The Effects of Copper and Zinc on Embryo Germination and Seedling Growth of Coconut *(Cocos nucifera L.)*

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ABSTRACT

The effects of Copper and Zinc on germination and initial seedling growth of coconut (*Cocos nucifera* L.) were studied using *in-vitro*-cultured embryos. The embryos were cultured in Eeuwens Y_3 medium by altering the $CuSO_4$ and $ZnSO_4$ concentration in culture media (0.5 – 1.0 µM $CuSO_4$ and 12.5 – 50 µM $ZnSO_4$ respectively) to get eight different combinations as treatments. The embryos were raised *in-vitro,* and their germination and growth of seedlings were measured after five months. The highest mean germination (68.5%), shoot height (26 cm), number of primary roots (3.02), secondary root growth (1.74), number of leaves (1.54) and fresh weight of shoots (16.7 g) were recorded with 2.0 µM $CuSO_4$ and 25.0 µM $ZnSO_4$, and the lowest response was recorded with $CuSO_4$ and $ZnSO_4$ free Y_3 medium (control) indicating that addition of Cu and Zn has significantly increased the germination, growth and development of seedlings. Doubling the concentration of either $CuSO_4$ or $ZnSO_4$ (2.0 µM $CuSO_4$ and 25.0 µM $ZnSO_4$ or 1.0 µM $CuSO_4$ and 50.0 µM $ZnSO_4$) resulted in significantly ($p<0.05$) higher germination and growth of seedlings. The reduction of $CuSO_4$ concentration to 0.5 µM (0.5 µM $CuSO_4$ and 25.0 µM $ZnSO_4$) resulted significantly ($p<0.05$) higher germination and shoot growth. The concentration 2.0 µM $CuSO_4$ and 50.0 µM $ZnSO_4$ recorded the next lowest values for germination and growth of seedlings indicating that doubling the concentration of $CuSO_4$ and $ZnSO_4$ had inhibition effect. Halving the concentration of $ZnSO_4$ (1.0 µM $CuSO_4$ and 12.5 µM $ZnSO_4$) or both $CuSO_4$ and $ZnSO_4$ (0.5µM $CuSO_4$ and 12.5 µM $ZnSO_4$) concentrations were not adequate to increase germination and growth of seedlings. The results indicated that 1.0 µM $CuSO_4$ and 50.0 µM $ZnSO_4$, 2.0 µM $CuSO_4$ and 25.0 µM $ZnSO_4$ and 0.5 µM $CuSO_4$ and 25.0 µM $ZnSO_4$ were the better combinations which gave positive effect on germination and seedling development. The results of this experiment can be used for further studies on micronutrients. Adopting better Cu and Zn combinations in embryo culture media can produce vigorous seedlings with higher shoot and root growth.

Keywords: Coconut, Copper, In-vitro, Seedling growth, Zinc

INTRODUCTION

Micronutrients play a significant role in physiological functions of the coconut palm. They act as coenzymes and catalysts of many life processes in the plant's biochemistry, synthesis and degradation of molecules, energy transfer, and transport of compounds within the plant. Micronutrients govern critical plant functions, and therefore, it is extremely important to recognise and provide sufficient amounts to overcome micronutrient deficiencies though they are required in minute quantities for growth and development. Coconut palms do not

response properly for external inputs such as fertiliser, irrigation and other management practices if the micronutrient availability is limited. Immature nut fall, premature decline, tapering, nut abnormalities, poor growth and low yield in coconut, susceptibility to fungal diseases, etc. are serious concerns of coconut growers that need to be addressed. Although there are no direct evidence to attribute micronutrient imbalances as the cause for various disorders of coconut palm, their influence can not be ruled out.

Due to continuous cultivation of coconut over two centuries, most of the coconut-growing soils in Sri Lanka are deficient in major nutrients, especially N, K and Mg (Tennakoon, *et al.,* 2010). To overcome such deficiencies, the Coconut Research Institute has given recommendations to apply N, P, K and Mg fertilisers for young and adult coconut palms in different agro-climates.

In the past, no incidences of micronutrient deficiencies in coconut were reported in Sri Lanka due to their adequate availability in the soil. However, there is a significant depletion of micronutrients in coconut-growing soils (Wijebandara and Somasiri, 2004). It is reported that a coconut palm yielding 75 – 110 nuts per year, looses nutrients through fallen fronds, inflorescences and harvested nuts nearly 7.21 g of Iron (Fe), 3.98 g of Manganese (Mn), 0.82 g of Copper (Cu), 2.78 g of Zinc (Zn) and 1.64 g of Boron (B) (Somasiri *et al,* 2003). Further, continuous application of fertilisers which contains only N, P, K and Mg, soil erosion and also exhaustion of soil organic matter over centuries have aggravated the micronutrient depletion in coconut-growing soils. At present, boron deficiency was observed in coconut palms in Sri Lanka. Analysis of soils and leaf samples collected from several parts of the coconut-growing areas revealed that, in some areas, Copper (Cu), Zinc (Zn) and Boron (B) levels are below the sufficiency range for coconut (Wijebandara and Somasiri, 2004; Anura, 2012,). This indicates the need for including micronutrients in fertiliser recommendations. Therefore, the objective of this study was to determine the effect of Cu and Zn on *in-vitro*-cultured embryo germination and seedling growth of coconut.

MATERIALS AND METHODS

In-vitro cultured embryos were used to study the effect of Cu and Zn levels on embryo germination and seedling development. Mature nuts (11-12 months after pollination) of the variety Tall x Tall were collected from the Isolated Seed Garden at Ambakelle. The embryos were excised from the endosperm of mature nuts using a cork borer and a scalpel and sterilised in 3% (v/v) sodium hypochlorite for 5 minutes followed by rinsing in 4 – 5 changes of sterile distilled water. The embryos were then cultured in glass test tubes (30 x 200 mm) containing 10 ml of pre-sterilised Eewens Y_3 (Eeuwens, 1978) liquid medium supplemented with different combinations of $CuSO_4$ and $ZnSO_4$ concentrations. The Y_3 medium without any $CuSO_4$ and $ZnSO_4$ was used as the control (T_1). The composition of $CuSO_4$ and $ZnSO_4$ in standard culture media was 1.0 µM and 25.0 µM respectively. The other tested treatments are given in Table 1.

Table 1 Concentration of CuSO4 ZnSO4 in culture media

Treatment	$CuSO_4$ (µM)	$ZnSO_4$ (µM)
T_1 (Control)	0	0
T_2	0.5	12.5
T_3	0.5	25.0
T_4	1.0	12.5
T_5	1.0	25.0
T_6	1.0	50.0
T_7	2.0	25.0
T_8	2.0	50.0

A Completely Randomised Design with 35 replicates in each treatment was used. Cultures were incubated for two months under dark conditions and then transferred to 16 hr photoperiod (Photosynthetic Active Radiation: about 75 µmol $m^{-2}s^{-1}$) at 28°C ± 1 Changing of culture media with the same levels of $CuSO_4$ and $ZnSO_4$ was done at four-week intervals by sucking out the old medium and adding about 10 ml of fresh medium. Data were collected over a period of 5 months. The number of embryos germinated (enlarged, sprouted), height of the seedling, number of leaves, number of primary roots, number of secondary root growth were measured every month by observing the embryos and seedlings, and after five months, the seedlings were taken out from the test tube and the fresh weight of the plants were measured. The embryos were considered germinated when the plumule sprouted and the radicle showed signs of emergence as reported by Danson *et al.* (2009).

The data on growth parameters were analysed using CATMOD procedure of the SAS software. The data on fresh weight of seedlings were analysed using one-way ANOVA. Mean comparisons were made using Dunnett's multiple comparison test ($p<0.05$).

RESULTS AND DISCUSSION

The cultured embryos developed into young seedlings containing about four scale leaves and two photosynthetic leaves within five months in all the tested treatments and showed higher germination percentage (>42.8%) than the control. The mean number of primary roots and secondary root development was also higher than that of the control.

The highest percentage germination of embryos, shoot height, number of primary roots, secondary root growth, number of leaves and fresh weight of seedlings were recorded in the presence of 2.0 µM $CuSO_4$ and 25.0 µM $ZnSO_4$ (Table 1) indicating that addition of Cu and Zn had increased the germination and growth and development of seedlings. Hall (2002) reported that Cu and Zn are utilised in the cellular metabolism because both these elements, mostly Zn, are present in many plant proteins. However, it was observed that even in the absence of Cu and Zn in the medium (control), a mean germination of 42.8% was recorded. This may be due to addition of very minute quantities of Cu and Zn to culture media as impurities through the other chemicals used in the experiment.

When the concentration either with $CuSO_4$ (T_7) or $ZnSO_4$ (T_6) in culture medium was doubled, significantly ($p<0.05$) higher mean germination %, number of leaves and fresh weight of seedlings, shoot height, number of primary roots and secondary roots of seedlings were observed (Table 1,Plate 1, 2 and 3). It revealed that increasing either Cu or Zn result in better germination, growth and development of seedlings.

The concentration 2.0 µM $CuSO_4$ and 50.0 µM $ZnSO_4$ recorded low percentage germination (45.0%) and the lowest growth of seedlings at higher concentration of $CuSO_4$ and $ZnSO_4$ indicated their inhibition effect. And also, low percentage germination and seedling growth was observed in the presence of 0.5 µM $CuSO_4$ and 12.5 µM $ZnSO_4$ indicating that those levels are not adequate to support germination and growth of seedlings. Although Cu and Zn are essential micronutrients (Hojiboland *et al.*, 2006; Clemens, 2006; Singh *et al.*, 2007a and Singh *et al.*, 2007b), they are highly toxic and biologically active at high concentrations (Kramer *et al.*, 2007). Ionic toxicity may be the cause for drastic effects of micronutrient salts on seed germination or it could be due to osmotic effect (Shaukat *et al.*, 1999). The reduction of seed germination can also be attributed to the alterations in selection permeability properties of cell membrane (Muhammad *et al.*, 2008). Nag *et al.* (1984) reported that toxic doses of Zn, increase the levels of a few oxidising enzymes like

peroxidase, IAA oxidase and ascorbic acid oxidase and a distinct inhibition in the activities of hydrolysing enzymes in rice seedlings bringing about a significant retardation in the growth pattern of rice.

Houshmandfar and Moraghebi (2011) also reported that the metal toxicity is an important factor governing germination and growth of plants. The studies on Cu and Zn toxicity on *Phaseolus vulgaris* and Mung bean also have revealed that seedling growth is significantly affected at high concentrations (Hojioland *et al.*, 2006).

The reduction in *Brassica juncea* L. seedling growth may be due to low water potential, hampered nutrient uptake and secondary oxidative stress (John *et al.*, 2009). The reason for reduced seedling growth under high $CuSO_4$ and $ZnSO_4$ treatment could be the reduction in meristematic cells present in this region and some enzymes present in cotyledons and endosperm. During seedling growth, hydrolysis of food reserves takes place by hydrolytic enzymes. The activities of hydrolytic enzymes might be affected and the food not reach to the radicle and plumule leading the reduction in seedling growth.

There was no significant difference in germination of embryos and growth parameters of the seedlings in the treatments of 1.0 µM $CuSO_4$ and 25.0 µM $ZnSO_4$ and 2.0 µM $CuSO_4$ and 50.0 µM $ZnSO_4$. Thus, it could be suggested that 1.0 µM $CuSO_4$ and 50.0 µM $ZnSO_4$, 2.0 µM $CuSO_4$ and 25.0 µM $ZnSO_4$ and 0.5 µM $CuSO_4$ and 25.0 µM $ZnSO_4$ are the better combinations which gives positive effect on germination and seedlings development.

Plate 1 Shoot height in T_6 (1.0 µM $CuSO_4$ and 50.0 µM $ZnSO_4$) and T_1 (Control) five months after incubation

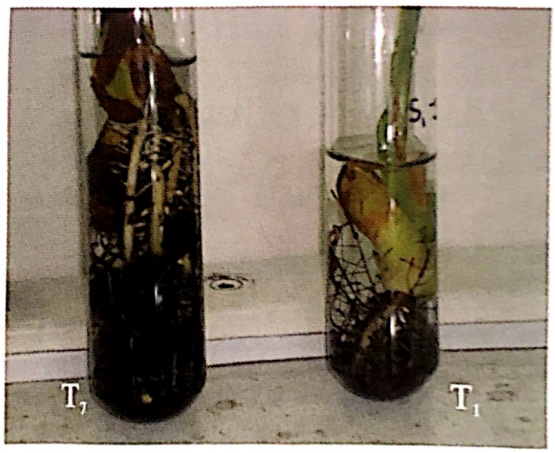

Plate 2 Primary root growth in T_7 (2.0 µM $CuSO_4$ and 25.0 µM $ZnSO_4$) and T_1 (Control) five months after incubation

Plate 3 Secondary root growth in T_7 (2.0 µM $CuSO_4$ and 25.0 µM $ZnSO_4$) and Control five months after incubation

Table 2 Means of germination of embryos and growth of seedlings five months after culturing of coconut embryos

Treatments			Germination (%)	Shoot height (cm) ± SE	No. of primary roots ± SE	No. of Secondary roots ± SE	No. of Leaves ± SE	Fresh weight of seedling (g) ± SE
	$CuSO_4$ (µM)	$ZnSO_4$ (µM)						
T_1	0	0	42.8	8.62 (±0.052)	1.22 (±0.039)	0.71 (±0.082)	0.51 (±0.013)	8.51 (±0.031)
T_2	0.5	12.5	45.5	17.1 (±0.037)	2.11 (±0.009)	1.51 (±0.063)	0.89 (±0.058)	14.6 (±0.052)
T_3	0.5	25.0	65.7***	25.1*** (±0.033)	2.25 (±0.006)	1.40 (±0.036)	1.28*** (±0.043)	16.4*** (±0.019).
T_4	1.0	12.5	51.4	16.1 (±0.039)	2.28 (±0.006)	1.14 (±0.048)	0.97 (±0.067)	10.9 (±0.029)
T_5	1.0	25.0	62.8	20.2 (±0.036)	1.88 (±0.015)	1.28 ±0.041)	1.20 (±0.047)	12.2 (±0.053)
T_6	1.0	50.0	65.7***	24.6*** (±0.033)	2.68*** (±0.099)	1.71*** (±0.025)	1.28*** (±0.043)	15.9*** (±0.038)
T_7	2.0	25.0	68.5***	26.0*** (±0.032)	3.02*** (±0.094)	1.74*** (±0.024)	1.54*** (±0.032)	16.7*** (±0.022)
T_8	2.0	50.0	45.0	16.0 (±0.039)	1.68 (±0.020)	1.02 (±0.055)	0.88 (±0.060)	9.32 (±0.031)
MSD value			**20.2**	**8.21**	1.08	**0.81**	**0.72**	**6.54**

Figures in parenthesis indicate the standard error MSD-Minimum Significant Difference,
*** Significant at the 0.05 level

The results of this experiment could be used for further studies on micronutrients, especially in adopting better Cu and Zn combinations in embryo culture media to produce vigorous seedlings with higher shoot and root growth which will lead to pot culture studies.

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