid cell division, differentiation and enlargement of meristematic cells resulting in early formation of shoot buds observed in the treated cuttings. Our results revealed that medium concentrations (6-8 mg L⁻¹) of exogenous BAP produced higher number of shoots than lower and higher BAP concentration. Low BAP concentration might not be enough to stimulate shoot bud formation while high concentration of BAP caused cell death. Evidence of programmed cell death has been reported at high exogenous BAP concentrations in *Epipremnum aureum* [10]. Generally, external application of BAP promoted leaf formation, shoot height, leaf area development and dry weight in treated plants compare with control plants, which could be due to elevated synthesis of chlorophylls a and b, carotenoids, total soluble sugars and proteins in BAP-treated plants (Table 2). Our results agreed with the recent report of Raji [26] who boosted shoot regeneration in *S. bialfrae* by a single external application of BAP powder on stem nodes. Earlier, Bessler [27] sprayed 50 mg/L BAP to achieve 30-fold increase in shoot formation of Tillandsias, a difficult to propagate horticultural plants. Also, Hasbullah [28] reported an increase in plant height of cowpea by 32% from a foliar application of combine 10.0 mg/L NAA and 10.0 mg/L BAP 9 weeks after planting. Similarly, Gaspera et al. [15] observed high rate of leaf formation following a foliar spray of BAP on *Cucurbita moschata*. Exogenous BAP has been reported to stimulate mobilization of photo assimilates, mineral nutrients and increase chloroplast activity [9].

The biological yield of the vegetable is the shoots which are harvested and consumed. External application of BAP could be a productive method as the vegetables partitioned more dry matter into the shoots than roots in all treated plants except those treated with 9 and 10 mg/L BAP. Previous reports have shown that a single BAP spray of some leaf vegetables during the first days after emergence increases total leaf area, fresh weight and dry weight [13,14]. Growth analysis revealed that enhanced dry weight partitioning to the aerial part by BAP foliar application in *Epipremnum aureu* did not result in leaf area rate increase, but high stem dry weight accumulation and low leaf area partitioning coefficient [9].

Chlorophylls and carotenoids are photosynthetic pigments essential in capturing light energy for carbon dioxide fixation. In this study, exogenous BAP enhanced chlorophylls and carotenoids synthesis

which may translate to high photosynthesis, carbohydrate, lipid and protein synthesis. High chlorophyll contents have been reported for BAP applied plants. Activities of enzymes in chloroplast have been observed to be increased by foliar application of BAP. Partier et al. [29] treated excised Cucurbita cotyledons with benzyladenine (BAP) and found a marked stimulation of Rubisco activity, enzyme protein content, and incorporation of labelled precursors into it, indicating cytokinin-stimulated synthesis of the enzyme phenolics are aromatic benzene ring compounds with one or more hydroxyl groups produced by plants mainly for protection against biotic and abiotic stresses. In the current study, exogenous BAP boosted phenolics synthesis suggesting that BAP treated would exhibit tolerance to abiotic and biotic stresses commonly encountered on the field. Foliar application of BAP on wheat cultivar AARI-11 increases drought tolerance by enhancing non-enzymatic antioxidants production and photosynthetic activity [30]. In addition, high contents of chl. a, chl. b and carotenoids observed in this study might responsible for enhanced total soluble sugars and soluble proteins in BAP-treated plants through enhanced photosynthesis and nitrogen metabolism. Foliar application of BAP in spinach has been reported to elevate the content of chl. a, b, carotenoids, total soluble protein and malondialdehyde [31].

Proximate contents of a vegetable determine the quality and value of the vegetable. Also, food and health benefits of a vegetable depend largely on its proximate and mineral elements contents, which will affect consumer demand for such vegetable. In the current report, external application of BAP decreased moisture content of the vegetable, however, crude fibre, crude protein, carbohydrate, ash and crude fat were enhanced. This situation could be accounted for by high content of photosynthetic pigments (Table 2) that enhanced carbohydrate synthesis and mineral absorption by plant roots which furnished materials for metabolisms of fat and proteins. It is noteworthy that mineral element content of BAP-applied plants was higher than the control plants. Improved root and shoot growths of BAP-applied plants, which increased synthesis of photosynthetic pigments and acquisition of essential mineral elements from the soil could explained high content of Na, K, P, Fe, Mg, Ca in BAP-treated plants than the control plants. This is because cytokinin regulates the ability of plants to take up various nutrients from the environment, including nitrogen, phosphorous, sulfur, and iron [32]. Also, the nutrient status of the plant regulates cytokinin function and hence the growth of the plant [32].

Conclusion

External application of BAP on stem-cuttings after planting reduced the number of days to emergence of shoot, improved shoot and root growths, enhanced physiological parameters, proximate contents and mineral element composition of plants raised from BAP-treated stem-cuttings compared with plants raised from untreated (control) stem-cuttings. Our results revealed that 60 mg L⁻¹ BAP is the optimum concentration as it promoted highest number of shoot per stem-cutting, total number of leaves, total leaf area, shoot height, dry weight, fresh shoot weight, shoot to root ratio, supported with highest synthesis of chlorophyll a and b, phenolics, total soluble sugars and

proteins. In addition, 60 mg L⁻¹ BAP favored highest partitioned of crude protein, fat, ash and carbohydrate in leaf tissue containing highest concentration of leaf K, Mg and Ca.

Conflict of Interest

Authors declare that there are no competing interests between individuals and organizations that can affect the publication of this work.

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References

- Schippers RR (2000) African indigenous vegetables: an overview of the cultivated species.
- Adebooye OC (2004) *Solanecio biafrae* (Oliv. & Hiern) C. Jeffrey. Grubben GJH, Denton OA (eds.). Plant Resources of Tropical Africa 2: Vegetables. Backhuys Publishers, Netherlands, pp: 469-471.
- Adebooye OC, Opabode JT (2004) Status of conservation of the indigenous leaf vegetables and fruits of Africa. African Journal of Biotechnology 3: 700-705.
- Adebooye OC (1996) Proximate composition and nutrient analysis of six selected leaf vegetables of southwest Nigeria. Ife J Agric 18: 56-62.
- Ajiboye BO, Ibukun EO, Edobor G, Ojo AO, Onikanni SA (2013) Chemical composition of *Senecio biafrae* leaf. Scientific Journal of Biological Sciences 2: 152-159.
- Opabode J, Akinyemiju O (2016) In vitro propagation of *Solanecio biafrae* and determination of genetic stability of plantlets using RAPD and ISSR markers. Journal of Horticultural Research 24: 29-36.
- Opabode JT, Adebooye OC (2005) Application of biotechnology for the improvement of Nigerian indigenous leaf vegetables. Afr J Biotechnol 4: 138-142.
- Kumar A, Purohit SS (2001) Plant physiology, fundamentals and applications. Agrobios India, pp: 45-56.
- Di Benedetto A, Galmarini C, Tognetti J (2015) Exogenous cytokinin promotes *Epipremnum aureum* L. growth through enhanced dry weight assimilation rather than through changes in partitioning. Amer J Exp Agric 5: 419-434.
- Benedetto A, Galmarini C, Tognetti J (2013) Changes in leaf size and in the rate of leaf production contribute to cytokinin-mediated growth promotion in *Epipremnum aureum* L. cuttings. The Journal of Horticultural Science and Biotechnology 88: 179-186.
- De Lojo J, Di Benedetto A (2014) Biomass accumulation and leaf shape can be modulated by an exogenous spray of 6-benzylaminopurine in the ornamental foliage plant, *Monstera deliciosa* (Liebm.). The Journal of Horticultural Science and Biotechnology 89: 136-140.
- Hosseini SM, Poustini K, Ahmadi A (2008) Effects of foliar application of BAP on source and sink strength in four six-rowed barley (*Hordeum vulgare* L.) cultivars. Plant Growth Regulation 54: 231-239.
- Coro M, Araki A, Rattin J, Mirave P, Di Benedetto A (2014) Lettuce and celery responses to both BAP and PBZ related to the plug cell volume. American Journal of Experimental Agriculture 4: 1103.
- Di Matteo J, Rattin J, Di Benedetto A (2015) Increase of spinach growth through the use of larger plug cell volume and an exogenous BAP spray. American Journal of Experimental Agriculture 6: 372.
- Della Gaspera P, Teruel J, Giardina E, Di Benedetto A (2016) Physiological and Technological Consequences of Benzyl Adenine (BAP) Application on Butternut Squash (*Cucurbita moschata* Duchesne ex Poir.) Productivity. Amer J Exp Agric 13: 1-11.
- Alizadeh O, Haghghi BJ, Ordoorkhani K (2010) The effects of exogenous cytokinin application on sink size in bread wheat (*Triticum aestivum*). African Journal of Agricultural Research 5: 2893-2898.
- Lichtenthaler HK, Wellburn AR (1985) Determination of total carotenoids and chlorophylls A and B of leaf in different solvents. Biol Soc Trans 11: 591-592.
- Julkunen-Tiitto R (1985) Phenolic constituents in the leaves of northern willows: methods for the analysis of certain phenolics. Journal of Agricultural and Food Chemistry 33: 213-217.
- Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72: 248-254.
- Yemm EW, Willis AJ (1954) The estimation of carbohydrates in plant extracts by anthrone. Biochemical Journal 57: 508.
- AOAC (1980) Official Methods of Analysis. 13th (edn.), Association of Official Analytical Chemists, Washington DC, pp: 20-28.
- AOAC (2005) Official Methods of Analysis. Association of Official Analytical Chemists, Washington DC, USA, pp: 45-56.
- AOAC (1990) Official methods of analysis. Association of Official Analytical Chemists, Washington, pp: 34-40.
- SAS S (2002) Guide SUS. Cary NC, USA: SAS Institute Inc.
- Argueso CT, Raines T, Kieber JJ (2010) Cytokinin signaling and transcriptional networks. Current Opinion in Plant Biology 13: 533-539.
- Raji IB (2016) Growth and physiological responses of *Solanecio biafrae* to external application of 6-benzylaminopurine and gibberellic acid. B. Agric thesis. Obafemi Awolowo University, Ile-Ife, pp: 43.
- Bessler B (1997) The use of 6-benzylaminopurine for rapid multiplication of tillandsias. HortScience 32: 256-258.
- Hasbullah NA, Lassim MM, Mazlan MA, Lood SZ, Kamarulzaman MLH (2014) Effect of BAP and NAA on growth optimization of *Vigna unguiculata* subsp *sesquipedalis*. Proceedings of International Conference on Latest Trends in Food, Biological & Ecological Sciences (ICLTFBE'14) Phuket, Thailand, pp: 34- 42.
- Parthier B, Lerbs S, Lehmann J, Wollgiehn R (1985) Cytokinin-controlled ribulose 1, 5-bisphosphate carboxylase gene expression in pumpkin cotyledons. Biologia Plantarum 27: 131.
- Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA (2009) Plant drought stress: effects, mechanisms and management. In Sustainable Agriculture, pp: 153-188.
- Aslam M, Sultana B, Anwar F, Munir H (2016) Foliar spray of selected plant growth regulators affected the biochemical and antioxidant attributes of spinach in a field experiment. Turkish Journal of Agriculture and Forestry 40: 136-145.
- Argueso CT, Ferreira FJ, Kieber JJ (2009) Environmental perception avenues: the interaction of cytokinin and environmental response pathways. Plant, Cell & Environment 32: 1147-1160.