

## INFLUENCE OF ZINC, COPPER AND MANGANESE ON DRY MATTER YIELD AND PHYSIOLOGICAL TRAITS OF THREE CASSAVA GENOTYPES GROWN ON SOIL MICRONUTRIENT DEFICIENCIES

ANON JANKET<sup>1</sup>, NIMITR VORASOOT<sup>1</sup>, THAWAN KESMALA<sup>1</sup> AND SANUN JOGLOY<sup>1,2\*</sup>

<sup>1</sup>Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Khon Kaen, 40002, Thailand

<sup>2</sup>Peanut and Jerusalem Artichoke Improvement for Functional Food Research Group, Khon Kaen University, Khon Kaen 40002, Thailand

\*Corresponding author's email: sjogloy@gmail.com

### Abstract

Deficiencies of one or more micronutrients is a common cause of low yield in cassava. The aims of this research were investigated the effects of foliar applications of zinc, manganese and copper on cassava plant growth in soils where these micronutrients are often limiting. Three cassava genotypes (Kasetsart 50, Rayong 9 and CMR 38-125-77) were treated with foliar fertilizer applications varying in Zinc, Copper and Manganese. SPAD chlorophyll meter readings, visual ratings of plant nutrient deficiencies, leaf area, harvest index, and plant height significantly improved as a result of the micronutrient foliar applications. Shoot, tuber and total biomass dry weights were also improved by the foliar sprays. Foliar spray of 2% ZnSO<sub>4</sub>·7H<sub>2</sub>O increased biomass yield in all cassava genotypes by 30.6% to 75.7% compared to application of soil fertilizers alone. The information from this study is now being used to develop appropriate management guidelines for cassava production under micronutrient deficiencies conditions.

**Key words:** *Manihot esculenta* Crantz, Foliar fertilization, Fertilizer management, Trace element, Mineral nutrition.

### Introduction

Cassava (*Manihot esculenta* Crantz.) is one of leading crops of the world, a staple food for more than half a billion people which is also used for animal feed, starch, flour and for bio-ethanol production (Zhou & Thomson, 2009; FAO, 2014). While cassava production at Thailand agricultural research centers and by its best farmers will exceed 50 tons ha<sup>-1</sup>, the average cassava yield in Thailand is 22.57 tons ha<sup>-1</sup> (Duangpatra, 2003; FAOSTAT, 2014; Office of Agricultural Economics, 2015). Cassava is known to be produced in infertile soils. Yet the long term continuous cultivation of cassava without simultaneously improving soil fertility will lead to the depletion of soil nutrients and a reduction in cassava yields (Panitnok *et al.*, 2013; Buasong *et al.*, 2014). Howeler's (1995) study noted that continuous long-term production of cassava without fertilizer applications caused yield reductions from 26-29 ton ha<sup>-1</sup> to 10-12 tons ha<sup>-1</sup>. Howeler's (1981) study noted that nutrient losses from a ton of cassava roots were 2.3 kg, 0.5 kg, 4.1 kg, 0.6 kg and 0.3 kg for nitrogen, phosphorus, potassium, calcium and magnesium, respectively. Other studies have reported that nutrient removal resulting from root harvest, was in the order of N > K > Ca > P > Mg > S > Fe > Mn > Zn > Cu > B (Howeler, 2002; Fageria *et al.*, 2010). The optimum pH range for cassava is 5.5-7.5, yet even within this range the availability of micronutrients in some soils may limit yield. Micronutrients such as zinc, manganese, iron, copper and boron are generally deficient in most cassava growing soils as these elements are decreased rapidly by precipitation or by soil adsorption, especially in high pH (Howeler *et al.*, 1982; Howeler, 2002; Lee & Saunders, 2003). In calcareous soils, copper, iron, manganese and zinc are rendered less available as these elements precipitate in soils to form carbonates or bicarbonates, and the elements are also less available in high organic matter soils (Achakzai *et al.*, 2010).

Cassava can also encounter zinc deficiency on both acid and alkaline soils (CAIT, 1984; Howeler, 2002), and it has been reported in many countries including Colombia, Indonesia, Malaysia, Australia, Mexico, Brazil and Nigeria (Asher *et al.*, 1980; Howeler, 2002). Severe copper deficiency, resulting in 30% yield reductions, has been reported on peat soils of southern Malaysia (Chew *et al.*, 1978; Howeler, 2002). Manganese deficiency has been reported in northeast Brazil, Colombia and Vietnam (Howeler, 2002). While foliar fertilizer applications of micronutrients have been successful in treating micronutrient deficiencies, (Panitnok *et al.*, 2013; Jabeen & Ahmad, 2011), the application of micronutrients to high pH soils is often not effective because the applied element is precipitated rapidly (CIAT, 1978).

During the growing season, plants require a balanced and sufficient supply of nutrients for maximum growth and yield. Yet, many cassava growers are unable to achieve high yields due to plant nutrient deficiencies. This study aims to investigate the effects of foliar application of zinc, manganese, copper and combinations of these elements on nutrient deficiencies in cassava.

### Material and Methods

#### Experimental design, treatments and crop management:

The 3×6 factorial experiment was arranged in randomized complete block design with three replications at the Khon Kaen University's agronomy farm, Khon Kaen province, Thailand (latitude 16°28'N, longitude 102°48'E, 200 m above sea level). This experiment was conducted from June 2, 2016 to September 2, 2016.

Factor A consisted of three cassava genotypes i.e. Kasetsart 50 (KU50), Rayong 9 (RY9) and CMR 38-125-77. KU50 cultivar was released by Kasetsart University, Thailand. RY9 and CMR38-125-77 were provided by the Department of Agriculture, Thailand. Factor B included 6

fertilizer applications consisting of no-fertilizer application (Fo), chemical fertilizer formula 15-7-18+Mg at the rate of 312.5 kg ha<sup>-1</sup> at 15 days after planting (DAP) (hereafter referred to as soil fertilizer), soil fertilizer with foliar applications of 2% MnSO<sub>4</sub>•4H<sub>2</sub>O at 15 DAP and 30 DAP, soil fertilizer with foliar applications of 2% ZnSO<sub>4</sub>•7H<sub>2</sub>O at 15 DAP and 30 DAP, soil fertilizer with foliar applications of 0.5% CuSO<sub>4</sub>•5H<sub>2</sub>O at 15 DAP and 30 DAP and soil fertilizer with foliar applications of 2% MnSO<sub>4</sub>•4H<sub>2</sub>O + 2% ZnSO<sub>4</sub>•7H<sub>2</sub>O + 0.5% CuSO<sub>4</sub>•5H<sub>2</sub>O at 15 DAP and 30 DAP. Chemical fertilizers were applied into the soil manually at the depth of 15 cm around the plants and covered with soil.

Stem cuttings of cassava were treated with Thiamethoxam (3-(2-chloro-thiazol-5-ylmethyl)-5-methyl-(1,3,5)-oxadiazinan-4-ylidene-N-nitroamine (25% WG), at the rate of 5 g 20 L<sup>-1</sup> for 15 minutes to prevent the cassava from mealy bug (*Phenacoccus manihoti*), then incubated under warm temperature for three days to stimulate bud germination. Prior to planting the soil was disked twice. The stem cuttings were planted in the plots of 2 × 3 m in size at spacing of 50 × 50 cm. The stem cuttings were inserted vertically into the flat soil to cover 2/3 of the length. Plots were hand weeded at 30 and 60 DAP. A mini-sprinkler irrigation system was used to keep plots well watered.

**Physicochemical properties:** Soil samples were collected in each replication before planting at the depths of 0-30 and 30-60 cm. Soil samples were air dried, mixed and analyzed to determine physical and chemical properties (Table 2).

**SPAD chlorophyll meter reading (SCMR) and plant height:** SCMR were recorded from the blade of the fourth or fifth fully expanded leaf from the top of the main stem of 5 randomly chosen plants in each plot at 30 and 60 DAP. Readings were taken between 10:00-11:30 am using a Minolta SPAD-502 meter, Tokyo, Japan. On six plants in each plot, plant heights were measured at final harvest (90 DAP) from the base of main stem to the highest leaf.

**Leaf area, leaf area index and specific leaf area:** Data were recorded for leaf area (LA), leaf area index (LAI) and specific leaf area (SLA) from five randomly chosen plants in each plot. Ten percent of each green leaf sample (by weight) were measured using a leaf area meter (LI 3100C Area Meter, LI-COR Inc., USA), and leaf samples were then oven-dried at 80°C for at least 48 hours or until the weights were constant. The leaves were weighted immediately after drying. SLA (cm<sup>2</sup> g<sup>-1</sup>) was calculated as the ratio leaf area (cm<sup>2</sup>) and leaf dry weight (g), and leaf area index (LAI) was calculated as the ratio leaf area (cm<sup>2</sup>) and ground area (cm<sup>2</sup>).

**Visual symptom of plant nutrient deficiencies:** Visual nutrient deficiency rating was evaluated 30 and 60 DAP for zinc, manganese and copper using a 0 to 5 nutrient deficiency scale for each nutrient (Table 1 and Fig. 1).

**Biomass determination and harvest index:** Six plants were harvested from the yield areas of each plot at 90 DAP to determine the dry weights of leaf, petiole, stem and storage root. Roots larger than 0.5 cm in diameter were determined as storage roots and storage root number per plant was recorded. Fresh weights were first recorded for leaf, stem, petiole and storage root, and ten percent by weight of each sample was oven-dried at 80°C for 72 hours or until the weight was constant and dry weight was recorded. Biomass production was calculated by combining the dry weight of shoot and storage roots. Harvest index (HI) was calculated as the ratio of storage root dry weight and total crop dry weight.

**Statistical analysis:** Data for each parameter were analyzed statistically using the Statistix 8 software program for a factorial experiment in a randomized complete block design (Gomez & Gomez, 1984). No symptom of manganese and copper deficiency were observed, and therefore no data on these elements are presented. Means were separated by Dunnett's test at 0.05 probability level (Chuang-Stein & Tong, 1995; Statistix8, 2003). Graphical presentations with standard deviations were accomplished using Microsoft Excel 2007.

**Table 1. Standard evaluation score of visual nutrient deficiencies in cassava.**

Score	Observation
<b>Zinc deficiency scores</b>	
0.	Normal growth, no leaf symptoms
1.	Nearly normal growth, but small white or pale yellow spots or patches appear in between the veins of leaves
3.	The lobes of leaves become narrow, most leaves chlorotic and curl upward
5.	Almost all leaves become necrotic and death of young plants
<b>Manganese deficiency scores</b>	
0.	Normal growth, no leaf symptoms
1.	Nearly normal growth, but small chlorosis or yellowing of middle leaves
3.	Uniform chlorosis in almost middle leaves, young leaves are small but not deformed
5.	Growth severely retarded, almost all leaves become completely necrotic and death of young plants
<b>Copper deficiency scores</b>	
0.	Normal growth, no leaf symptoms
1.	Nearly normal growth, but uniform chlorosis of upper leaves and deformity of the young leaves with leaf tips and margins bending up-or downward
3.	Necrosis of the tips, petioles of fully expanded leaves long and bending down
5.	Almost all leaves become necrotic and death of young plants

Modified from CAIT (1985); Howeler (2002)

**Table 2. Soil physicochemical in the experimental fields at the depths of 0-30 cm and 30-60 cm.**

Soil parameter	0-30 cm.	30-60 cm.	Average	Rating <sup>1</sup>
Total N (%)	0.02	0.02	0.02	Very low
Available P (mg kg <sup>-1</sup> )	81.02	76.22	78.62	Very high
Exchangeable K (mg kg <sup>-1</sup> )	44.40	42.61	43.51	Low
Exchangeable Ca (mg kg <sup>-1</sup> )	383.93	409.91	396.92	Medium
Total Mg (mg kg <sup>-1</sup> )	33.17	33.17	33.17	Low
S (mg kg <sup>-1</sup> )	54.88	51.48	53.18	Medium
Exchangeable Na (mg kg <sup>-1</sup> )	54.59	56.25	55.42	-
Total Fe (mg kg <sup>-1</sup> )	21.27	19.42	20.35	Medium
Total Mn (mg kg <sup>-1</sup> )	4.98	3.53	4.26	Very low
Total Zn (mg kg <sup>-1</sup> )	0.58	0.60	0.59	Low
Total Cu (mg kg <sup>-1</sup> )	0.08	0.07	0.08	Very low
Total B (mg kg <sup>-1</sup> )	2.01	1.90	1.96	High
pH (1:1 H <sub>2</sub> O)	7.01	7.12	7.07	High
Electrical conductivity (dS/m)	0.05	0.06	0.06	Medium
Organic matter (%)	0.44	0.43	0.44	Very low
Cation exchange capacity (cmol kg <sup>-1</sup> )	3.32	4.31	3.82	-
<b>Texture class</b>	<b>Loamy-sand</b>	<b>Loamy-sand</b>		-

<sup>1</sup> Classification /of soil chemical characteristics according to the nutritional requirements of cassava (Howeler, 2002; Panitnok *et al.*, 2013)

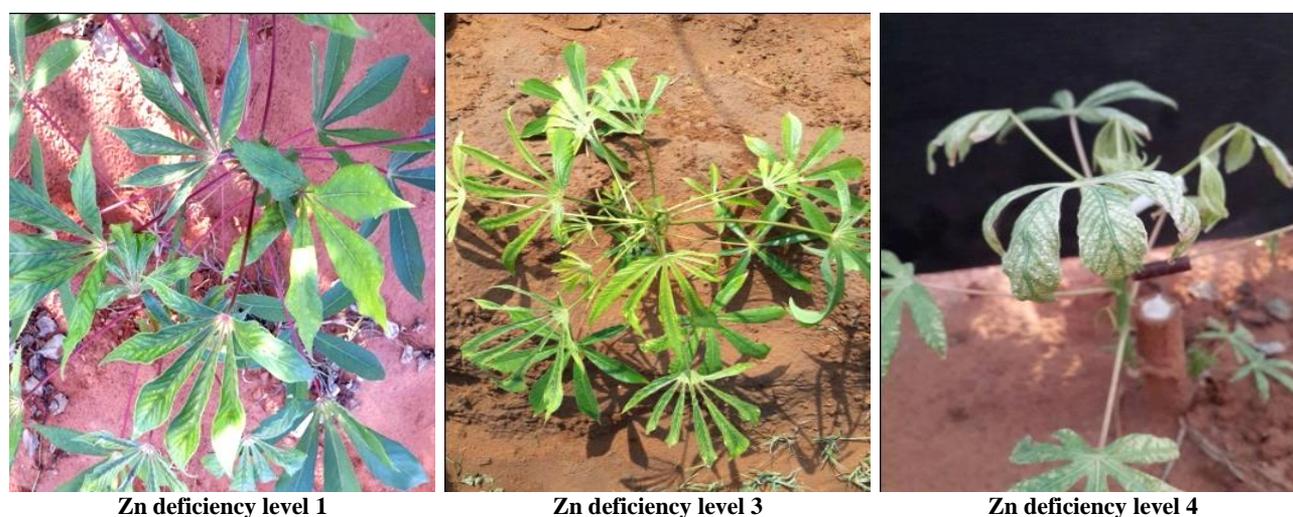


Fig. 1. Standard evaluation score of visual nutrient deficiencies in cassava.

## Results

**Soil physicochemical properties:** The soil type in experimental site was a Yasothon series (Yt), which is common in Northeastern Thailand. The soil pH was 7.07 and the soil texture was a loamy sand (Table 2). Most soil chemical and physical properties such as total nitrogen (0.02%), exchangeable potassium (43.51 mg kg<sup>-1</sup>), total magnesium (33.17 mg kg<sup>-1</sup>), manganese (4.26 mg kg<sup>-1</sup>), zinc (0.59 mg kg<sup>-1</sup>), copper (0.08 mg kg<sup>-1</sup>), organic matter (0.44%) and cation exchange capacity (3.82 cmol kg<sup>-1</sup>) were low and very low when compared with nutrient requirement for cassava (Howeler, 2002). However, exchangeable calcium (396.92 mg kg<sup>-1</sup>), total sulfur (53.18 mg kg<sup>-1</sup>), iron (20.35 mg kg<sup>-1</sup>) and electrical conductivity (0.06 dS m<sup>-1</sup>) were medium, whereas available phosphorus (78.62 mg kg<sup>-1</sup>) and total boron (1.96 mg kg<sup>-1</sup>) were high (Howeler, 2002).

## Differences of genotypes and fertilizer treatment:

Cassava varieties were significantly different for shoot, tuber and total BM dry weights, tuber number, SLA ( $p \leq 0.01$ ), HI and SCMR at 30 DAP ( $p \leq 0.05$ ) except for LA, plant height, LAI, Zn deficiency score and SCMR at 60 DAP, whereas, the differences among fertilizer application were significant for most characters except for tuber number, SLA, HI, plant height, Zn deficiency score and SCMR at 60 DAP. The interactions between genotype and fertilizer application were significant for shoot and tuber dry weights, tuber number, SLA and HI. However, the mean squares for the interactions were lower than those of genotype and fertilizer application main effects. As the interactions between genotype and fertilizer application were significant for dry weight, data were analyzed separately (Table 3).

Soil applied fertilizers did not affect any measured parameter when compared to the untreated control (Tables 4 and 5). However, significant differences among foliar applied fertilizer treatments were observed for shoot, tuber and total BM dry weights in all genotypes. Overall, CMR38-125-77 had the highest shoot, tuber and biomass yields. When grown without additional fertilizer CMR38-125-77 had tuber yields of 1,410 kg ha<sup>-1</sup> and when a foliar fertilizer containing Zn were applied these yields increased to as high as 1,875 kg ha<sup>-1</sup>. The genotype KU50 also responded to Zn containing fertilizers, averaging 1,110 kg ha<sup>-1</sup> without fertilizers and up to 2,409 kg ha<sup>-1</sup> when a zinc containing fertilizer was foliar applied. RY9 had both the lowest yields without and with foliar applied Zn fertilizers, averaging 789 kg ha<sup>-1</sup> and 1,135 kg ha<sup>-1</sup> respectively (Table 4). Foliar fertilizer application of Zn had high tuber yield also had high shoot and total biomass dry weights, with the most responsive variety being KU50's shoot and total BM dry weights increases ranging from 67.9% to 75.7%. Changes in shoot and total BM dry weights for RY9 and CMR38-122-77 ranges from 58.3% to 60.5 % and 30.6% to 37.4%, respectively. Yet, foliar applications of Zn, Cu and mixtures did significantly increase tuber number per plant ranging from 27.5% to 75.9% in KU 50, but they did not significantly affect number of tuber per plant in RY9 and CMR 38-125-77. In KU50, the highest number of tuber per plant was observed in treatment with foliar application of Zn. When grown without additional fertilizer KU50 had tuber number of 4.45 tubers per plant and when a foliar fertilizer containing Zn was applied these number of tuber increased to as high as 9.73 tubers per plant.

**Effect of fertilizer applications on plant growth:** Soil applied fertilizers did not affect any measured parameter when compared to the untreated control. In addition, neither soil or foliar applied fertilizer treatments did not significantly affected plant height (which ranged from 103.8 cm to 107.8 cm) at 90 DAP (Fig. 2). Yet, foliar applications of micronutrients did significantly increase SCMR in the upper leaves at 30 DAP (ranging from 35.53 to 41.29) (Fig. 3). However, by 60 DAP SCMR were statistically the same across all treatments (ranging from 40.01 to 49.68). Leaf area indexes improved with the addition of foliar applied micronutrients, with the most responsive variety being KU50's LAI increases ranging from 60.1 to 99.7%. (Table 5). Changes in LAI for RY9 and CMR38-122-77 ranges from -2% to 44.9 % and -1.7% to 41%, respectively.

**Visual ratings of nutrient deficiencies:** Nutrient deficiency ratings were taken at 30 and 60 DAP. While the soil test levels for Cu and Mn were very low, however, visual symptoms of deficiencies were rarely seen. Zn deficiencies, however, were clearly observed at 30 DAP and to a lesser extent at 60 DAP (Table 5). The reduction in visual Zn deficiency at 60 DAP was observed in all treatments including the control. KU50 was the variety which visually seemed to be the most sensitive to Zn deficiency, and also the line most responsive to foliar applications of Zn; whereas CM38-125-77 was the least sensitive.

## Discussion

Micronutrients play a critical role for growth and yield of cassava, which is widely grown under poor soil fertility and micronutrient deficient conditions. In this experiment, foliar application of micronutrients were effective in relieving these deficiencies. We also noted that as the season progressed, the micronutrient deficiencies tended to be less evident (Fig 4). These results suggested that expansion of the plant root system into soils with a more favorable pH or micronutrient content may have helped, as was also noted by Howeler (2002). As in an earlier study by Panitnok *et al.*, (2013), soil and foliar fertilizer treatments had little effect on plant height. Panitnok *et al.*, (2013), speculate that plant height may be more of a genetic and climatic response than to plant nutrition. Zn is an essential element needed in many enzymatic reactions, including dehydrogenases, proteinases, and peptidases, to aid molecular configurations between an enzyme and a substrate (Römheld & Marschner, 1991; Swietlik, 1999; Fereria *et al.*, 2010).

Similar responses in cassava tuber growth to Zinc have been reported by Ali & Elkader (2014), and positive responses in other tuber crops, such as potato, have been documented by Ahmed *et al.*, (2011) and Mousavi *et al.*, (2007). In our study multiple application of micronutrients increased shoot DW and total BM in KU50 and RY9, yet less in CMR38-125-77. Panitnok *et al.*, (2013) suggests that differences among genotypes might be due to the differences in plant characteristics.

The increase in HI in KU50 caused by Zn, Cu and combination of these nutrients was associated with high tuber number and tuber dry weight (Tables 4, 5). In cassava, partitioning of photosynthetic assimilates from leaves to storage roots is high at tuber bulking period during 180-300 DAP, and these traits also have high genotype by environment interaction as shown in Table 3, and in Alves (2002). Reports of the potential benefits of micronutrient sprays is often inconsistent because of differences in plant species, stage of plant growth, soil and experimental conditions. Antagonistic effect between Zn and Mn, have been reported, as have synergistic effects between Zn and Cu. (Labanauskas & Puffer, 1964; Tariq *et al.*, 2007; Mousavi *et al.*, 2012).

In conclusion, the application of soil fertilizers alone did not significantly cassava yields. Yet, foliar applications of micronutrients, zinc in particular, did increase tuber and biomass yields, and these results. The field site was high in calcium and in soil pH, making many micronutrients like Zn poorly available at best, and thus the need for and success of foliar applications. Overall, the genotype CMR38-125-77 had the highest tuber and biomass yields. Yet KU50 was very responsive to foliar applications of Zinc resulting in yields equal to CMR38-125-77. In fields similar to our test field, foliar applications of 2% ZnSO<sub>4</sub>•7H<sub>2</sub>O, should aid cassava growth, development and yield. Soil applications of other fertilizers should also aid plant growth and help lower soil pH over time.

Table 3. Mean squares for growth parameters, physiological traits, zinc deficiency score and SCMR of three cassava genotypes tested with 6 fertilizer treatments.

Source of variation	DF	Shoot DW (kg ha <sup>-1</sup> )	Tuber DW (kg ha <sup>-1</sup> )	Total BM (kg ha <sup>-1</sup> )	Tuber no. (no. plant <sup>-1</sup> )	LA (cm <sup>2</sup> )	SLA (cm <sup>2</sup> g <sup>-1</sup> )	LAI	HI	Height (cm)		Zn DS		SCMR	
										30 DAP	60 DAP	30 DAP	60 DAP	30 DAP	60 DAP
Replication	2	697450	221067	319513	3.11	17360000	3848.9	2.77	0.0037	3109	1.13	20.17	119.76	54.98	
Genotypes (A)	2	1396000**	4918548**	3195000**	17.12**	18290000ns	26910.4**	2.92ns	0.0236*	184ns	6.51ns	0.79ns	565.35*	26.27ns	
Fertilizer (B)	5	2452000**	832559**	3327000**	3.80ns	9047000**	623.6ns	14.48*	0.0013ns	24.9ns	10.79**	3.10ns	90.50*	4.21ns	
AxB	10	3321246*	733185**	3284084ns	6.58**	22210000ns	1969.0*	3.56ns	0.0062*	95.8ns	1.41ns	0.74ns	24.65ns	5.23ns	
Error	34	1448082	98399	1819512	1.51	12080000	754.9	1.93	0.0008	209	3.14	1.26	18.41	5.87	
<b>CV (%)</b>		<b>15.84</b>	<b>21.04</b>	<b>14.84</b>	<b>22.05</b>	<b>22.68</b>	<b>8.29</b>	<b>22.69</b>	<b>16.92</b>	<b>13.72</b>	<b>130.18</b>	<b>122.68</b>	<b>11.42</b>	<b>4.96</b>	

DF= Degree of freedom, LA= Leaf area, SLA= Specific leaf area, LAI= Leaf area index, HI= Harvest index, ZnDS = Zinc deficiency score, SCMR= Spad chlorophyll meter reading. ns, \*, \*\* = Non-significant and significant difference at p<0.05 and p<0.01 respectively. DAP= Days after planting

Table 4. Shoot dry weight, tuber dry weight, total biomass production (BM), tuber number per plant, leaf area (LA) and specific leaf area (SLA) at 90 days after planting of three cassava genotypes tested with 6 fertilizer treatments.

Fertilizer treatment	Shoot dry weight (kg ha <sup>-1</sup> )			Tuber dry weight (kg ha <sup>-1</sup> )			Total biomass (kg ha <sup>-1</sup> )		
	KU50	RY9	CMR8-125-77	KU50	RY9	CMR8-125-77	KU50	RY9	CMR8-125-77
Fo	5342±176	5060±553	6941±365	1110±156	789±217	1410±124	6452±327	5849±729	8351±409
(15-7-18+Mg)	5722±886	5450 ±946	7746±442	1112±91	793±228	1842±120	6834 ±799	6243±1139	9588 ±348
(15-7-18+Mg)+ Mn	8445±1274*	6959±1237	6991±967	1489±134	959±67	1711±221	9934±1377*	7918±1180	8702 ±987
(15-7-18+Mg)+ Zn	9609±1897*	8747±452*	10644±1560*	2409±197*	1135±226	1875±113	12008±1655*	9882±647*	12519±1480*
(15-7-18+Mg)+ Cu	7281±428	6505±327	9961±289	1890±344	1051±44	1953±181	9171±1035	7556 ±306	11914±488*
(15-7-18+Mg)+(Mn+Zn+Cu)	8395±846*	8739±1860*	8392±399	2367±289*	950±231	2409±321*	10762±1056*	9689±1586*	10801±919

**Increase or reduction percentages**

(15-7-18+Mg)	7.1	7.7	11.6	0.2	0.5	30.6	5.9	6.7	14.8
(15-7-18+Mg)+ Mn	47.6	27.7	-9.7	33.9	20.9	-7.1	45.4	26.8	-9.2
(15-7-18+Mg)+ Zn	67.9	60.5	37.4	116.6	43.1	1.8	75.7	58.3	30.6
(15-7-18+Mg)+ Cu	27.2	19.4	28.6	70.0	32.5	6.0	34.2	21.0	24.3
(15-7-18+Mg)+(Mn+Zn+Cu)	46.7	60.3	8.3	112.9	19.8	30.8	57.5	55.2	12.7

**Tuber no. (no. plant<sup>-1</sup>)**

Fertilizer treatment	Tuber no. (no. plant <sup>-1</sup> )			Leaf area (cm <sup>2</sup> plant <sup>-1</sup> )			Specific leaf area (cm <sup>2</sup> g <sup>-1</sup> )		
	KU50	RY9	CMR8-125-77	KU50	RY9	CMR8-125-77	KU50	RY9	CMR8-125-77
Fo	4.45 ±0.6	4.58±1.6	4.28±0.9	9918±897	12372±3703	13930±1285	298±11	401±46	333±12
(15-7-18+Mg)	5.53±1.5	5.23±1.0	4.78±0.5	9962 ±1897	13310 ±2836	14659±1827	301±5	360 ±10	307±23
(15-7-18+Mg)+ Mn	5.33±0.7	6.08±2.2	5.30±1.4	17273±2823	13555±3465	14401±2678	303±15	345 ±23	335±28
(15-7-18+Mg)+ Zn	9.73±1.8*	4.99±0.5	4.80±0.8	19863±3555*	19265±2081*	18877 ±4228	321±14	378 ±16	295±25
(15-7-18+Mg)+ Cu	7.05±0.4*	5.40±1.3	4.30±0.4	16303±4437	13035 ±2984	20658±506*	306±21	347 ±31	341±13
(15-7-18+Mg)+(Mn+Zn+Cu)	7.25±0.7*	4.98±1.9	5.98±1.9	15910±2979	17025±2668	17125±1152	273±24	370 ±19	350±10

**Increase or reduction percentages**

(15-7-18+Mg)	24.2	14.2	11.7	0.4	7.6	5.2	1.0	-10.2	-7.8
(15-7-18+Mg)+ Mn	-3.6	16.3	10.9	73.4	1.8	-1.8	0.7	-4.2	9.1
(15-7-18+Mg)+ Zn	75.9	-4.6	0.4	99.4	44.7	28.8	6.6	5.0	-3.9
(15-7-18+Mg)+ Cu	27.5	3.3	-10.0	63.7	-2.1	40.9	1.7	-3.6	11.1
(15-7-18+Mg)+(Mn+Zn+Cu)	31.1	-4.8	25.1	59.7	27.9	16.8	-9.3	2.8	14.0

Values are the mean ± SD. of three replications.

Percentage changes, for the treatment with application of soil chemical fertilizer alone compared to no-fertilizer application (Fo), and for foliar fertilization group compared to application of soil chemical fertilizer alone.

\* = significant different from application of soil chemical fertilizer alone by Dannett's test at p<0.05.

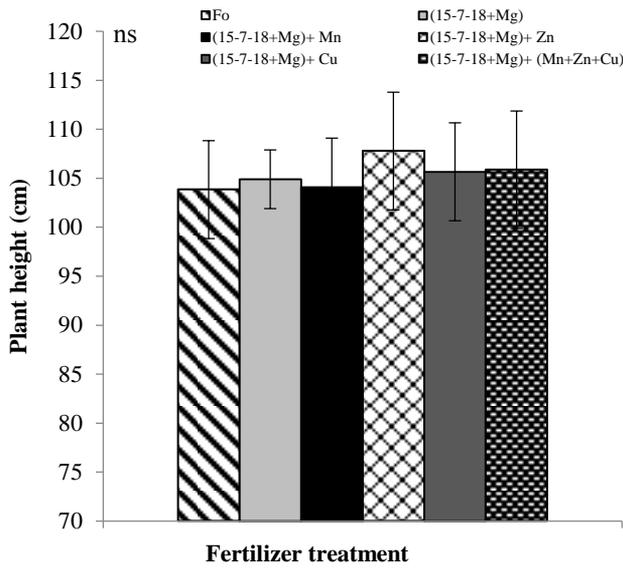


Fig. 2. Plant height (cm) at 90 days after planting of three cassava genotypes tested with 6 fertilizer treatments. ns = non significant difference at  $p \leq 0.05$ .

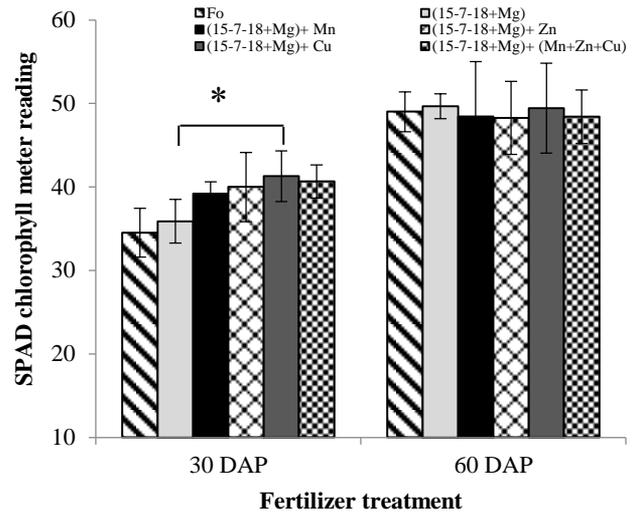


Fig. 3. SPAD chlorophyll meter reading of upper leaves at 30 and 60 days after planting (DAP) of three cassava genotypes tested with 6 fertilizer treatments. \* = significant different from application of soil chemical fertilizer alone by Dannett's test at  $p \leq 0.05$ .

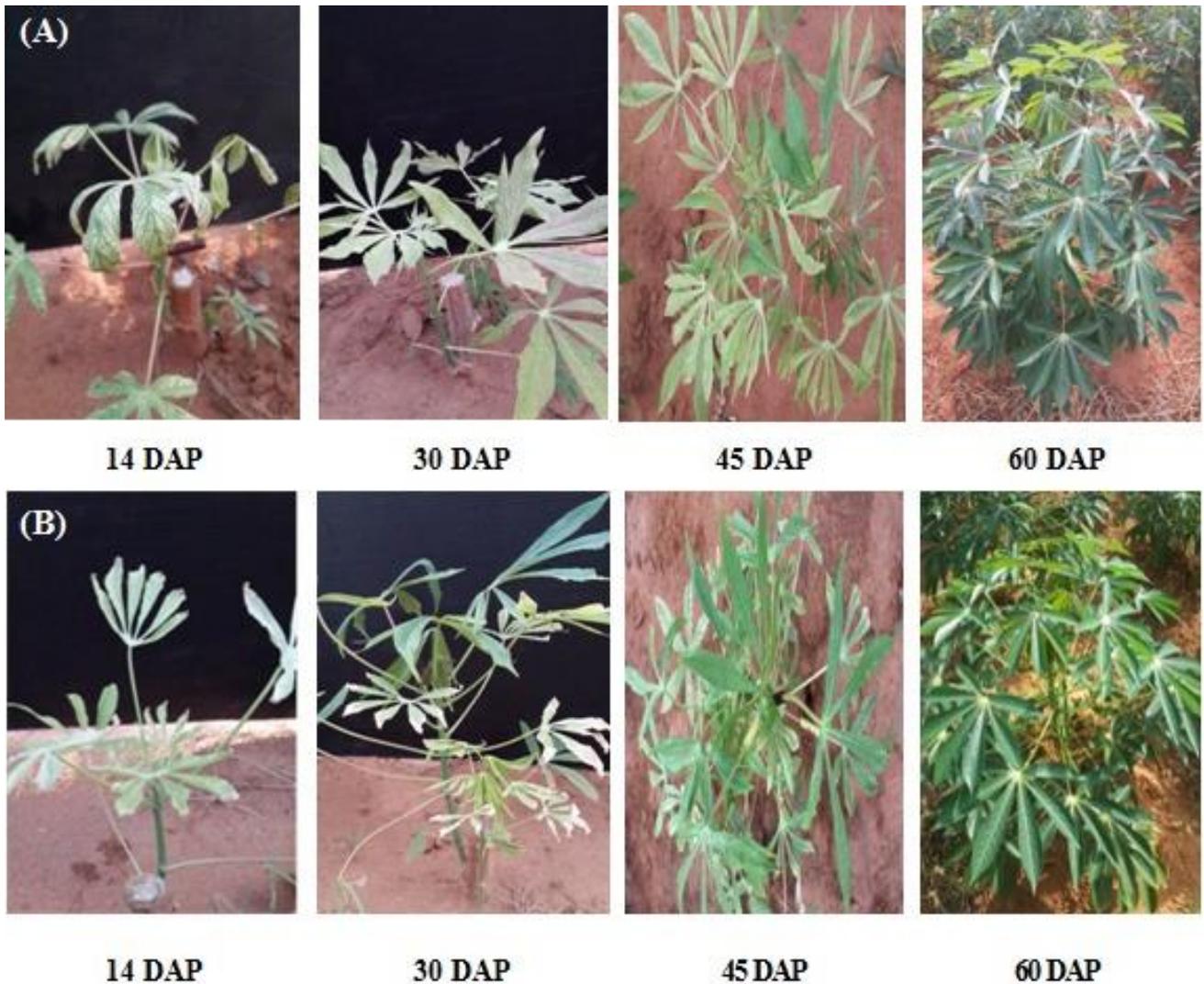


Fig. 4. Visual nutrient deficiency under no fertilizer application (Fo) (A) and under application of soil fertilizer formula 15-7-18+Mg and sprayed 2%  $ZnSO_4 \cdot 7H_2O$  (B) during crop growth. DAP= days after planting.

## Acknowledgements

The research was partially supported by The Royal Golden Jubilee Ph.D. Program, the National Science and Technology Development Agency (NSTDA), Thailand, Assistance in conducting the work was also received from Plant Breeding Research Center for Sustainable Agriculture, Khon Kaen University. Grateful acknowledgment is also made to the Thailand Research Fund (TRF) for providing financial supports to this research through the Senior Research Scholar Project of Professor Dr. Sanun Jogloy. The Thailand Research Fund (TRF) (Project no. 5780003), Khon Kaen University, and Faculty of Agriculture, Khon Kaen University were also acknowledged for providing financial support for manuscript preparation activities.

## References

- Achakzai, A.K.K., S.A. Kayani and A. Hanif. 2010. Effect of salinity on uptake of micronutrients in sunflower at early vegetative stage. *Pak. J. Bot.*, 42(1): 129-139.
- Ahmed, A.A., M.M.H. Abd El-Baky, M.F. Zaki and F.S. Abd El-Aal. 2011. Effect of foliar application of active yeast extract and zinc on growth, yield and quality of potato plant (*Solanum tuberosum* L.). *J. App. Sci. Res.*, 7(12): 2479-2488.
- Ali, R.A.M. and D.Y. Elkader. 2014. Influence of application systems of K<sub>2</sub>SO<sub>4</sub> and foliar application of micronutrient mixtures on cassava grown in sandy soil. *Alexandria Sci. Exchange J.*, 35(4): 315-324.
- Alves, A.A.C. 2002. Cassava Botany and Physiology. In: (Eds.): Hillocks, R.J., M.J. Thresh and A.C. Bellotti. *Cassava: Biology, Production and Utilization*. CAB International, Wallingford, pp. 67-89.
- Asher, C.J., D.G. Edwards and R.H. Howeler. 1980. Nutritional disorders of cassava, Dept Agric, Univ. Qld, St. Lucia, Qld, Australia.
- Buasong, A., L. Narangajavana, J. Thitamadee and N. Punyasuk. 2014. Correlation of fertilizer application, growth and nutrient transporter gene expressions in Thai cassava. The 26<sup>th</sup> annual meeting of the Thai society for biotechnology and international conference. Mae Fah Lunag University, Chiang Rai, Thailand, pp. 203-209.
- Centro Internacional de Agricultura Tropical (CIAT). 1984. Annual Report 1984. Cali, Colombia.
- Centro Internacional de Agricultura Tropical (CIAT). 1985. Annual Report 1985. Cali, Colombia.
- Centro Internacional de Agricultura Tropical (CIAT). 1978. Annual Report 1978. Cali, Colombia.
- Chew, W.Y., K. Ramli and K.T. Joseph. 1978. Copper deficiency of cassava (*Manihot esculenta* Crantz) on Malaysian peat soil. *MARDI Reseach Bullentin.*, 6(2): 208-213.
- Chuang-Stein, C. and D.M. Tong. 1995. Multiple comparisons procedures for comparing several treatments with a control based on binary data. *Stat Med.*, 14(23): 2509-2522.
- Duangpatra, P. 2003. Methodology of the development of research and development projects by using logical framework approach. Lecture for Course KU SLUSE. Kasetsart University. (in Thai)
- FAO, 2014. FAOSTAT. Available at <http://faostat.fao.org/site/339/default.aspx> (verified December 5, 2016).
- Fergeria, N.K., V.C. Baliger and C.A. Jones. 2010. Growth and mineral nutrition of field crops. CRC Press, Florida.
- Gomez, K.A. and A.A. Gomez. 1984. Statistical procedures for agricultural research (2<sup>nd</sup> Ed.). John Wiley and Sons, New York.
- Howeler, R., D.G. Edwards and C.J. Asher. 1982. Micronutrient deficiencies and toxicities of cassava plants grown in nutrient solutions. I. Critical tissue concentrations. *J. Plant Nut.*, 5(8): 1059-1076.
- Howeler, R.H. 1981. Mineral nutrition and fertilization of cassava. Series 09EC-4, CIAT, Cali. Colombia.
- Howeler, R.H. 1995. Agronomy research in the Asian Cassava Network—Towards better production without soil degradation. In: (Ed.): Howeler, R.H. *Cassava Breeding, Agronomy Research and Technology Transfer in Asia. Proc. 4<sup>th</sup> Regional Workshop*, held in Trivandrum Kerala, India. Nov 2-6, 1993. pp. 368-409.
- Howeler, R.H. 2002. Cassava mineral nutrition and fertilization. In Hillocks R.J., M.J. Thresh, and A.C. Bellotti (Eds.) *Cassava: Biology, Production and Utilization*. CAB International, Wallingford, pp. 115-147.
- Jabeen, N. and R. Ahmad. 2011. Effect of foliar-applied boron and manganese on growth and biochemical activities in sunflower under saline conditions. *Pak. J. Bot.*, 43(2): 1271-1282.
- Labanauskas, C.K. and R.E. Puffer. 1964. Effect of foliar application of manganese, zinc, and urea on 'Valencia' oranges yield and foliage composition. *Proc. Amer. Soc. Hort. Sci.*, 84: 158-164.
- Lee, M.K. and J.A. Saunders. 2003. Effects of pH on metals precipitation and sorption: Field bioremediation and geochemical modeling approaches. *Vadose Zone J.*, 2: 177-185.
- Mousavi, S.R., M. Galavi and G. Ahmadvan. 2007. Effect of zinc and manganese foliar application on yield, quality and enrichment on potato (*Solanum tuberosum* L.). *Asi. J. Pla. Sci.*, 6(8): 1256-1260.
- Mousavi, S.R., M. Galavi and M. Rezaei. 2012. The interaction of zinc with other elements in plants: a review. *Intl. J. Agr. Cro. Sci.*, 4(24): 1881-1884.
- Office of Agricultural Economics. 2015. Available at <http://www.oae.go.th/oaenew/OAE/index.html> (verified December 10, 2016).
- Panitnok, K., S. Chaisri, E.D. Sarobol, S. Ngamprasitthi and P. Chaisir. 2013. The combination effects of zinc, magnesium, sulphur foliar fertilizer management on cassava growth and yield and yield grown on map bon, coarse-loamy variant soil. *Proc. Soc. & Beh. Sci.*, 91(2): 288-293.
- Röemheld, V. and H. Marschner. 1991. Function of Micronutrients in Plants. In: *Micronutrients in Agriculture, Soil Science Society of American Inc.* (Ed.): Mortvedt, J.J. Madison, Wisconsin, pp: 297-328.
- Statistix 8. 2003. Statistix8: analytical software user's manual. Tallahassee, Florida.
- Swietlik, D. 1999. Zinc nutrition in horticultural crops. *Hortic. Rev.*, 23:109-178.
- Tariq, M., M. Sharif, Z. Shah and R. Khan. 2007. Effect of foliar application of micronutrients on the yield and quality of sweet orange (*Citrus sinensis* L.). *Pak. J. Biol. Sci.*, 10(11): 1823-1828.
- Zhou, A. and E. Thomson. 2009. The development of biofuels in Asia. *Appl. Energy.*, 86: 11-20.