

Full Length Research Paper

The effect of gibberellins on sprouting of cuttings and quality of bush tea (*Athrixia phylicoides* DC)

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A study was conducted to determine the effect of gibberellins on sprouting of cuttings and subsequent quality of bush tea (*Athrixia phylicoides* DC). The study was conducted under a 40% shade net with light/shading as a gradient. Treatments consisted of gibberellins (Progibb 40%) applied at various rates as follows: 0, 1, 2, 3 and 4%, arranged in a randomized complete block design (RCBD) and effected on 18 February, 2008. Treatments were applied until run-off and the experiment was harvested for analysis three months after transplanting. Total polyphenols were determined using Folin Ciocalteau method, total tannins were determined using the Vanillin HCL method and total antioxidants were determined using the trolox equivalent antioxidant capacity (TEAC) assay. The results of this study showed favourable response of bush tea growth to increased rates of gibberellin application. For growth parameters such as plant height, number of branches, leaf area and fresh biomass, best results were recorded at 3 and 4% gibberellin application rates. Chemical attributes in the form of total polyphenols, and total antioxidants tended to decline with increasing gibberellin application rate. However, tannin content peaked at 2% gibberellin application rate. Overall, gibberellin application improved bush tea growth and chemical attributes.

Key words: Gibberellins, total polyphenols, total tannins, total antioxidants.

INTRODUCTION

Bush tea (*Athrixia phylicoides*DC) is an indigenous tea of South Africa. Nine of the 14 species in the genus *Athrixia* are found in South Africa (Leistner, 2000). Bush tea has been predominantly used for many years for treating boils, cleansing or purifying blood, bad acne, infected wounds and cuts, skin eruption, and for bathing by traditional communities (Roberts, 1990). The usage of *Athrixia* tea has declined over time with the availability of commercially produced teas but the plant is considered to have economic potential as herbal infusion (Mudau et al., 2007). According to the research done on 68 of the 92 people interviewed in rural places in Wolkberg region of the Limpopo Province, who still consume the tea, only 8% used it for medicinal properties (McGaw et al., 2007). In urban surveys conducted in Soweto, Mamelodi and Marabastad (Gauteng Province), 83 out of 150 people who use the plant indicated that they would buy it if it

were available for purchase in stores (McGaw et al., 2007). There is therefore need for development of technologies for large scale production of bush tea if it is to be commercialized.

Gibberellins are a family of endogenous growth regulators in plants that are involved in nearly all stages of plant growth and development (Phillips, 1998). Gibberellins are implicated in germination, leaf expansion, bolting, flower induction, flower development, seed set and fruit development (Davies, 1995). Liang et al. (1996) reported gibberellins to be effective in stimulating flushing of the tea plant (*Cammellia sinensis*) and increasing tea leaf yield. Kagira (1975) reported that gibberellic acid stimulated auxillary bud activity, inhibited extension growth of the apical meristems and had an effect on tea leaf chemical composition and quality. Liang et al. (1996) also reported that application of gibberellins was beneficial to green tea quality. They also reported that application of gibberellins improved the content of amino acids, vitamin C and tea catechin index (flavanols and flavanolgallates). These authors also reported that gibberellins reduced the content of tea polyphenols and

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the ratio of tea polyphenols to amino acids. In herbal teas, quality is best determined by the presence of certain chemical compounds (polyphenols and tannins) in the leaves.

Polyphenols have been shown to possess a wide range of biological and pharmaceutical benefits including intestinal and breast cancer prevention (Celestino and Augustin, 2000). Chemicals such as polyphenols and tannins are known to be potential indicators of quality as they are antioxidant in nature. The quality as determined by these and other compounds is important as they are likely to determine the ultimate price of bush tea when it becomes commercialized (Mudau et al., 2007).

Information on the effect of gibberellins on growth and chemical composition of bush tea is lacking. Therefore, the objective of this study was to investigate the effects of gibberellins on sprouting of bush tea cuttings and quality of bush tea.

MATERIALS AND METHODS

The experimental site and planting materials

The trial was conducted at Madzivhandila College of Agriculture. The planting materials for the experiment, made up of mature bush tea stock plants, were collected from Mudzidzidzi village, next to Tshatshingo Potholes in November 2007 and planted at Madzivhandila College in a 40% shaded nursery. Selection of the planting materials was made on the basis of true-to-name and type, free of disease and insect damage, and in good physiological state. During cultivation, to stimulate rapid and prolific rooting of cuttings, plants were cut to about 7 to 8 cm long and were treated with 0.3% IBA (Seradix No.2) (Bayer Pretoria, South Africa) and planted in seedling trays on a mist bed, supplied with a misting system operating through misting nozzles. The mist bed was 3 m long, 1.5 m wide and 0.5 m high. Irrigation was done 3 times a day except on rainy days.

Rooted cuttings (seedlings) were ready and were transplanted directly into 20 bags after two and half months. The medium used during transplanting was pine bark and sand in a ratio of 2:1, respectively. In an attempt to achieve optimum growth, the growing bush tea plants in plastic bags were treated with N, P and K at rates of 300, 300 and 200 kg/ha, respectively (Mudau et al., 2007) two weeks after transplanting.

Treatments and experimental design

The experiment was conducted under a 40% shade net in a randomized complete block design (RCBD) with light/shading as a gradient. Treatments consisted of gibberellins (Progibb 40%) applied at various rates as follows: 0, 1, 2, 3 and 4% and were effected on 18 February, 2008. Treatments were applied until run-off and the experiment was harvested for analysis three months after transplanting.

Data collection and analysis

Growth parameters collected and recorded were plant height, number of branches, biomass, as well as leaf area. Total polyphenols, tannins and total antioxidants were analyzed.

Determination of total polyphenol content

Methanol was used as the extraction solvent for the determination of total polyphenols. Duplicates of 2 g of tea were extracted using 40 ml of the solvent as follows: an amount of 20 ml of methanol was added to 2 g of sample in centrifuge tubes and the sample were vortexed and mixed every 10 min for 2 h to improve extraction efficiency. The samples were then centrifuged at 3500 rpm for 10 min (25°C) using centrifuged tubes and decanted. Each sample residue was rinsed once with 20 ml of solvent, vortexed and mixed for 5 min, centrifuged as earlier mentioned and decanted. Two supernatants were combined and used for analysis. The Folin Ciocalteau method (Singleton and Rossi, 1965), modified by Waterman and Mole (1994), was used to determine total polyphenols in black tea extracts. This method is based on the reducing power of phenolic hydroxyl groups (Hahn et al., 1984) which react with the phenol reagent to form chromogens that can be detected spectrophotometrically. In brief, methanol extract (0.5 ml) was added to a 50 ml volumetric flask containing distilled water and mixed. Folin Ciocalteau phenol reagent (2.5 ml) was then added and mixed with methanol and water, followed by 7.5 ml sodium carbonate solution (20 g/100 ml) within 1 to 8 min after the addition of the Folin Ciocalteau phenol reagent. The contents were mixed and the flask was made up to volume with distilled water and thoroughly mixed. Absorbance of the reactants was read after 2 h at 760 nm using UV-visible Genesys 20 Spectrophotometer (ThermoFisher, Maryland, USA). Catechin was used as standard to prepare a standard curve and results were expressed as mg equivalents/100 mg of samples on dry mass basis.

Determination of tannins

The vanillin HCl method of Prince et al. (1978) was used for the determination of tannins. This method is based on the ability of flavonoids to react with vanillin in the presence of mineral acids to produce a red colour that is measured spectrophotometrically. The extracts and reagents were maintained at 30°C in a thermostat-controlled water bath before mixing the reactants. The methanolic extract (1 ml) was added to 5 ml vanillin reagent (4% HCl in methanol and 0.5 ml vanillin in methanol) and mixed. Sample blanks were done with 4% HCl in methanol, replacing vanillin reagent. The reactants were maintained at 30°C and absorbance read at 500 nm after 20 min. Absorbance reading of the blanks was subtracted from those of the samples. Catechin was used as a standard and results were expressed as mg catechin equivalents/100 mg sample on dry mass basis.

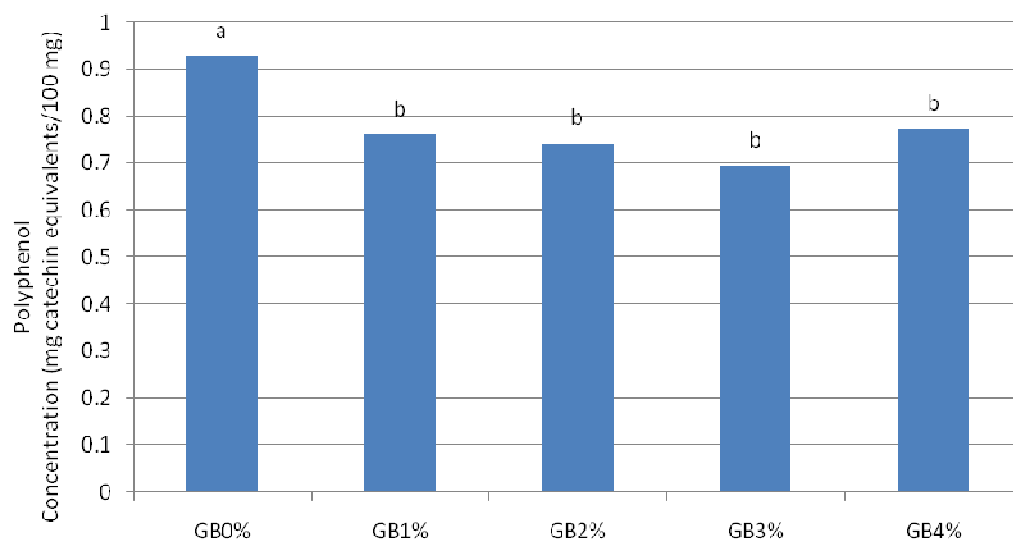
Determination of antioxidants activity

Antioxidant activity of the extracts was determined using trolox equivalent antioxidant capacity (TEAC) assay as described by Awika et al. (2004). TEAC is a spectrophotometric technique that measures the relative ability of hydrogen-donating antioxidants to scavenge the ABTS⁺ radical cation chromogen in relation to that of trolox, the water soluble vitamin E analogue which is used as an antioxidant standard. The ABTS⁺ was produced by mixing equal volume of 8 mM ABTS with 3 mM potassium persulfates prepared in distilled water and allowed to react in the dark for at least 12 h at room temperature before use. The ABTS⁺ solution was diluted with a phosphate buffer solution (pH 7.4) prepared by mixing 0.2 M of NaH₂PO₄, 0.2 M NaHPO₄ and 150 mM NaCl in 1 L of distilled water, with pH adjustment using NaOH where necessary. This solution was made fresh for each analysis. The ABTS⁺ solution (2900 µl) was added to the methanol extracts of tea (100 µl) in a test tube and mixed. Absorbance readings (at 734 nm) were taken after 30 min (for the samples) and 15 min (for the standard) of the initial

Table 1. Growth characteristics of bush tea in response to different rates of gibberellin application.

Applied gibberellin (%)	Plant height (cm)	Number of branch/plant	Leaf area/plant (cm ²)	Fresh biomass/plant (g)	Dry biomass/plant (g)
0	32.5±2.35 ^b	26.2±9.47 ^c	261.8±22.96 ^{ab}	26±3.95 ^{ab}	10±5.11 ^a
1	36.99±2.35 ^{ab}	63.0±9.47 ^{ab}	231.4±22.96 ^{abc}	23.27±3.95 ^{ab}	19.91±5.11 ^a
2	31.6±2.35 ^b	36.5±9.47 ^{bc}	222.6±22.96 ^{bc}	16.887±3.95 ^b	7.19 5.11 ^a
3	40.03±2.35 ^a	57.4±9.47 ^{ab}	293.1±22.96 ^a	30.361±3.95 ^a	14.157±5.11 ^a
4	39.32±2.35 ^a	66.8±9.47 ^a	186.2±22.96 ^c	29.9±3.95 ^a	13.091±5.11 ^a

*Means followed by the same letters in the same column are not significantly different at 5% probability level.

**Figure 1.** Total polyphenol concentrations of bush tea at different rates of gibberellins application.

*Means denoted by the same letter are not significantly different at the 5% level probability.

mixing of the samples and standard, respectively. The results were expressed as μM trolox equivalents/g of sample on dry mass basis.

Statistical analysis

Data was subjected to analysis of variance (ANOVA) using PROC GLM (General linear model) procedure of SAS version 8.0. (SAS Institute Inc., 1999).

RESULTS AND DISCUSSION

Effect of gibberellins on sprouting (growth) of bush tea cuttings

Results in Table 1 shows that there was no linear or quadratic response on plant height, number of branches, leaf area, fresh and dry biomass after the application of gibberellins at different rates. However, there was a tendency of high concentration of gibberellins at 3% to

increase plant height and fresh biomass. The number of branches also increased at 4% gibberellins. The leaf area increased on application of 3% gibberellin concentration. Increasing rates of gibberellins application enhanced plant growth as shown by an increase in the quantity of many growth parameters measured. For plant height, number of branches, leaf area and fresh biomass, most increments were recorded at 3 and 4% application rates. Similar results were reported by Philips (1998) and Liang et al. (1996) who reported gibberellins to be effective in promoting leaf expansion and stimulating flushing of tea plant and increasing tea leaf yield, respectively.

The effect of gibberellins on quality of bush tea

Gibberellin application decreased the concentration of total polyphenols as compared to zero application of gibberellins (Figure 1). The highest concentration at 0% application rate was 0.928 mg catechin equivalents/100

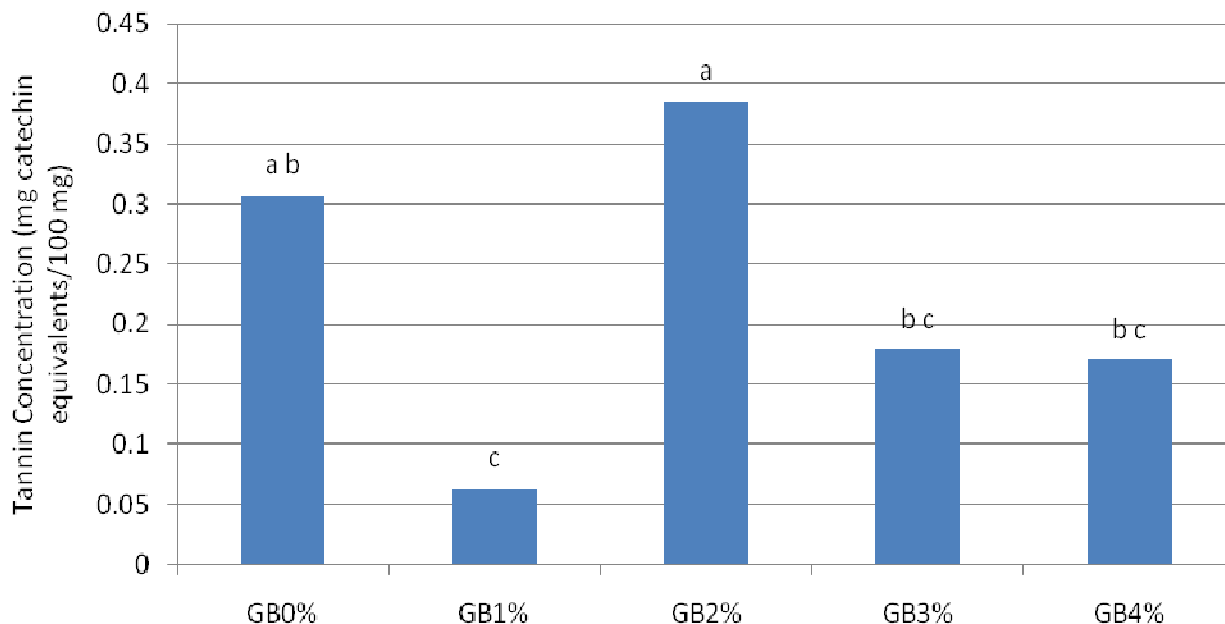


Figure 2. Tannin concentrations of bush tea at different rates of gibberellin application. *Means denoted by the same letter are not significantly different at the 5% level probability.

mg, making the difference between the highest total polyphenol concentration at 0% rate and the lowest total polyphenol concentration at 3% application rate to be 0.233 mg catechin equivalents/100 mg.

A decrease in tea polyphenols was also reported by Liang et al. (1996) who showed a significant effect of gibberellins on *Camellia sinensis* by improving content of amino acids, vitamin C and tea catechin index, and reducing the content of tea polyphenols. Their findings suggested that gibberellins stimulated the synthesis and accumulation of amino acids but inhibited accumulation of polyphenols and caffeine in tea plants. In this study, the application of gibberellins suppressed the positive health benefits of reducing the incidence of skin, lung, stomach and liver cancer that are associated with the presence of polyphenols in bush tea. High polyphenol content is associated with good quality of tea.

Tannin concentration showed neither quadratic nor linear response to gibberellins application as reflected in Figure 2. The highest concentration (0.385 mg catechin equivalents/100 mg) was realized at 2% application rate and the lowest concentration (0.0627 mg catechin equivalents/100 mg) at 1% gibberellin application rate. The difference in tannin concentration between 2 and 1% gibberellin application rates was 0.322 mg catechin equivalents/100 mg. Thus, application of 2% gibberellins had potential to produce bush tea with bitter taste and astringent flavor (tannin distinctiveness), whilst higher rate reduced this quality parameter.

Figure 3 shows that application of gibberellins increased total antioxidants as compared to zero gibberellin application (GB0%). The lowest concentration was 0.419

μM trolox equivalents/g at GB 0% application rate and the highest content was 24.45 μM trolox equivalents/g at GB 1% application rate. Difference in total antioxidant content between the highest and lowest was 24.031 μM trolox equivalents/g

The results from this study have shown gibberellin application to have a favourable effect on growth of bush tea, with 3 and 4% showing the best favourable results. The results from this study also indicated a declining total polyphenol and antioxidant content with increasing gibberellin application rate, while tannins peaked at 2% application rate.

Viable bush tea commercialization will require that bush tea be cultivated or grown in a large scale. Only bush tea grown on a large scale will guarantee the availability of the plant with consistency in quality (Mudau et al., 2007). While many trials on agronomic practices and bioactivity were conducted in a nursery setup, it is recommended that in future, this trial should be reciprocated on a field setup. This will help assess the performance of bush tea, in response to application of the plant growth regulator, in a field environment, and if successful will guarantee the large scale production of tea imperative to commercialization of bush tea.

Conclusion

The experimental results have generally shown favourable response of bush tea growth to increased rates of gibberellin application. For most of the growth parameters including plant height, number of branches, leaf area and

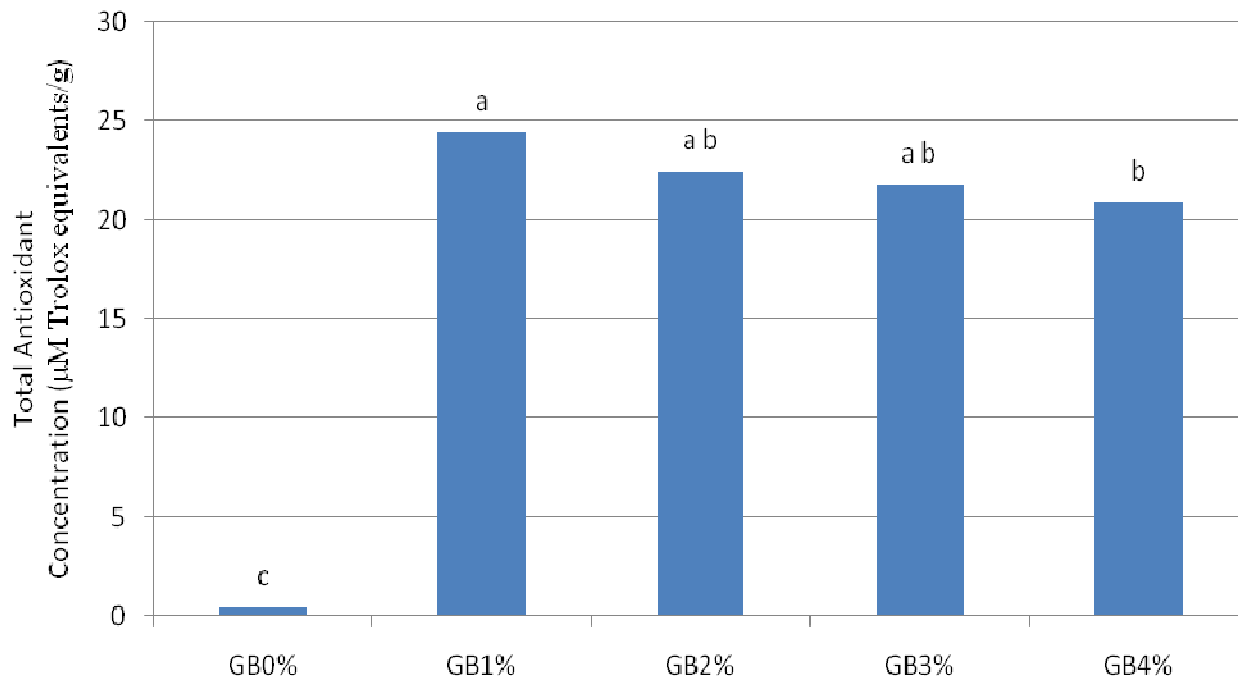


Figure 3. Total antioxidant concentrations of bush tea at different rates of gibberellins application. *Means denoted by the same letter are not significantly different at the 5% level probability.

fresh biomass, best results were recorded at 3 and 4% gibberellin application rates.

Chemical attributes in the form of total polyphenols and total antioxidants however, tended to decline with increasing gibberellin application rate. Tannin content however tended to peak at 2% gibberellin application rate. Future trials could attempt to determine the appropriate timing and rates of application of gibberellins to bush tea.

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