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Original article

Vegetative propagation, biochemical and antioxidants characteristics of *Antidesma bunius* L. Spreng in eastern Himalayas, India

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Summary

Introduction – ***Antidesma bunius* L. Spreng is an important dietary and medicinal component edible fruit trees of the eastern Himalayas, India. However, the non-availability of quality planting materials and little information on biochemical and antioxidant properties of this species hinder its commercialisation. Therefore, a study was conducted to determine the biochemical and antioxidant properties of the fruits, and to find out a propagation technique for rapid multiplication and conservation of the fruit tree.** *Materials and methods* – **Ripened fruits were used for carrying out morphological, biochemical and antioxidant analysis. One-year-old healthy shoots were used to study the response of stem cuttings to different concentrations of Indole butyric acid (0, 1,000, 2,000 and 3,000 ppm).** *Results* – **Maximum fruit weight (198.05 ± 0.14 mg), fruit yield (37.82 ± 4.91 kg tree^{-1}), total soluble solid (13.63 ± 0.53 °Brix), total sugar (13.51 ± 0.14%), crude fibre (6.64 ± 0.07%), anthocyanin (421.76 mg C3GE 100 g^{-1}), total flavonoid (3.76 mg QE 100 g^{-1}), total phenolic content (140.17 mg GAE 100 g^{-1}) and FRAP (10.34 ± 0.16 mg AAE g^{-1}) were recorded in genotype Umpowin. While titratable acidity (1.44 ± 0.01%), vitamin C (28.28 mg 100 g^{-1}) and total ash (13.11 ± 0.33%) was highest in genotype Liarkhla. Stem cutting had maximum response at IBA 3,000 ppm which reduced the days to sprouting by 22.3% but increased the number of primary roots, root length, fresh weight roots and plant survival (78%).** *Conclusion* – **Fruits showed high biochemical and antioxidant properties which can be incorporated in dietary and as alternative sources of edible colour. Stem cutting using IBA 3,000 ppm can help in rapid multiplication and conservation of this fruit tree species.**

Keywords
wild edible fruits, variation, genotypes, hormones, multiplication

Significance of this study

What is already known on this subject?

- Indole butyric acid is the most effective rooting hormone in propagation of different fruit trees species. Its dosage and effectivity vary with species.

What are the new findings?

- *Biochemical and antioxidants:* The fruits are rich in total soluble solid, total sugar, crude fibre, anthocyanin, total flavonoid, total phenolic content and FRAP.
- *Rapid multiplication technique:* The optimum IBA concentration (3,000 ppm) promotes early sprouting, improves root performance and plant survival.

What is the expected impact on horticulture?

- The rich sources of biochemical and antioxidant properties of this fruit can be incorporated in diet and as alternative sources of edible colour. Stem cutting using IBA 3,000 ppm can help in rapid multiplication and conservation of this fruit tree species.

Introduction

Antidesma bunius L. Spreng belongs to the Phyllanthaceae family and is believed to be native of Southeast Asia including the lower Himalayas of India and northern Australia (GRIN, 2021). The fruit are found growing wild as well as semi-cultivated in the subtropical areas of the eastern Himalayas, India. The plant is found in the primary and secondary evergreen to deciduous vegetation and around human habitation. In the eastern Himalayas range, the plant is reported to be existing in all the Himalayas states of India, particularly, the north eastern states. In addition to eastern Himalayas of India, this fruit tree is reported to be found in Sri Lanka, and southeastern Asia to the Philippines, Solomon Islands and northern Australia (GRIN, 2021). The *Antidesma* species found in the eastern Himalayas range of India are *A. bunius* L. Spreng, *A. acidum*, *A. diandrum* Heyne ex Roth., *A. ghaesembilla* Gaertn. and *A. khasianum* Hook. f. However, the fruit of *A. bunius* L. Spreng is the most economically important among the tribal population. The fruits are small, oval shaped when ripe, attractive bright red to deep purple colour with a distinct blend of sweet and sour and distinct flavour. The fruits are available during July–August in the local markets whose cost varies, ₹ 70–120 per kg. Ethnobotanically, whole fruits of *A. bunius* L. Spreng are edible including seed, consumed as fresh or processed as tea, jam, jelly or red-wine. Traditionally, the fruit juice extracted from *A. bunius* L. Spreng is possessing an attractive red to deep purple colour. Fruit is reported to be a rich source of bioactive compounds displaying distinct pharmacological properties such as antibacterial properties (Lizardo *et al.*, 2015), α-glucosidase inhibitory activities (Lawang *et al.*, 2012),

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antidiabetic properties (El-Tantawy *et al*., 2015) and notable antioxidant properties (Belina-Aldemita *et al*., 2013), anti-apoptotic and anti-inflammatory effects in human breast epithelial cells (Puangpronpitag *et al*., 2011), reduced blood pressure and improved the hemodynamic status of induced hypertensive rats (Chaikham *et al*., 2016). Fruits of *A. bunius* L. Spreng contain high nutritional components; carbohydrates, organic acids, vitamins, anthocyanins, flavonoids, phenolic acids, proteins and minerals (Butkhup and Samappito, 2008). Therefore, this species may be considered as an important medicinal-fruit tree due to their rich sources of bioactive substances and medicinal properties. However, there is no information generated so far on this fruit tree from the eastern Himalayas of India.

In the last decades, the species are rarely observed in the forest and around habitat areas which might be due to reckless harvesting of fruits, fire wood, lack of improved varieties, non-availability of multiplication techniques and poor recognition of these species in horticultural promotion and conservation programs. Therefore, a study was undertaken to determine the morphology, biochemical and antioxidants, and vegetative propagation techniques of this fruit tree so that interest may take up for its popularization as fruit crop of the future.

Materials and methods

Experimental site and plant materials

Wild edible fruit of *A. bunius* L. Spreng grown in the forests and/or in the backyards were collected from Umpowin (25°44'09"N, 92°08'01"E, elevation 836 m a.m.s.l.), Liarkhla (25°44'64"N, 92°04'33"E, elevation 894 m a.m.s.l.), and Namdong (25°40'02"N, 92°19'00"E, elevation 987 m a.m.s.l.) of Meghalaya, India for the study. The plant was identified through fresh materials and herbarium collating using India Biodiversity Portal (https://indiabiodiversity.org/species/show/7533). The collected fruits were analysed for fruit morphology, biochemical and antioxidant attributes at Horticulture Laboratory, ICAR Research Complex for NEH Region, Umiam, Meghalaya, India, during 2019–2020.

Following the characterization of fruits, an experiment on response of stem cutting of *A. bunius* L. Spreng to growth regulators was conducted in Horticulture Experimental Farm of the Institute located at 25°41'91''N and 91°55'15''E,) with an altitude of 995 m a.m.s.l. The average total annual rainfall during this period was 2,684 mm, of which more than 90% was received during May–October. Mean minimum and maximum temperature vary from 6.6 °C in January to 29.06 °C in August. Relative humidity ranges between 51.1% during winter and 90.13% during rainy season. One-year-old healthy and disease-free, pencil thickness shoots of 20–25 cm length were collected from Umpowin, Ri-Bhoi district of Meghalaya in July 2019–2020. The collected shoot was sprayed with benomyl (500 ppm), incised at 20–25 cm length, and kept in a shade net (75%). The basal ends of cutting were immersed up to a depth of 2 cm into different concentrations of rooting hormone IBA (Indole butyric acid at 1,000, 2,000 and 3,000 ppm) and distilled water (I0 – control) for 15 sec. Ethanol (75%) diluted with distilled water was used to dissolve the IBA. The treated cuttings were planted in media composed soil + river sand + FYM, 1:1:1 /v:v:v). The substrate was mixed thoroughly and filled in a polybag (size 15 cm × 25 cm) leaving 3 cm top space, and planting was done uniformly about 6 cm depth. The plants were transferred to shade net house (75%) and irrigated immediately. Plant protective measures were taken including the alternate application of Imidacloprid or dimethoate and Bavistin or copper oxychloride at 0.2% each after two weeks of planting, to protect against pest and disease incidence. No fertilizer was applied to plants during the study. Substrates were watered to drip point before planting. The cuttings were uprooted and washed immediately in running water for removal of adhering dirt and cleaned with tissue paper.

Morphological analysis

Physiological mature fruit of each genotype were used for carrying out the morphological, biochemical and antioxidant analyses (Figure 1). Fruits samples were washed and kept at room temperature for 10 min to remove the adhering water before analysis. Parameters, *viz*., weight of fruit, pulp and seeds, were determined using electronic balance (Adiar Dutt-1620C) and dimension of fruits were measured using digital caliper (Code 1108-150).

Determination of biochemical attributes

Biochemical parameters such as total soluble solids (TSS) were determined using a hand-held refractometer (HI 96801) and titratable acidity, vitamin C, reducing sugars and total sugars were analyzed according to Rangana (1997), total ash as per method (AOAC, 1990), and crude fiber content according to Sadasivam and Manickam (1992).

Determination of functional attributes and antioxidant activity

1. Preparation of fruit extract. One gram pulp of each fruit was extracted with 10 mL methanol at ambient temperature and incubated for 1 h at room temperature with continuous magnetic stirring at 200 rpm. After 1 h, the samples were centrifuged at 1,000*g* for 20 min and the supernatant was collected and stored at -20 °C until analysis (Vega *et al*.,

Figure 1. Genotypes of *Antidesma bunius* L. Spreng, a) Umpowin; b) Namdong; c) Liarkhla.

2016). The aliquot was used for assessments of total phenolic content, total anthocyanin, total flavonoids, DPPH free radical scavenging capacity and FRAP reducing power.

2. Determination of total phenolic content. The crude extracts were estimated for total phenolic content using Folin-Ciocalteu procedure as per the method of Singleton and Rossi (1965) modified by Keskin-Šašić *et al.* (2012). 0.2 mL of extract was transferred to tubes containing 1 mL Folin-Ciocalteu reagent (1:10 v/v distilled water) and incubated for 10 min. After 10 min, 0.8 mL of sodium carbonate solution (7.5%) was added. The mixture was vortex for 15 sec and allowed to stand for 30 min at room temperature for colour development. The absorption was measured at 743 nm in Labinda UV-visible spectrophotometer (Model UV 3200) and the total phenolic content was expressed as mg gallic acid equivalent per 100 g of fruit extract (mg GAE 100 g^{-1}).

3. Determination of total anthocyanin. Total anthocyanin was determined by the pH-differential method (Lako *et al.*, 2007) which measures the color difference of two samples obtained by reacting samples with a buffer solution at two different pH levels. 0.4 mL of extract solution was taken and 3.6 mL of the corresponding buffer, potassium chloride (0.025 M) buffer solution (pH 1.0) was added to one of the vials and sodium acetate (0.4 M) buffer solution (pH 4.5) to the other vial. The diluted samples were placed in a dark room for 20 min. The absorbance of each solution was taken against a blank in a cuvette with 1 cm path length at 510 nm and 700 nm wavelength using UV-Vis spectrophotometer. Anthocyanin pigment concentration was expressed as cyanidin-3-glucoside equivalents per 100 g (C3GE 100 g^{-1}) and calculated as follows:

$$\text{Anthocyanin pigment (mg/g)} = \frac{A \times MW \times DF \times 1000}{\epsilon \times l} \times 1{,}000$$

where:
A = (A510 nm – A700 nm) $_{pH\ 1.0}$ – (A510nm – A700nm) $_{pH\ 4.5}$;
MW (molecular weight) = 449.2 g mol^{-1} for cyanidin-3-glucoside (cyd-3-glu);
DF = dilution factor established in D;
L = path length in cm;
ϵ = 26,900 molar extinction coefficients for cyd-3-glu; and
1,000 = factor for conversion from g to mg.

Measurement of total flavonoids

The total flavonoid content of extracts was estimated using aluminium chloride ($AlCl_3$) colourimetric assay as previously described by Zhishen *et al.* (1999). 1 mL of sample extract was taken and 0.3 mL of 5% $NaNO_2$ was added. After 6 min, 0.3 mL of 10% $AlCl_3$ solution was added. This mixture was allowed to stand for another 5 min at room temperature. To the mixture 2 mL of 1M NaOH was added and the volume was making up to 5 mL with distilled water and allowed to stand for 15 min at room temperature. Then absorbance was measured at 510 nm (UV-visible spectrophotometer, Model UV 3200). Total flavonoid content was presented as mg quercetin equivalent per 100 g (mg QE 100 g^{-1}).

Measurement of DPPH free radical scavenging activity

The free radical scavenging activity of the fruit extracts was estimated with the stable radical DPPH (1,1-diphenyl-2-picrylhydrazyl) as described by Shen *et al.* (2010). Different volume for each sample (0.2, 0.4, 0.6, 0.8, 1.0 mL) was transferred to test tubes and the final volume was made up to 1 mL with methanol to which 3 mL of freshly prepared DPPH solution (0.1 mM in methanol) was added. The mixtures were then thoroughly mixed and allowed to stand for 30 min in the dark. Measurement of absorbance was done at 517 nm (UV-visible spectrophotometer, Model UV 3200). The equation [DPPH radical: DPPH scavenged (%) = {(Ac – At)/Ac} × 100] was used to calculate the capability to scavenge, where Ac is the absorbance of the control reaction and At is the absorbance in presence of the sample of the extracts. The antioxidant activity of the extract was expressed as IC_{50} (the concentration of fruit sample required to decrease the absorption at 517 nm by 50%). The IC_{50} value was expressed as the concentration in mg of extract per mL that inhibited the formation of DPPH radicals by 50%. Ascorbic acid (5–25 µg mL^{-1}) was used as positive control.

Measurement of FRAP reducing power

The reducing power of the extracts was assessed as per the method of Oyaizu (1986). About 100 µL of fruit extracts was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and 1% potassium ferricyanide (2.5 mL). This mixture was incubated at 50 °C for 20 min, to which 2.5 mL aliquots of trichloroacetic acid (10%) was added. The content was centrifuged at 3,000 rpm for 10 min. The upper layer of the solution (2.5 mL) was extracted and mixed with 2.5 mL distilled water and 0.5 mL of freshly prepared ferric chloride solution (0.1%). Then measurement of absorbance was recorded at 700 nm (UV-visible spectrophotometer, Model UV 3200) and the reducing power was expressed in terms of ascorbic acid equivalent (AAE) in mg per g of extract (mg AAE g^{-1}).

Rooting performance

Observations such as the number of primary roots, root length, root fresh weight and number of leaves were recorded at 90 days after planting (DAP). Days to sprouting were recorded as when the sprout appears. The success of rooting cuttings (%) was calculated by dividing the number of rooted cuttings by a total number of cuttings.

Statistical analysis

The replicated data ($n = 3$) for biochemical and antioxidants were analysed using one-way ANOVA ($p < 0.05$) and data were presented as mean ± SE. The experiment on propagation was conducted in a completely randomized design (CRD) with five replications comprising ten plants per replication. The analysis of variance (ANOVA) was conducted at a 5% level of probability. Pearson correlation was analysed to establish a relationship among variables. All the data were subjected to SPSS (16.0) software for analysis.

Results and discussion

Morphological and quality parameters

Result showed significant variation of fruit and seed morphological among genotypes of *A. bunius* L. Spreng (Table 1). The highest fruit weight (198.05 ± 0.14 mg), fruit length (7.08 ± 0.24 mm), fruit diameter (6.65 ± 0.19 mm), pulp thickness (2.70 ± 0.31 mm), pulp weight (164.03 ± 0.01 mg), seed weight (34.02 ± 0.03 mg $fruit^{-1}$), seed length (5.47 ± 0.11 mm), juice content (5.21 ± 0.24 mL) and fruit yield (37.82 ± 4.91 kg $tree^{-1}$) were recorded in genotype Umpowin. However, genotype Liarkhla had higher seed diameter (4.05 ± 0.15 mm) and seed thickness (2.73 ± 0.32 mm) over other genotypes. It indicated that the fruit of *A. bunius* L. Spreng is among the smallest known fruits of the eastern Himalayas, India, with

Table 1. Morphological characteristics of fruits of *A. bunius* L. Spreng.

Genotypes	No. of fruits/ bunch	Fruit weight (mg)	Fruit length (mm)	Fruit diameter (mm)	Pulp thickness (mm)	Pulp weight (mg)	Seed weight/ fruit (mg)	Seed length (mm)	Seed diameter (mm)	Seed thickness (mm)	Juice content (mL)	Fruit yield (kg tree^{-1})
Umpowin	76.4±6.8a	198.1±0.14a	7.1±0.24a	6.6±0.19a	2.7±0.31a	164.0±0.01a	34.0±0.03a	5.5±0.11a	3.9±0.13ab	2.6±0.09a	5.2±0.24a	37.8±4.91a
Namdong	65.6±4.7c	183.4±0.06ab	6.8±0.16ab	6.3±0.22ab	2.5±0.11b	154.5±0.02b	28.8±0.2c	5.6±0.12a	3.7±0.15b	2.4±0.16a	4.9±0.29b	30.0±1.87b
Liarkhla	72.2±7.7b	194.7±0.08a	6.9±0.15a	6.5±0.30a	2.4±0.41b	163.1±0.02a	31.6±0.4b	5.6±0.21a	4.1±0.15a	2.7±0.32a	5.0±0.21b	35.1±5.83ab

Mean (n = 20) with ± S.E. followed by different letters in each column are significantly different from each other according to Tukey's test (p < 0.05).

Table 2. Biochemical characteristics of fruits of *A. bunius* L. Spreng.

Genotypes	Moisture (%)	TSS (°Brix)	Titratable acidity (%)	Maturation index (%)	Total sugar (%)	Reducing sugar (%)	Crude fibre (%)	Total ash (%)	Dry matter (%)
Umpowin	82.7±3.37a	13.6±0.53a	1.12±0.03c	12.2±0.26a	13.5±0.14a	1.8±0.04a	6.6±0.07a	11.5±0.23b	21.7±0.42a
Namdong	79.6±1.15bc	11.6±0.04b	1.31±0.03b	8.8±0.19b	12.6±0.13c	1.5±0.03b	5.5±0.06c	8.8±0.29c	19.9±0.20b
Liarkhla	80.8±0.52b	13.0±0.12a	1.44±0.01a	9.0±0.05b	13.1±0.07b	1.7±0.04ab	5.7±0.02b	13.1±0.33a	21.1±0.58a

Mean (n = 3) with ± S.E. followed by different letters in each column are significantly different from each other according to Tukey's test (p < 0.05).

respect to fruit weight as reported by Rymbai *et al*. (2014, 2016a, b, 2019, 2020); *Prunus nepalensis* (3.98–7.91 g), *Elaeagnus latifolia* (13.0–16.4 g), *Myrica esculenta* (7.0–14.13 g), *Baccaurea sapida* (11.02–12.60 g), *Pyrus pashia* (6.57–44.25 g), *Calamus meghalayensis* (11.46–17.25 g), *Prunus undulata* (2.86–5.62 g), *Docynia indica* (25.27–42.26 g), *Viburnum foetidum* (0.25–0.33 g). However, the fruit weight of *A. bunius* L. Spreng per infructescence (76.40 g) and per tree (37.84 kg) showed promising, considering the whole fruits are edible, less incidence of pest and disease and high market value of the fruit (₹ 70–120 per kg). In addition, fruits of *A. bunius* L. Spreng possess appealing pigmentation of both pulp and juice which can be a good potential source of natural edible colour in food industry.

Biochemical characteristics

The biochemical characteristics of fruits contribute to the consumer perception of quality traits including those associated with taste, mouth feeling and appearance. Genotypes of *A. bunius* L. Spreng had significant variation for biochemical traits (Table 2). Genotype Umpowin had higher moisture content (82.69 ± 3.37%), total soluble solid (13.63 ± 0.53%), maturation index (12.18 ± 0.26%), total sugar (13.51 ± 0.14%), reducing sugar (1.79 ± 0.04%), crude fibre (6.64 ± 0.07%) and dry matter (13.11 ± 0.33%), while genotype Liarkhla recorded maximum titratable acidity (1.44 ± 0.01%) and total ash (13.11 ± 0.33%). The variation in biochemical properties among genotypes might be due to genetic makeup of the genotypes. It shows that genotypes of *A. bunius* L. Spreng had higher biochemical properties over other fruits reported from the region, including *P. nepalensis* (crude fibre, 2.5%), *E. latifolia* (TSS 11.9%), *Myrica esculenta* (TSS, 5.7–6.5%) (Rymbai *et al*., 2016a, b, 2020). The total soluble solids and titratable acidity are two important factors that determine the fruit quality including the taste, sweetness, and maturity indices of the fruit and its suitability for processing. Therefore, a blend of TSS and acidity improve the fresh consumption as well as processing and value addition and can be promoted for different value-added products such as ready to serve (RTS), wine, *etc*.

Antioxidant properties

Our results indicated significant variations in antioxidant properties as depicted in Figure 2. The highest anthocyanin (421.76 ± 4.88 mg 100 g^{-1}), total flavonoid (3.76 ± 0.13 mg QE 100 g^{-1}), total phenolic content (140.17 ± 3.02 mg GAE 100 g^{-1}) and FRAP (10.34 ± 0.16 mg AAE g^{-1}) was recorded in genotype Umpowin. While, vitamin C (28.28 ± 0.37 mg 100 g^{-1}) and DPPH radical scavenging activity with an IC_{50} value of 156.73 µg mL^{-1} was recorded lowest in genotype Umpowin, whereas the IC_{50} value of ascorbic acid was 12.29 µg mL^{-1}. These variations could be attributed to various intrinsic and extrinsic factors, such as genetical and environmental factors. Results indicated that the vitamin C of *A. bunius* L. Spreng (26.37 mg 100 g^{-1}) were higher than majority of the fruits reported; *E. latifolia* (16–19.2 mg 100 g^{-1} pulp), *M. esculenta* (17.6–28.2 mg 100 mL^{-1} pulp), and *P. pashia* (1.22 mg g^{-1} pulp), *Docynia indica* (14.8–17.5 mg 100 g^{-1}) (Rymbai *et al*., 2016a, 2019). *A. bunius* L. Spreng also contain higher anthocyanin content (421.76 mg 100 mL^{-1} juice) comparatively over Sohiong (358.86 mg 100 g^{-1}, Rymbai *et al*., 2014), *E. latifolia* (16.2 mg 100 g^{-1} pulp) (Rymbai *et al*., 2020). Similarly, FRAP value of *A. bunius* L. Spreng (10.34 ± 0.16 mg AAE g^{-1}) were higher than those value reported in promising wild edible fruits ranged, 0.0518 ± 0.49 to 0.111 ± 0.00 mg AAE g^{-1} by

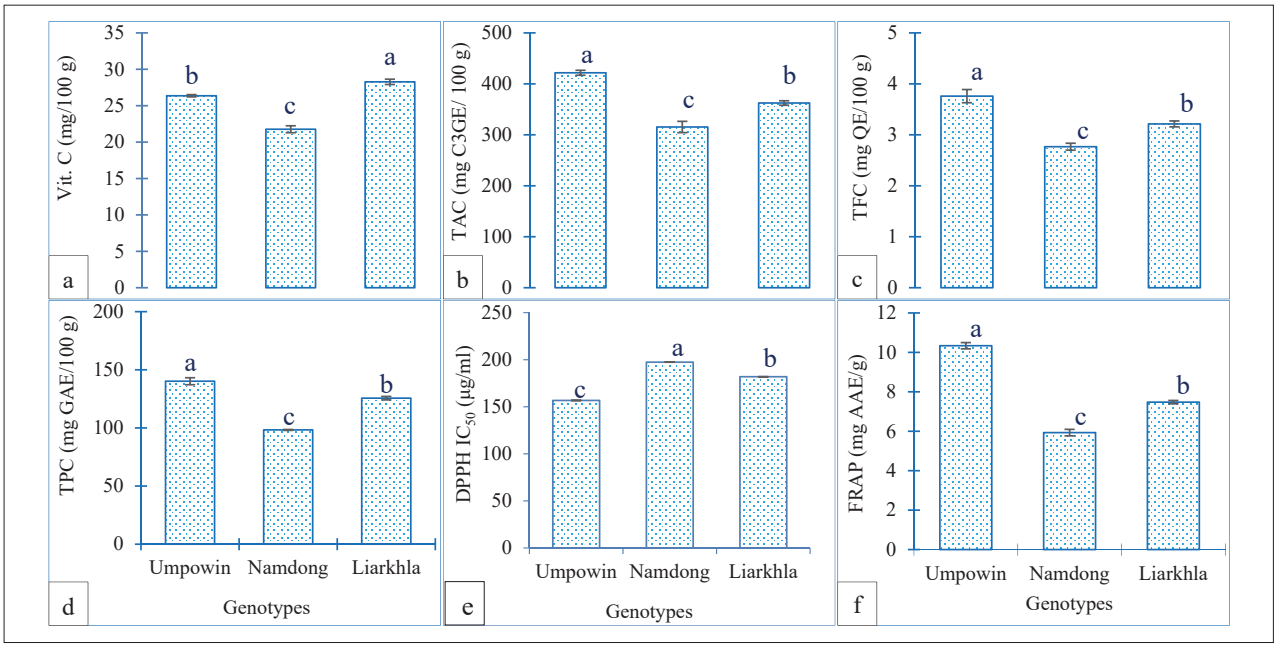

FIGURE 2. Antioxidant properties of fruits (*A. bunius* L. Spreng). a) Vit C (vitamin C); b) TAC (total anthocyanin content); c) TFC (total flavonoid content); d) TPC (total phenolic content); e) DPPH [1,1-diphenyl-2-picrylhydrazyl, IC_{50}: Half-maximal inhibitory concentration (Ascorbic acid IC_{50}, 12.29 µg mL^{-1})]; f) FRAP (ferric reducing antioxidant power). Mean value ($n = 3$) ± S.E. followed by different letters on each bar indicate significant difference from each other according to Tukey's test ($p < 0.05$).

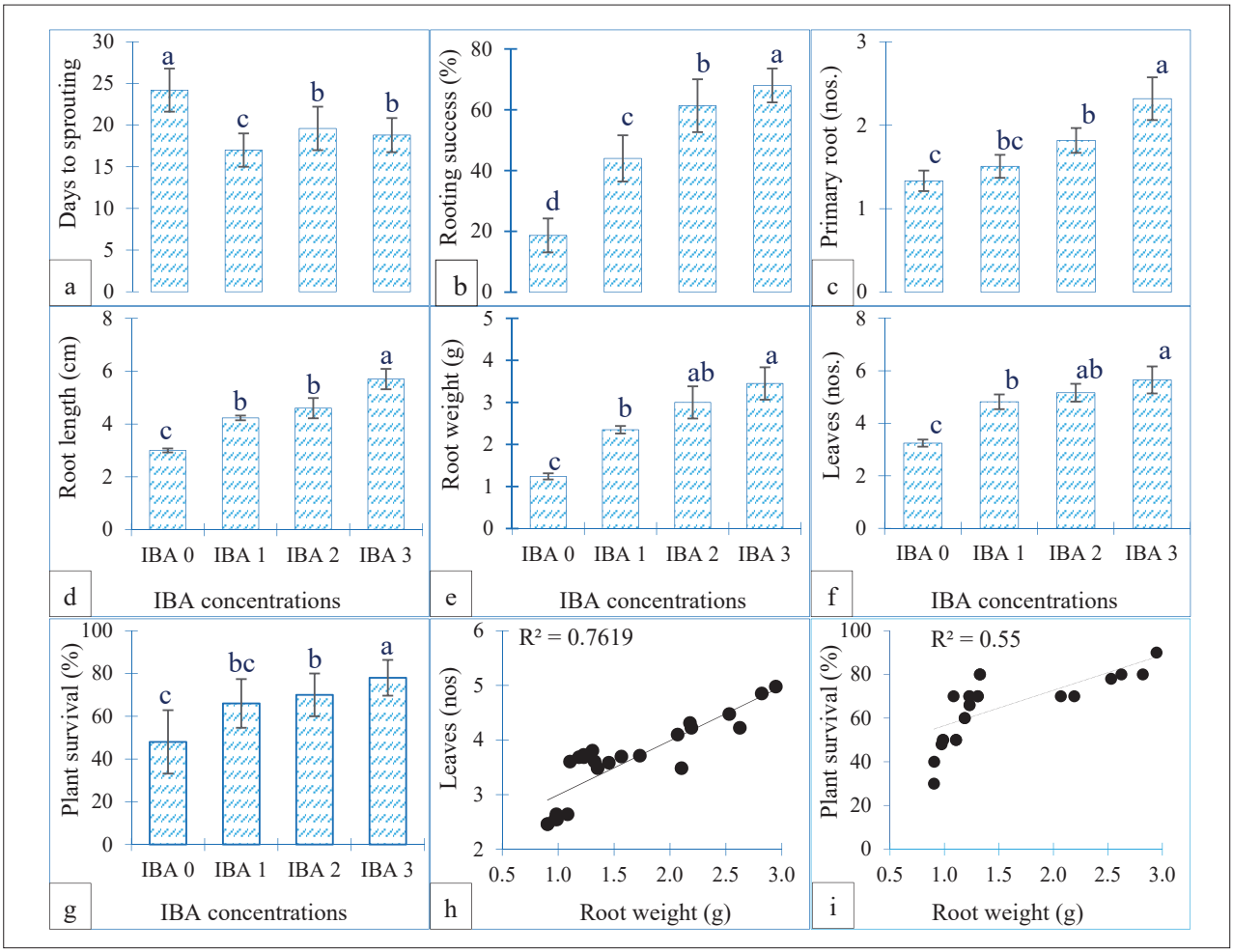

FIGURE 3. Response of stem cutting of *A. bunius* L. Spreng to various IBA concentrations a) days taken to sprouting; b) rooting success (%); c) number of primary roots; d) primary root length; e) root weight; f) number of leaves; g) plant survival (%) and correlation among rooting attributes (h–i). Mean value of five replications (each replication consisted of 10 plants) ± S.E. followed by different letters on each bar indicate significant difference from each other according to Tukey's test ($p < 0.05$).

Mahadkar *et al*. (2013). It is clear that most of the underutilized fruits showed strong reducing capability as compared to other fruit crops which might be probably due to the presence of high polyphenols which are responsible for their antioxidant activity (Khabade *et al*., 2012). In addition to minor fruits, antioxidants of *A. bunius* L. Spreng also exceeding those of commercial fruit crops for vitamin C (*Citrus sinensis,* 10.13 mg 100 g^{-1}; *Ananas comosus*, 6.40 mg 100 mg^{-1}; *Malus domestica*, 7.94 mg 100 mg^{-1}; *Prunus persica,* 5.92 mg 100 mg^{-1}; Gupta and Prakash, 2009), total anthocyanin (sweet cherry, 44.19 mg 100 g^{-1} f.w. by Oancea *et al*., 2013), total flavonoids (*Prunus mahaleb,* 1.24 g kg^{-1}, Blando *et al*., 2015) and total phenolics content (pineapple, 47.9 mg GAE 100 g^{-1}; banana, 7.2–18.9 mg GAE g^{-1} d.w.; papaya, 57.6 mg GAE 100 g^{-1} (Luximon-Ramma *et al*., 2003; Bennett *et al*., 2010). Our result exhibited lesser DPPH values than those reported in commercial fruits (grapes, 0.79 IC_{50} mg mL^{-1}; pineapple, 0.83 IC_{50} mg mL^{-1}; guava, 1.71 IC_{50} mg mL^{-1} by Singh *et al*., 2012). It is an established fact that the lower IC_{50} values specify greater antioxidant activity (Matuszewska *et al*., 2018). These constituents may find new applications in functional foods, pharmaceuticals and industrial products, and as an alternative source of antioxidants and natural food colourants (Rymbai *et al*., 2013). Furthermore, the incorporation of this fruit may enrich the diet nutritionally and be helpful in preventing the different degenerative diseases due the total anti-oxidant activity of fruit results from a synergism between the various bioactive compounds and reactive oxidative species scavenging intermediates (Rymbai *et al*., 2013).

Response of stem cutting to growth regulators

Results showed a significant effect of IBA concentrations on rooting attributes of stem cutting (Figure 3a–g). Days to sprouting was minimum in IBA 3,000 ppm (18.8 ± 2.05 days) and maximum in IBA control (24.2 ± 2.59). IBA 3,000 ppm had highest rooting success (68.03 ± 5.58%), number of primary roots (2.32 ± 0.26 per rooted cutting), root length (5.70 ± 0.39 cm) and root weight (3.45 ± 0.39 g), number of leaves (5.65 ± 0.51 per rooted cutting) and plant survival (78 ± 8.37%). Result indicates that IBA 3,000 ppm reduced the rate of sprouting by 22.3%, however, increased rooting success, number of primary roots, root length and fresh weight by 228.6%, 73.8%, 90.0% and 178.2%, respectively as compared to control. The result is contradicting to research finding that root production is more receptive to lower concentrations of IBA in *Holarrhena pubescens* Wall. (Baul *et al*., 2010). Totaan (2019) also reported that IBA had significantly increased shoot length and shoot number but had no influence on root length, root number and percent survival at IBA concentrations (500, 1,000 and 1,500 ppm) in *A. bunius* L. Spreng. However, our result showed that IBA (3,000 ppm) had significantly influenced the root and shoot characteristics of stem cutting in *A. bunius* L. Spreng. The optimal IBA concentration (3,000 ppm) might have stimulated the hydrolysis of reserved carbohydrates rapidly resulting in enhancing the supply of food materials to tissue, thus generating new cells, accelerating respiratory activity, cell division and cell wall elongation (Hartmann *et al*., 2002). Furthermore, this process induces a re-differentiation of mature parenchyma cells into cambial tissue that intricated the root initiation and formation leading to a higher number of adventitious roots, root length, root weight and rooting success (Rymbai and Reddy, 2010a, b). It was observed that the number of leaves and plant survival at IBA 3,000 ppm were increased by 74.12% and 62.50%, respectively, over control. This could be attributed to the vigorous root system and better performance of shoots as showed by a strong correlation of leaves number with root fresh weight (r = 0.762) and plant survival (r = 0.628) (Figure 3h–i). Our results are in agreement with the findings of Rymbai *et al*. (2012) in guava, and of Upadhyay and Badyal (2007) in pomegranate.

Conclusion

The present result exhibited a significant variation among genotypes for physico-chemical and antioxidant properties. Genotype Umpowin showed higher yield, biochemical and antioxidant activities which may find its potential application in functional food. A rapid multiplication technique of *A. bunius* L. Spreng using stem cutting with IBA 3,000 ppm has significantly higher rooting attributes and plant survival, which may facilitate its wide cultivation and conservation.

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