African Journal of Plant Science Vol. 5 (4), pp. 268-272, April 2011
Available online at http://www.academicjournals.org/ajps
ISSN 1996-0824 ©2011 Academic Journals

Full Length Research Paper

Effect of benzyladenine (BA) on fruit set and mineral nutrition of morula (*Sclerocarya birrea* subspecies *caffra*)

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Accepted 23 February, 2011

A field experiment was conducted to evaluate the effects of benzyladenine (BA) on fruit set and mineral nutrition of morula (*Sclerocarya birrea* subspecies *caffra*). Benzyladenine was applied when the fruitlets were 8 to 10 mm in diameter at concentrations of 0, 50, 100 or 150 mg/l. Application of BA to morula fruit trees significantly ($p < 0.001$) reduced fruit set of morula trees by between 48 to 67%. Morula tree sprayed with BA had significantly higher leaf and fruit mineral content than control trees. The fruit calcium (Ca) ($p < 0.05$), magnesium (Mg) ($p < 0.05$), nitrogen (N) ($p < 0.01$) and potassium (K) ($p < 0.001$) increased significantly with increasing BA concentrations. The results showed that BA can be used as a chemical agent to reduce crop load and improve the morula tree mineral uptake and mineral partitioning to the leaves and fruits.

Key words: *Sclerocarya birrea* subspecies *caffra*, benzyladenine, fruit set, mineral content.

INTRODUCTION

Morula (*Sclerocarya birrea* subspecies *caffra*) is a drought tolerant, deciduous tree. It belongs to the family anacardiaceae as the mango (*Mangifera indica*), cashew nut (*Anarcardium occidentale)* and pistachio nut (Roodt, 1998; Van-Wyk et al., 1997). The name *Sclerocarya* is derived from a greek word *"skleros"* for hard and "*karyon*" for nut or kernel in illusion to a large, woody kernel of the fruit (Roodt, 1998). Morula is widely distributed in the African continent from Senegal to Sudan, Ethiopia, Swaziland and Southern Africa; where caffra species is dominantly found (Lost Crops of Africa, 2010). This tree has been introduced in Mauritius, Reunion, Australia, India, Oman and it is grown as an experimental crop in Israel (Hall, 2002). In the African continent, morula plays an important socio-economic role. It is a multipurpose tree with highly nutritive fruits which can be consumed fresh or commercially processed (Venter and Venter, 1996; Van-Wyk et al., 1997). The commercial products of morula have been marketed in Southern Africa over the last 20 years (Hall, 2002). This includes popular morula liquer-Amarula from South Africa which is marketed internationally, 'Marulaan' wine in Zambia (Leakey, 1999) and pasteurised juice in Botswana (Taylor and Kwerepe, 1995). In Namibia morula oil is extracted and exported as a cosmetic product (Hall, 2002).

The aim of fruit production is to attain commercially marketable fruits of a normal size, highly nutritive value, desirable flavour and appearance. This is achieved through manipulation of fruiting behaviour (Jackson, 1985) of which includes management practices such as pruning and fruit thinning (hand thinning, mechanical thinning and chemical thinning). Fruit thinning provides a more flexible technique for adjusting the actual fruit load thereby reducing the within fruit competition for carbohydrates hence improving the fruit quality (Horscroft and Sharples, 1987; Byers and Carbaugh, 1991). Chemical thinning is the common technique mostly used by commercial farmers, it involves use of chemical agents to remove part of the crop (flowers/fruitlets). These compounds are mostly hormone type materials such as naphthaleneacetic acid (NAA) and

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naphthaleneacetamide (NAAm) [auxin], benzyladenine (BA) [cytokinin], ethephon [ethylene] (Edgerton, 1981). BA is a synthetic cytokinnin which has the ability to hasten cell division, promote carbohydrate metabolism and create new source-sink relationships hence leading to increased sink strength and fruit size at harvest (Dyer 1990; Emongor and Murr, 2001).

Almost half of the 300 useful plants in Southern Africa are food plants and only a few have been explored for their commercial use, yet they are the most resources which can improve sustainable agriculture (FAO, 2002). Morula is one of the most important food plants that require be well utilizing and conserving as it provides diverse benefits to most African households at subsistence and commercial level. The morula fruit as the main product has been used as dietary food product for over 10000 years (Hall, 2002). The tree is exceptionally drought resistant, it sets too many fruits and this leads to a heavy crop load per cluster (Fox and Norwood, 1982; Booyers, 1996). Thus in a heavy fruiting season, a single morula tree can produce between 21000 and 91000 fruits (Welford, 2008). The heavy fruit set results in low quality fruits in terms of size, mineral content, colour and soluble solids content (Faust, 1989). It is therefore ideal for researchers to discover technologies that can reduce morula crop load and improve fruit quality and mineral nutrition of morula fruits desirable for commercial marketing both locally and internationally. BA is chemical thinning agent mainly used in fruit production for improving fruit quality mostly through increased cell division and enlargement (Stoppa, 1999). The objective of this study was to evaluate the effect of BA on fruit set and mineral nutrition of morula (*Sclerocarya birrea* subspecies *caffra*).

MATERIALS AND METHODS

Experimental site

A field experiment was conducted at veld product research and development site at Gabane in Botswana, at an altitude of 1110 m above sea level, from August 2008 to May 2009. Soils are shallow sands with poor phosphorus values for most agricultural crops (De Wilt and Nachtengaele, 1996). Mean annual rainfall is 500 mm but erratically distributed (Campbell, 1990). The orchard was planted in January 1995 on rootstocks of superior phenotypes of morula selected from different areas in Botswana.

Experimental design

Single, whole tree plots were used in all trials in randomized complete block design with six replications. The treatments were 0, 50, 100 or 150 mg/L of BA.

Cultural practices

Clearing and pruning of trees was done, random tree selection was then carried out and tree trunk circumference recorded at 10 cm above graft union. Before full bloom, 2 limbs per tree, 10 to15 cm in circumference (measured at 5 cm from the crotch angle), were selected and tagged, circumference recorded and blossom clusters counted. The selected limbs were from opposite sides of the tree and met the following criteria: well exposed to sunlight; free from severe or unusual pruning cuts; and bloom representative of the entire tree. Full bloom was considered to be a day when 80% of spur bloom flowers on 3 to 4 year old wood are open on the north side of the tree and bees are active. BA in the form of Maxcel^{R} (Vaalent Biosciences USA) was applied to the whole tree as dilute spray to runoff with a pressurized knapsack sprayer at 0, 50, 100, or 150 mg/L BA [Valent Biosciences USA; liquid concentrate containing 19 g a.i/L (w/w) benzyladenine]. Control trees of morula were sprayed with tap water.

Fruit set determination

After natural drop was completed (January), all fruit persisting on two tagged limbs per tree were counted using a counter. Fruit number per centimeter limb circumference was calculated.

Mineral analysis

Midshoot leaves (leaf 5 and 6) from current season growth were collected in the first week of January 2009. The samples were oven-dried at 66°C to constant weight (72 h). Seven days before commercial harvest time, 10 representative fruits per tree were also harvested for mineral determination. Fruit epidermis and mesocarp were cut finely from 10 samples per tree and the mixture oven dried at 66°C to constant weight for three days. The dried samples were ground using a sieve of size 2 and 1.25 g composite sample digested in 20 ml sulphuric acid (98%) and 4 ml hydrogen peroxide (30%) in a BD block at 330°C for 7 h.

Nitrogen (N) was determined through distillation and titration using the micro kjeldahl method (AOAC, 1990). Phosphorus (P) was determined calorimetrically using sodium phenol and ammonium molybdate plus ascorbic acid method (AOAC, 1990). The absorbance was read on the UV Visible Spectrophotometer (Model of spectrophotometer). Calcium (Ca), magnesium (mg) and potassium (K) were determined by atomic absorption spectrophotometry (Varian SpectrAA 300). Data was expressed as total mineral content in mg/g on dry weight basis.

Statistical analysis

Analysis of variance was performed on the data collected using general linear model (PROC GLM) procedure of Statistical Analysis System (SAS, 2009, Carey, NC) program package. Appropriate regression models were used to examine the response of morula fruits to increasing benzyladenine concentration. Multiple comparisons among means was done using protected least significant difference (LSD) at P = 0.05. Proc univariate procedure was carried out on residuals to support assumptions of normality made.

RESULTS

Fruit set

Benzyladenine application significantly ($p < 0.0001$) reduced fruit set of morula compared to the control trees. Fruit set decreased with increasing BA concentrations

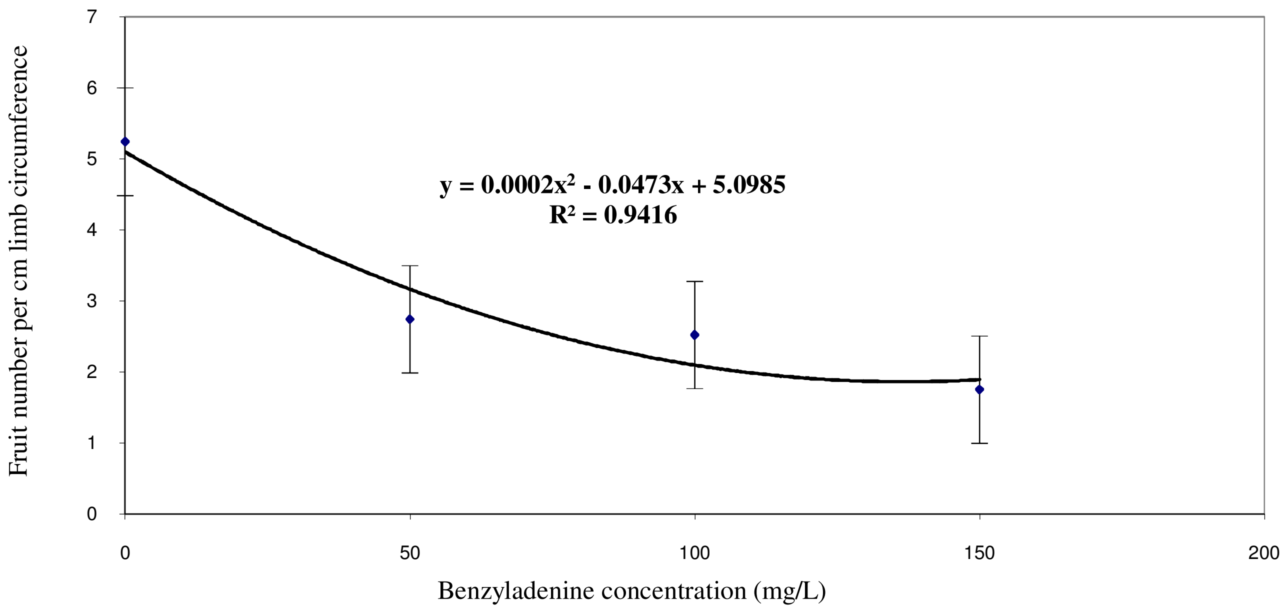

Figure 1. Effect of benzyladenine on fruit set of morula; I represent standard error bars.

and the response was quadratic (Figure 1). The reduction in fruit set by BA ranged between 48 to 67%. However, there was no significant difference between 50, 100 and 150 mg/L BA in their ability to reduce fruit set of morula trees (Figure 1).

Fruit and leaf mineral content

Application of benzyladenine at 50, 100 or 150 mg/L to morula trees significantly increased the morula fruit mineral content compared to the control trees (Table 1). The fruit calcium (Ca) ($p < 0.001$), magnesium (Mg) ($p < 0.05$), nitrogen (N) ($p < 0.01$) and potassium (K) ($p < 0.001$) were significantly increased with increasing BA concentration reaching the highest mineral content at 150 mg/l BA (Table 1). There were no significant differences in fruit P, Na, Ca, Mg, N and K content from trees sprayed with either 100 or 150 mg/L BA (Table 1). With respect to fruit P, K and Na morula content, there were no significant differences among the BA concentrations in their ability to influence P, K and Na translocation to the fruit (Table 1). In comparison to other minerals calcium had the highest response to BA applications with fruit content between 10 to 23% compared to the control, thus the overall increase of 13% and potassium had the lowest response compared to other mineral elements ranging from 11 to 13% thus an overall increase of 3% (Table 1). The percentage increase of phosphorus was between 15 to 24%, while Na percentage increase was between 10 to 20% (Table 1).

Similar to fruit mineral content, BA application to morula trees increased the leaf mineral content (Table 2). Application of 150 mg/L BA significantly increased the morula tree mineral uptake of P, Na, Ca, Mg, N and K compared to control trees (Table 2). However, application of 50 or 100 mg/L BA did not significantly increase the leaf content of P, Na, N and K compared to control (Table 2). There were no significant differences with respect to leaf P, Na, N, and K contents in trees sprayed with either 50 or 100 mg/L BA (Table 2). With respect to leaf Ca and Mg content, application of 100 mg/L BA increased significantly their contents compared to leaf content in the control trees (Table 2).

DISCUSSION

Fruit set

Based on the results, BA reduced the fruit set of morula fruit trees and the response was quadratic. However, there was no significant difference between 100 and 150 mg/L BA in their ability to reduce crop load or fruit set. Similar results were reported by Emongor (1995), in Empire apples where application of 50, 100 and 200 mg/L BA significantly reduced fruit set with a quadratic response. Elfving and Cline (1993) using several apple cultivars reported a linear relationship between BA concentration and reduction of fruit set. BA has been reported to be effective in thinning fruit trees and its effectiveness varies among different species/cultivars, fruitlet size, time of application and application rates (Greene and Autio, 1989; Greene et al., 1992; Elfving and Cline, 1993; Ferree, 1996, Emongor and Murr, 2001). It has been reported that in some apple cultivars,

Table 1. Morula fruit mineral content (mg/g) as affected by benzyladenine.

BA (mg/L)	Phosphorus (mg/g)	Sodium (mg/g)	Calcium (mg/g)	Magnesium (mg/g)	Nitrogen (mg/g)	Potassium (mg/g)
0	0.47b	0.90b	1.20b	1.18b	3.55b	10.29b
50	0.55ab	1.00ab	1.35b	1.31ab	3.75b	11.58a
100	0.62a	1.12a	1.59a	1.48a	3.77b	11.61a
150	0.57a	1.05a	1.64a	1.54a	4.21a	11.79a
Significance	**	**	***	*	**	***
LSD	0.1	0.14	0.23	0.27	0.35	0.29

Significance: * 0.05, ** 0.01, *** 0.001, **** 0.0001. Means with the same letter are not significantly different from each other.

Table 2. Morula leaf mineral content (mg/g) as affected by benzyladenine.

Benzyladenine (mg/L)	Phosphorus (mg/g)	Sodium (mg/g)	Calcium (mg/g)	Magnesium (mg/g)	Nitrogen (mg/g)	Potassium (mg/g)
0	0.38b	0.96b	1.76b	3.22c	3.80b	7.22b
50	0.45b	1.04b	2.07b	3.41bc	3.84b	7.40b
100	0.52b	1.22b	2.52a	3.67ab	3.85b	8.83b
150	0.78a	1.67a	2.89a	3.94a	4.17a	16.00a
Significance	**	***	****	**	*	****
LSD	0.23	0.33	0.4	0.44	0.29	3.4

Significance: * 0.05, ** 0.01, *** 0.001, **** 0.0001 .Means with the same letter are not significantly different from each other.

BA effectively thins fruits at the concentration of 50 to 150 mg/L while at concentrations above 150 mg/L it may not thin or may over thin them (Elfving and Cline, 1993; Emongor, 1995; Ferree, 1996; Emongor and Murr, 2001).

The reduction of fruit set may be induced by the increase in ethylene production in the BA-treated fruitlets and leaves (Greene et al., 1992; Emongor and Murr, 2001; Stopar, 2002). Ethylene releasing compounds have been shown to be very effective in the formation of abscission layer in fruits such as cherries, apples, walnuts, macadamia nuts and tangerines (Arteca, 1996). This may explain morula fruitlet abscission and dropping a few days after BA application. The formation of the abscission layer can also be due to fruitlet sensitivity to ethylene, interference of BA with the synthesis, transport or action of auxins and thereby leading to the abscission of fruitlets (Emongor and Murr, 1999).

Mineral nutrition

Generally fruiting reduces growth of all parts of the plant but with a greater effect on the roots (Forshey, 1986). This reduces development, strength and activity of the roots and hence reducing the supply of reserves and essential growth regulators such as water, plant growth regulators (since others such as cytokinins are synthesized in the roots) and the mineral elements. Due to enough supply of assimilates in response to BA application, the root activity was improved and hence promoting the translocation of minerals to the sinks (fruits and leaves) and other growth regulators. This correlates well with the current results of this study in which BA significantly increased the mineral content of morula leaves and fruits compared to the control (untreated) trees.

In some trees especially heavy fruiting cultivars, it has been found that fruiting reduces the root growth, development and activity which affect the mineral nutrient uptake (Wright, 1985). This implies that with a reduced crop load the root activity was increased, and thereby enhancing their ability to transport minerals to the sinks. Because the fruits are stronger sinks than the leaves, the results of the current study showed that they had a greater amount of total minerals compared to the leaves. Apart from the reduction in fruit set, BA also increased mineral nutrient mobilization, a phenomenon known as the cytokinin-induced nutrient mobilization (Taiz and Zeiger, 2002). The nutrients will move from the site of production and/or storage (roots and/or leaves) to the site of utilization (leaves/ fruits).

The effect of BA on the increased potassium mineral content in both the leaves and fruits may have influenced the increased uptake of other minerals (N, P, Mg, Na, Ca). Potassium plays an important role in osmo-regulation and translocation of nutrients into plant cells

(Atwell et al., 1999). The uptake of minerals through the transpiration stream is driven by diffusion/ mass flow through the osmotic gradient, thus water availability in the transpiration stream is a major drive for mineral translocation. Calcium also plays a role in the uptake of nitrogen and other minerals, due to their role in the osmotic balance. The increased phosphorus content might have also increased uptake of other minerals because through cell division, phosporus stimulates the formation and growth of roots (Marschner, 1995). The rate of root elongation and surface area of the roots are important factors for root activity because larger surface area is vital for mineral uptake from the soil and their translocation to the leaves and fruits. Phosphorus might have improved the ability of plants to absorb water and nutrients.

Conclusions

Application of BA to morula trees decreased fruit set and increased mineral nutrition of both fruits and leaves of morula. This suggests that BA can be used as a chemical thinning agent to manipulate fruiting behavior of morula fruit trees to reduce crop load and improve the fruit quality in terms of mineral nutrition of both the leaves and fruits.

ACKNOWLEDGEMENTS

Special thanks are conveyed to my mother Violet. M. Moatshe for the financial support, my brother William and uncle Francis Moatshe for being my research assistants, Botswana College of Agriculture, University of Botswana and Veld Products Research and Development for use of their facilities.

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