EFFECTS OF MACRONUTRIENT DEFICIENCIES ON THE GROWTH AND VIGOUR OF *KHAYA IVORENSIS* SEEDLINGS

V Jeyanny*, AG Ab Rasip, K Wan Rasidah & Y Ahmad Zuhaidi

Forest Research Institute Malaysia, 52109 Kepong, Selangor Darul Ehsan, Malaysia

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JEYANNY V, AB RASIP AG, WAN RASIDAH K & AHMAD ZUHAIDI Y. 2009. Effects of macronutrient deficiencies on the growth and vigour of *Khaya ivorensis* seedlings. Nutrient deficiency may adversely affect the growth and quality of seedlings in forest nurseries. The objectives of this study were to determine the initial effects of macronutrient deficiency on the growth and vigour of *Khaya ivorensis* seedlings. One-month-old *K. ivorensis* seedlings were transplanted into plastic pots filled with acid washed sand. Six treatments were assigned to the seedlings—one control (T1) containing complete nutrient solution, and five treatments T2–T6, each lacking one of the following macronutrients N, P, K, Ca and Mg respectively. Watering with 100 ml of the specified nutrient solution or distilled water was done on alternate days. All treatments comprising eight replications were laid out in a complete randomized design. The visual deficiency symptoms and seedling growth were recorded monthly. Plant tissue nutrient content and dry weight were determined six months after transplanting. All treatments except for control induced deficiency symptoms. Macronutrient deficiencies induced visual deficiency symptoms, changes in seedling growth and tissue nutrient concentration. Lack of Mg and K adversely affected the tissue nutrient content of the seedlings.

Keywords: Nursery, insufficient nutrient, symptoms, seedling growth, tissue content

JEYANNY V, AB RASIP AG, WAN RASIDAH K & AHMAD ZUHAIDI Y. 2009. Kesan kekurangan makronutrien terhadap pertumbuhan dan kesuburan anak benih *Khaya ivorensis*. Kekurangan nutrien mungkin mendatangkan kesan yang buruk pada pertumbuhan dan kualiti anak benih di tapak semaian hutan. Kajian ini bertujuan untuk menentukan kesan awal kekurangan makronutrien terhadap pertumbuhan dan kesuburan anak benih *Khaya ivorensis*. Anak benih *K. ivorensis* berusia satu bulan ditanam di dalam pasu plastik mengandungi pasir yang dibersihkan dengan asid. Enam rawatan diberi kepada anak benih—kawalan (T1) yang mengandungi larutan nutrien yang lengkap, dan lima rawatan yang lain, T2–T6, iaitu setiap satu rawatan masing-masing kekurangan satu makronutrien sama ada N, P, K, Ca atau Mg. Penyiraman dengan 100 ml larutan nutrien spesifik atau 100 ml air suling dijalankan pada selang setiap hari. Setiap rawatan mempunyai lapan ulangan dan disusun mengikut reka bentuk rawak lengkap. Gejala kekurangan yang dapat dilihat dan pertumbuhan anak benih direkod setiap bulan. Kandungan nutrien tisu tumbuhan dan berat kering ditentukan enam bulan selepas penanaman. Semua rawatan kecuali kawalan menunjukkan gejala kekurangan. Kekurangan makronutrien menunjukkan gejala kekurangan, perubahan dalam pertumbuhan anak benih dan perubahan dalam kandungan nutrien tisu. Kekurangan Mg dan K mendatangkan kesan yang buruk pada kandungan nutrien tisu anak benih.

INTRODUCTION

Reforestation by establishing forest plantations in Malaysia was undertaken to compensate the dwindling timber source from natural forests. Shortage of timber supply and the long wait for yield had initiated the search for potential fast-growing species. Several exotic species such as *Acacia mangium*, *Paraserianthes falcataria* and *Khaya ivorensis* were introduced for the establishment of short-rotation forest plantation (MTIB 2007). *Khaya ivorensis* (African mahogany) was introduced in Malaysia in the 1950s (Ahmad Zuhaidi *et al.* 2006) and it has adapted well to the local climatic conditions. This exotic and yet promising species from the Meliaceae family is highly sought after by the furniture industries for producing panels, cabinets, superior joinery and other decorative works (Ahmad Zuhaidi *et al.* 1999). In its native land Cote de' Ivoire, Africa, it

* E-mail: jeyanny@frim.gov.my

is used in anti-malaria treatments (Agbedahunsi *et al.* 2004). *Khaya ivorensis* wood is characterized as medium hardwood with air-dry density of 560 kg m^{-3}, and was reported as having a market value of between 425 and USD570 tonne^{-1} (Ahmad Zuhaidi *et al.* 2006). Although studies of nutrient translocations (Kadir *et al.* 1998) and significant growth at different localities (Ahmad Zuhaidi *et al.* 1999) have been reported, studies on macronutrient requirements in the nursery stage are still limited. The importance of establishing good stands of forest tree species nurtured from the seedling stage is becoming apparent in recent years (Kumar 1999, Xu & Timmer 1999, Ong *et al.* 2002).

The present study was done to determine the effects of macronutrient deficiencies on the growth and vigour of *K. ivorensis* seedlings and to identify deficiency symptoms.

MATERIALS AND METHODS

The study was conducted for a period of six months in a greenhouse at the Forest Research Institute Malaysia. The temperature of the greenhouse ranged from 29 to 32 °C, light intensity at 22% penetration and relative humidity at 77%. Coarse river sand was passed through a 0.6–2 mm sieve to remove debris and large clay particles (Resh 1994). The sand was washed with water, and soaked in 18% hydrochloric acid and 1% oxalic acid for five days to remove organic matter and nutrient residues (Hewitt 1966). The acid washed sand was leached with running tap water and finally rinsed with distilled water and tested for residual acid and chloride before pot preparation. The pH and nutrient concentrations of the river sand are shown in Table 1. The C, N, P, K, Ca and Mg content of the acid washed sand were lower than 0.5% and suitable for treatment applications. The washed river sand was transferred to 15 cm diameter × 23 cm high plastic experimental containers of 0.4 m^3 volume for treatment application. One-month-old healthy seedlings of *K. ivorensis* with uniform height and stem diameter were transferred to experimental containers. Seedlings were supplied with Hoagland No. 2 solution for 10 days before treatment application. The chemical composition of macro- and micronutrients of Hoagland No. 2 solution are shown in Tables 2 and 3. The six treatments used were as follows:

(1) Complete Hoagland No. 2 nutrient solution (control) [T1]
(2) Nutrient solution lacking N (T2)
(3) Nutrient solution lacking P (T3)
(4) Nutrient solution lacking K (T4)
(5) Nutrient solution lacking calcium Ca (T5)
(6) Nutrient solution lacking Mg (T6)

Each treatment had eight replications and all pots were laid out in a complete randomized design. The visual deficiency symptoms were recorded based on unusual growth patterns and coloration. Measurements of seedling height, stem diameter and number of leaves were made once every month. However, results obtained on the first (initial stage) and sixth month (final stage) after transplanting were reported. The dry weight of seedlings was measured at the end of the experiment. Foliar analysis of N, P, K, Ca and Mg were carried out based on standard soil chemistry laboratory practice. Leaves were dried at 70 °C for 48 hours (Quoreshi & Timmer 2000) and ground to fine powder before analysis. Foliar total nitrogen was determined by dry combustion method (Carter & Gregorich 2008), and total P, K, Ca and Mg contents were determined using the dry ashing and nitric acid digestion method (Yeoh 1975), followed by determination of element concentration on inductively coupled plasma (ICP) spectrometer. Log transformation was done for count data of leaves before analysis. All other variables except for deficiency symptoms were analysed using unpaired *t*-test to determine the effects of treatments (T2–T6) against control

Table 1 pH and nutrient concentrations of river sand

Treatment	pH	Organic C (%)	N (%)	Avail P (mg kg^{-1})	K (%)	Ca (%)	Mg (%)
Unwashed river sand	7.2	0.22	0.04	1.50	Trace	Trace	Trace
Acid washed sand	7.0	trace	0.02	0.31	Trace	Trace	Trace

Table 2 Chemical composition of 100× strength macronutrient stock solutions

Solution	Source	g l^{-1}
Complete Macronutrient solution	KNO_3	59.1
	$MgSO_4.7H_2O$	49.2
	H_3PO_4	3.8
	$Ca(NO_3)_2$	65.6
Minus N	$MgSO_4.7H_2O$	49.2
	H_3PO_4	3.8
	$CaCl_2.2H_2O$	58.8
Minus P	KNO_3	59.1
	$MgSO_4.7H_2O$	49.2
	$Ca(NO_3)_2$	65.6
Minus K	$Ca(NO_3)_2$	65.6
	H_3PO_4	3.8
	$MgSO_4.7H_2O$	49.2
Minus Ca	KNO_3	59.1
	H_3PO_4	3.8
	$MgSO_4.7H_2O$	49.2
Minus Mg	KNO_3	59.1
	H_3PO_4	3.8
	$Ca(NO_3)_2$	65.6

Table 3 Chemical composition of 1000× strength micronutrient stock solutions

Solution	Source	g l^{-1}
Boron	H_3BO_3	0.28
Manganese	$MnSO_4.H_2O$	1.54
Zinc	$ZnSO_4.7H_2O$	0.22
Copper	$CuSO_4.5H_2O$	0.08
Molybdenum	$(NH_4)_6Mo_7O_{24}.4H_2O$	0.02
Iron	Fe-EDTA	0.01

pots (T1) using SAS System release 8.12 (SAS Institute Inc. 2001). Nutrient solution and water were supplied in the morning and evening for the first 10 days of treatment. All pots were supplied with 100 ml of specified nutrient solution on alternate days. On the other days, 100 ml of distilled water were added (Gopikumar & Varghese 2004). Pest and disease control was performed on all seedlings to keep the seedlings healthy. The pH of control pots was maintained between 5 and 6 and electrical conductivity, (EC) between 2 and 3 mmhos cm^{-1} to facilitate suitable growing conditions for the seedlings.

RESULTS

Visual deficiency symptoms

The seedlings treated with complete Hoagland No. 2 nutrient solution (T1) were healthy and produced dark green normal shaped foliage throughout the experiment. The symptoms of N deficiency (T2) appeared two months after transplanting. During the initial stages, the older leaves changed colour to pale green (Figure 1). Towards the end of the experiment, tissue death was observed on leaf edges.

Initially, mild chlorosis was observed on leaves of T3, which were P deficient. However, leaves were fairly dark green at the end of the six months (Figure 2). Symptoms of K deficiency are shown in Figures 3a and b. Close observations showed yellow patches forming on these leaves two months after transplanting (Figure 3b). Plants which were deprived of Ca (T5) showed necrosis symptoms on young leaves (Figure 4a). The leaves turned pale yellow and severe stunting was observed (Figure 4a). The presence of brown lesions was evident at later stages (Figure 4b). Magnesium deficiency symptoms with interveinal chlorosis were noticed from the second month onwards (Figures 5a and b).

Seedling growth

The treatment effects on the growth of *K. ivorensis* seedlings are shown in Table 4. During the initial period, treatments lacking macronutrients were significantly different compared with the control for seedling growth variables except for number of leaves. Nevertheless, all variables related to seedling growth were affected significantly at the end of the experiment. The number of leaves and dry weight were significantly affected in T2 compared with the control at the final stage. The dry weight of seedlings in T2 was 45% lower than the control (T1) at this stage. The overall seedling growth of T3 was not significantly affected compared with the control. About 30% reduction in stem diameter was observed after one month in the T4 seedlings compared with the control. Seedlings not receiving Ca (T5) grew less in height (19%) and up to 24% less diameter compared with the control at the end of the experiment. In T6, seedling height was the same as the control at the end of the study, while the average stem diameter was reduced to

Figure 1 Symptoms of nitrogen deficiency compared with the control

Figure 2 Symptoms of phosphorus deficiency compared with the control

Figure 3a Symptoms of potassium deficiency compared with the control

Figure 3b Yellow patches in between veins of older leaves on potassium-deficient seedlings

Figure 4a Symptoms of calcium deficiency compared with the control

Figure 4b Brown lesions on calcium-deficient seedlings

Figure 5a Symptoms of magnesium deficiency compared with the control

Figure 5b Interveinal chlorosis on magnesium-deficient seedlings

Table 4 Effects of nutrient deficiencies on *Khaya ivorensis* seedling growth

Growth	Stage	T1 Control	T2 Minus N	T3 Minus P	T4 Minus K	T5 Minus Ca	T6 Minus Mg
Height (cm)	Initial	17.2 (0.61)	18.1 (0.90)	19.0 (0.68)	19.2 (1.16)	21.0** (0.66)	20.5* (1.35)
	Final	31.4 (1.71)	29.5 (1.66)	31.8 (5.63)	29.4 (1.72)	25.4* (1.58)	28.8 (2.28)
Stem diameter (mm)	Initial	2.1 (0.08)	2.1 (0.11)	2.0 (0.15)	1.5** (0.08)	1.8** (0.05)	1.7** (0.09)
	Final	5.8 (0.21)	5.8 (0.22)	5.9 (1.01)	5.2 (0.63)	4.4** (0.36)	4.0** (0.45)
No. of leaves	Initial	2 (0.04)	2 (0.04)	2 (0.04)	2 (0.04)	2 (0.03)	2 (0.05)
	Final	4 (0.16)	3 ** (0.15)	3 (0.55)	4 (0.19)	3 (0.40)	3** (0.33)
Total dry matter yield (g)	Final	12.58 (1.69)	6.93** (0.88)	16.10 (0.89)	14.03 (1.91)	8.34 (2.14)	6.12** (1.11)

Figures in parentheses are standard errors.
* = significant at 0.05 level, ** = significant at 0.01 level

4 mm, a 31% reduction against the control (Table 4). Seedlings not receiving Mg (T6) was also affected, as indicated by the poor leaf growth, which was 25% reduction as compared with the control. This simultaneously led to a reduction in dry weight (51%) compared with the control.

Nutrient concentration

The effects on tissue nutrient concentration of *K. ivorensis* are shown in Table 5. Treatments lacking macronutrients were initially significantly different compared with the control for K, Ca and Mg nutrient concentrations. However, towards the end of the study, N, P, K, and Mg were affected significantly compared with the control. The tissue concentration at the end of the experiment of N, P, K and Mg in T2 were all significantly lower compared with the control. The decrease in N and P alone was 72 and 96% respectively, in contrast with the control. Seedlings lacking P (T3) showed a profound reduction in P concentration (77%) on the 6th month.

Table 5 Effects of nutrient deficiencies on nutrient content of *Khaya ivorensis* seedlings

Nutrient content (mg g^{-1})	Stage	T1 Control	T2 Minus N	T3 Minus P	T4 Minus K	T5 Minus Ca	T6 Minus Mg
N	Initial	23.7 (0.05)	22.2 (0.09)	24.5 (0.02)	23.5 (0.06)	22.3 (0.05)	18.5 (0.21)
	Final	22.3 (0.03)	6.3** (0.07)	13.5** (0.15)	17.6** (0.05)	18.1** (0.11)	17.1** (0.10)
P	Initial	1.2 (0)	1.2 (0.01)	1.2 (0.01)	1.2 (0.01)	1.3 (0)	1.3 (0)
	Final	2.6 (0)	0.1* (0.02)	0.6** (0.01)	3.4** (0.01)	2.9 (0.02)	2.5 (0.05)
K	Initial	24.0 (0.06)	25.3 (0.07)	24.7 (0.06)	21.5* (0.07)	25.5 (0.04)	23.2 (0.10)
	Final	33.2 (0.11)	21.4** (0.09)	26.4** (0.16)	8.3** (0.04)	38.3 (0.29)	26.2* (0.20)
Ca	Initial	3.4 (0.05)	4.3 (0.01)	3.9 (0.01)	5.1* (0.01)	4.9 (0)	3.6 (0.01)
	Final	3.4 (0.19)	0.2 (0)	0.1 (0)	3.3 (0.01)	1.0 (0.01)	6.9 (0.05)
Mg	Initial	4.0 (0.03)	4.3 (0.01)	3.9 (0.01)	5.1* (0.01)	4.9 (0)	3.4 (0.01)
	Final	1.6 (0)	1.3* (0)	1.4 (0)	4.8* (0)	2.6** (0.01)	1.0** (0.01)

Figures in parentheses are standard errors.
* = significant at 0.05 level, ** = significant at 0.01 level

In addition, the final N and K concentrations also decreased compared with the control. T4 showed a reduction in K concentration (10%) compared with T1 during the initial stage. The K concentration further declined four folds compared with the control at the end of the study period. The N, P and Mg tissue concentrations were also statistically affected in T4 compared with the control at the end of the experiment. The level of N was 21% lower than the control for T4. On the other hand, P and Mg concentrations were 31 and 200% higher respectively compared with the control for the same treatment. The seedlings which were deprived of Ca (T5) showed a reduction in N (19%) and an increment of Mg (63%) concentration respectively. The Mg content in T6 was lower than the control (38%) at the final stage.

DISCUSSION

Macronutrient elements are important for the normal growth of *K. ivorensis* seedlings. Information on the deficiency of macronutrients may provide additional knowledge to foresters and nursery operators for the production of healthy and vigorous seedlings for forest plantation activities. Some elements such as N, P, K and Mg are mobile, thus the symptoms can appear first on older leaves. Calcium is relatively immobile and deficiency symptoms become evident in younger leaves (Taiz & Zeiger 2002).

The yellowing of mature leaves for nitrogen deficiency is due to inadequate supply of N for chloroplast protein synthesis (Gopikumar & Varghese 2004). The prolonged deficiency led to tissue death as observed in Figure 1. Similar observations were also reported in *Calamus manan* seedlings using sand culture (Raja Barizan & Aminah 1991). Since nitrogen plays an integral part in photosynthesis, which increases dry matter yield, deficiencies related to N deter the vegetative growth of plants, resulting in lower dry matter yield and foliage. Nitrogen concentration reduced to 6.3 mg g^{-1} from an initial content of 22.0 mg g^{-1} whereby any amount, which remained initially was used for amino acid assimilation (Havlin *et al.* 1998).

P deficiency (T3) in *K. ivorensis* seedlings was minimal during the initial months. Mild chlorosis was observed and leaves were fairly dark green. P deficiency was reported to increase the chlorophyll content of leaves, which resulted in dark green foliage of soybean leaves due to the retardation of cell and leaf expansion (Fredeen *et al.* 1989). Similar results were also reported by Silva and Beyl (2005) in wheat. However, the conventional symptom of purple bronzing as reported by Dell *et al.* (1995) was not apparent in this study. At the end of the experiment, the P content in the seedlings were reduced to 77%. However, no apparent growth retardation was observed due to lack of P. The sufficient levels of P reported in plants were 1 to 5 mg g^{-1} (Marschner 1995). The study showed an intermediate value of 0.6 mg g^{-1} (Table 5) which did not affect the seedling growth of *K. ivorensis*. Nevertheless, there were positive interactions whereby the increase of P simultaneously increased N and K in this study. Our findings were similar to studies by Ebdon *et al.* (1999) which confirmed that these nutrients had a combined effect on plant tissue concentration.

The K deficiencies were seen as yellow patches on leaves at later stages. Potassium is important for phloem transport, osmotic balance, photosynthesis (Tripler *et al.* 2006) and as enzyme activators (Dell *et al.* 1995). Leaf yellowing, similar to this study, was also reported in *Picea engelmanii* (conifer) seedlings by Van Den Driessche and Ponsford (1995). The stem diameter of K deficient seedlings was only affected initially and no changes were observed at the end of the experiment. However, the sharp drop of K content (four folds) in T4 was eminent at the final stage, probably due to the utilization of K in adenosine triphosphate (ATP) production (Marschner 1995). Low K content also gave high concentrations of P and Mg in K deficient seedlings. Antagonistic effects of K and Mg (Gopikumar & Varghese 2004), and K and P (Aldana 2005) were also reported as plant interactions which determine the tissue nutrient concentration.

Calcium deficient seedlings (T5) were recognized by the discoloration of young leaves and brown lesions. The cell wall stability of plants depends heavily on Ca and at deficient levels, this would increase the ability of pathogens to invade the plant and secrete harmful enzymes (Marschner 1995), leading to damage. Havlin *et al.* (1998) reported that the lack of Ca would lead to the failure of terminal bud and shoot development, causing plants to cease growth. Thus, the Ca deficient plants were severely affected with regard to stem diameter and seedling height. They also reported that the N and Mg contents of Ca deficient plants are related positively. However, in this study, only N showed positive relationship.

The seedlings which were deficient in Mg displayed characteristic chlorotic patterns between veins in older leaves. Fassbender (1988) also reported interveinal chlorosis in *Shorea leprosula* and *Shorea roxburghii* seedlings suffering from Mg deficiency. The omission of Mg resulted in poor seedling growth with regard to stem diameter, foliage, dry weight and tissue concentration of Mg. The investigations by Houcheng *et al.* (2008) in Chinese cabbage also reported growth retardation due to magnesium deficiency, while Lamb and Murphey (2008) observed that the reduction in stem diameter of Mg deficient silver maple seedlings is due to the secondary effect of low chlorophyll content.

CONCLUSIONS

Lack of N, P, K, Ca and Mg in seedlings were manifested as visual deficiency symptoms and changes in seedling growth and nutrient tissue concentration. Magnesium plays an important role in seedling growth of *K. ivorensis*, whereas potassium affects the nutrient tissue content. The physiological symptoms can be used as guidelines for diagnosing nutrient deficiencies of *K. ivorensis* seedlings in nurseries and plantations.

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