CHAPTER 5

Effect of fertilizer application combining with AMF mixed with EDB on growth, nutrient uptake and level of IAA in *C. alismatifolia* Gagnep.

5.1 Introduction

AMF are important for plant health, nutrient uptake, survival rate and conservation of soil structure. AMF procure and transport phosphate and other nutrients from soil to plant root, the host plant, in turn, provides fixed carbon (Harrison, 1999). Thereafter, EDB have been isolated from a number of plants in which significant biological N_2 fixation has been demonstrated. Although EDB have the potential to promote, it is still uncertain what their interaction is with other beneficial organisms, e.g., AMF, and how this might affect plant growth (Gutiérrez-Miceli *et al*., 2008).

Curcuma is of economic importance for Thailand. Rhizomes are exported to many countries around the word. *Curcuma* production uses abundant chemical fertilizers, so the alternative method is to reduce chemical fertilizers. Thus, AMF and EDB have high potential for application in many crops. They can reduce the need for

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

fertilizer N and P, incluing increased income from high yields and reduced fertilizer costs (James and Olivares, 1998; Kennedy *et al*., 2004).

AMF was highly effective on growth as explained, in Chapter 3, so it was selected for mixing with the best two EBD as described in Chapter 4. Therefore, the research in this chapter was focused on the effects of AMF mixed with EDB application on growth and development, nutrient uptake and level of IAA in *C. alismatifolia* Gagnep.

5.2 Materials and methods

5.2.1 Plant materials and experimental treatments

Rhizomes of *C. alismatifolia* with the diameter of 2 cm and 4-5 storage roots were grown in black plastic pots containing sand: rice husk: rice husk charcoal at the ratio of 1 : 1 : 1 under natural environment. A selected AMF (*Glomus claroideum*) in Chapter 3 was added in this experiment into growing media with the rate of 200 spores/plant. Plants were subjected to experimental treatments with two factors. The first factor was the two levels of fertilizers application, i.e., at 0.0 g/pot and 7.5 g/pot. The second factor was four types of microorganism application, i.e., 1) using only AMF 2) adding with AMF+ECS203 (the highest of N_2 fixation isolate) 3) adding with ECS202 (the highest of IAA production isolate) and 4) adding with AMF+ ECS202+ECS203. After the 1st leaf had fully expanded, four microorganism treatments were added into plants again with the same method and concentration (10^6cell/ml). Fertilizer application treatments were supplied with 15-15-15 until flowering twice a month at 7.5 g/pot then added with 13 13-21 until senescence twice a month at 7.5 g/pot, compared with non-fertilizer treatment.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

5.2.2 Data collection

Plant height and number of leaves/plant were measured at every 2 weeks after planting (WAP) until flowering (12 WAP). Fresh and dry weight of rhizome, storage roots, fibrous roots, new rhizome, leaves and inflorescences were measured at 12 WAP. Chlorophyll content was measured at 12 WAP using chlorophyll meter (Spad-502, Minolta, Japan). The total leaf area was measured using an LI3100 Area meter (Lincoin Nebraska, USA) at 12 WAP.

Diffusible IAA mesurement

The leaves were cut off at 12 WAP (3-4 leaves) into falcon test tubes (one plant per tube), which contained 5.0 ml of 0.10 M phosphate buffer at pH 6.2. The tubes were placed in ice boxes and incubated in the dark at 22 $^{\circ}$C and 100% RH for 20 hrs. Then, the leaves were removed and the tubes were kept at -20 $^{\circ}$C until use for the IAA analysis by Gruber and Bangerth method (1990).

Inflorescence quality

The quality attributes of inflorescence, i.e., peduncle length, inflorescence length, inflorescence width, number of green bracts and number of pink bracts at flowering stage were recorded at 12 WAP.

Nitrogen, Phosphorus and Potassium analysis

Nitrogen (N) concentration, phosphorus (P) concentration in each organ were analysed by modified method of Ohyama *et al.*, 1991 and potassium (K) concentration was determined by Atomic Adsorption Spectrometers (Mizukoshi *et al*., 1994) at flowering stage (12 WAP) and harvest (26 WAP).

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Rhizome quality

The quality of rhizome, i.e., diameter of rhizome, diameter of storage roots, number of rhizomes per cluster, number of storage roots, length of storage roots, fresh and dry weight of rhizome and storage roots were measured at harvest.

5.2.2.4 Statistical analysis

Experimental design was a factorial in Completely Randomized Design (CRD) with 2x4 factorial treatments, four replicates per treatment. Means of each pair of data combinations within the same stage were analyzed using a statistical analysis program, the Statistic 8 (SXW Tallahassee, FL, USA). The least significant difference (LSD) was used to interpret significant difference among the means ($p<0.05$).

5.3 Results

5.3.1 Plant growth

Plant height was measured from the base of the pseudostem to the top of leaf tip when assembling all leaves together. The result showed that the average height (Figure 5.1) and number of leaves per plant increased rapidly from 2 WAP until 12 WAP (Table 5.1) and then increased gradually until the flowering stage.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

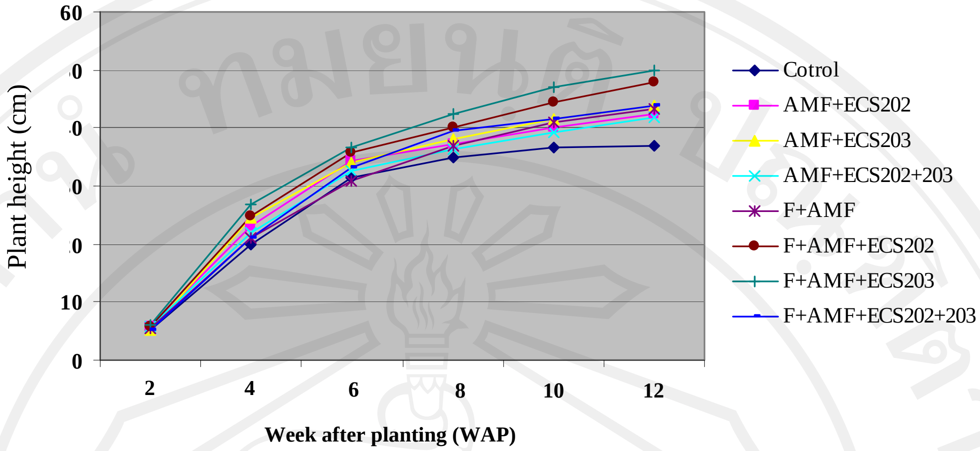

Figure 5.1 Effects of fertilizer rates and AMF mixed with EDB treatments on plant height of *C. alismatifolia* from 2 - 12 WAP

Main effects

The fertilizer rate at 7.5 g/pot was significantly produced greater effects on plant height, chlorophyll content, total leaf area and IAA concentration than the treatment of 0 g/pot (non-fertilizer). The plant height increased to a maximum of 46.2 cm when fertilizer was applied at the rate of 7.5 g/pot at 12 WAP, while it only 41.2 cm in fertilizer rate at 0 g/pot was attained. The average number of leaves/plant was not statistically different (Figure 5.1 and Table 5.1).

Plant height, total leaf area and IAA concentration of the AMF+ECS203 gave the best result compared with the others but AMF+EDB treatments did not affect the number of leaves/plant and chlorophyll content (Figure 5.2 and Table 5.1). However, AMF+ ECS202 had highest IAA concentration, followed by AMF+ECS202+ECS203 treatment.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Interaction between factors

The interaction between two factors was determined at 12 WAP. There was an interaction between fertilizer rates and AMF+EDB treatments on plant height, chlorophyll content, total leaf area and IAA concentration (Table 5.2). Applying fertilizer at 7.5 g/pot and adding with AMF+ECS203 gave the best of plant height growth than other treatments. The result also indicated that adding AMF+EDB with or without fertilizer application gave higher chlorophyll content than non-fertilizer application and adding with only AMF. Total leaf area was the greatest in treatment of 7.5 g fertilizer application rate plus AMF+ECS203. Leaf diffusible IAA was greatest in the treatment of 7.5 g fertilizer application plus ECS202 (Table 5.2).

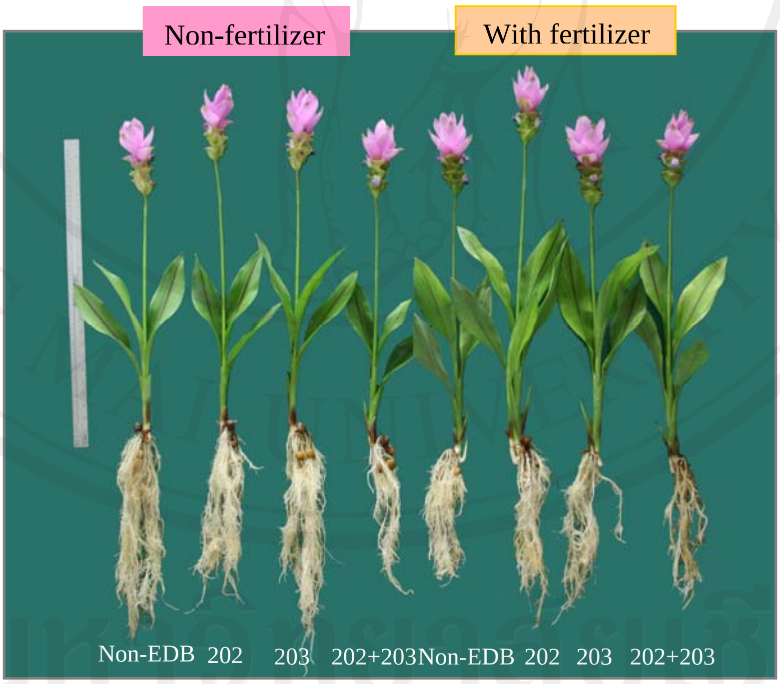

Figure 5.2 Effects of fertilizer rates and AMF mixed with EDB treatments on growth of *C. alismatifolia* at 12 WAP (flowering stage)

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Table 5.1 Effects of fertilizer rates and AMF mixed with or without EDB treatments on growth and leaf diffusible IAA (pg/leaf/20hr) of *C. alismatifolia* at 12 WAP.

Factors		Plant height	Number of leaves/plants	Chlorophyll contents	Total leaf area	IAA concentration
Fertilizer rates	0.0 g/pot	41.2 b	3.6	45.6 b	261.38 b	344.98 b
	7.5 g/pot	46.2 a	3.7	48.6 a	363.48 a	439.74 a
F-test		*	ns	*	*	*
$LSD_{0.05}$		1.03	0.17	2.59	32.48	12.72
AMF+EDB	Non-EDB	40.2 d	3.6	44.2	263.80 c	339.20 d
	ECS202	45.1 b	3.6	47.9	322.36 ab	435.38 a
	ECS203	46.9 a	3.8	49.0	364.36 a	380.67 c
	ECS202+203	42.8 c	3.6	44.2	299.19 bc	414.19 b
F-test		*	ns	ns	*	*
$LSD_{0.05}$		1.46	0.24	3.67	45.94	18.00

Means within the factor in the same columns followed by different characters showed significant difference between treatments by LSD test at *P* <0.05.

The symbol “ns” is : not significantly different and “*” is significantly different at the *P*<0.05

Table 5.2 The combination of fertilizer rates and AMF mixed with or without EDB treatments on growth and leaf diffusible IAA (pg/leaf/20hr) of *C. alismatifolia* at 12 WAP.

Fertilizer	EDB	Plant height	Number of leaves/plants	Chlorophyll content	Total leaf area	IAA concentration
0.0 g/pot	AMF+Non	37.0 d	3.5	39.0 b	243.60 d	272.62 f
0.0 g/pot	AMF+ECS202	42.3 bc	3.6	48.2 a	260.53 d	381.99 d
0.0 g/pot	AMF+ECS203	43.9 b	3.8	47.7 a	269.12 cd	340.35 e
0.0 g/pot	AMF+ECS202+203	41.7 c	3.6	47.2 a	272.26 cd	384.94 d
7.5 g/pot	AMF+Non	43.3 bc	3.6	49.5 a	284.00 cd	405.77 cd
7.5 g/pot	AMF+ECS202	47.9 a	3.6	47.6 a	384.19 b	488.77 a
7.5 g/pot	AMF+ECS203	49.8 a	3.8	50.2 a	459.61 a	420.98 bc
7.5 g/pot	AMF+ECS202+203	43.8 b	3.6	47.2 a	326.11 bc	443.44 b
F-test		*	ns	*	*	*
$LSD_{0.05}$		2.06	0.33	5.18	64.96	25.45

Means within the factor in the same columns followed by different characters showed significant difference between treatments by LSD test at *P* <0.05.

The symbol “ns” is : not significantly different and “*” is significantly different at the *P*<0.05

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright © by Chiang Mai University
All rights reserved

5.3.2 Inflorescence quality

Main effects

Inflorescence length, inflorescence width and number of pink bracts were significantly higher when plant was applied with fertilizer rate at 7.5 g/pot than non-fertilizer treatment (Figure 5.2 and Table 5.3). On the other hand, the number of green bracts was greatest in non fertilizer treatment (Table 5.3).

Addition of AMF+EDB gave significant difference in all parameters of inflorescence qualities. The adding of AMF+ECS203 gave highest results in peduncle length, inflorescence length, inflorescence width and number of pink bracts except number of green bracts (Figure 5.2 and Table 5.3).

Interaction between factors

The interaction between fertilizer application and AMF+EDB adding was found in the parameters of inflorescence length, inflorescence width and number of pink bracts. The fertilizer application plus AMF+ECS203 adding gave the best of inflorescence length, inflorescence width and the number of pink bracts than the other treatments. However, there was no interaction between fertilizer rates and EDB treatments on peduncle length and number of green bracts (Table 5.4).

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

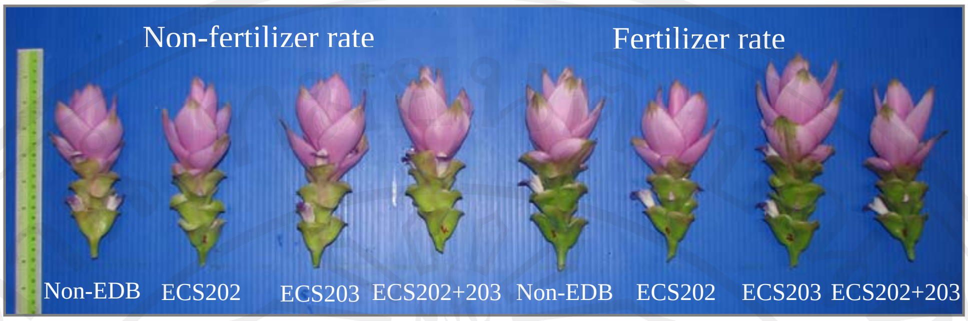

Figure 5.3 Effects of fertilizer rates and AMF mixed with EDB treatments on quality of inflorescence of *C. alismatifolia* at 12 WAP (flowering stage)

Table 5.3 Effects of fertilizer rates and AMF mixed with or without EDB treatments on inflorescence quality of *C. alismatifolia* at 12 WAP.

Factors		Inflorescence quality				
		Peduncle length (cm)	Inflorescence length (cm)	Inflorescence width (cm)	Number of green bracts	Number of pink bracts
Fertilizer rate	0.0 g/pot	51.14	14.90 b	5.18 b	9.0 a	11.2 b
	7.5 g/pot	52.51	16.73 a	5.96 a	8.8 b	11.8 a
F-test		ns	*	*	*	*
$LSD_{0.05}$		1.50	0.52	0.32	0.25	0.37
AMF+EDB	Non-EDB	47.77 b	15.82 b	5.46 b	9.3 a	11.3 b
	ECS202	51.16 b	15.45 b	5.23 b	8.9 b	11.4 b
	ECS203	56.30 a	16.61 a	6.26 a	8.8 b	12.1 a
	ECS202+203	52.07 b	15.37 b	5.34 b	8.6 b	11.2 b
F-test		*	*	*	*	*
$LSD_{0.05}$		2.18	0.74	0.45	0.35	0.52

Means within the factor in the same columns followed by different characters showed significant difference between treatments by LSD test at $P<0.05$.

The symbol “ns” is : not significantly different and “*” is significantly different at the $P<0.05$

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Table 5.4 The combination of fertilizer rates and AMF mixed with or without EDB treatments on inflorescence quality of *C. alismatifolia* at 12 WAP.

Fertilizer	EDB	Inflorescence quality				
		Peduncle length (cm)	Inflorescence length (cm)	Inflorescence width (cm)	Number of green bracts	Number of pink bracts
0.0 g/pot	AMF+Non	46.18	14.45 d	4.82 d	9.3	10.7 d
0.0 g/pot	AMF+ECS202	51.58	14.82 cd	5.04 cd	9.2	11.4 bcd
0.0 g/pot	AMF+ECS203	55.03	15.20 bcd	5.46 c	8.7	11.5 bc
0.0 g/pot	AMF+ECS202+203	51.77	15.12 bcd	5.40 cd	8.8	11.2 bcd
7.5 g/pot	AMF+Non	49.36	17.20 a	6.10 b	9.3	11.9 b
7.5 g/pot	AMF+ECS202	50.74	15.92 b	5.42 cd	8.6	11.4 bcd
7.5 g/pot	AMF+ECS203	57.56	18.02 a	7.05 a	8.7	12.6 a
7.5 g/pot	AMF+ECS202+203	52.37	15.78 bc	5.28 cd	8.4	11.1 cd
F-test		ns	*	*	ns	*
$LSD_{0.05}$		3.00	1.04	0.63	0.50	0.72

Means within the factor in the same columns followed by different characters showed significant difference between treatments by LSD test at *P* <0.05.

The symbol “ns” is : not significantly different and “*” is significantly different at the *P*<0.05

5.3.3 Fresh weight of *C. alismatifolia* at 12 WAP

Main effects

The result suggested that fertilizer rate at 7.5 g/pot significantly increased fresh weight of each organ, i.e., rhizome , new rhizome, leaves and inflorescence compared with using 0 g/pot fertilizer application, except storage roots fresh weight (Table 5.5).

Application of AMF plus EDB gave the significant difference in rhizome FW, new rhizome FW and leaf FW as shown in Table 5.5. Adding AMF+ECS202 and AMF+ECS203 resulted in more fresh weight of rhizome, new rhizome and leaves than the others. However, storage roots FW, fibrous root FW and inflorescence were not significantly different among treatments.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Interaction between factors

The interaction between fertilizer rates and AMF+EDB treatments had effects on new rhizome and leaf fresh weight (Table 5.6). Treatments of fertilizer mixed with AMF, AMF+ESC202, AMF+ECS203 each isolates gave the highest average of new rhizome than non-fertilizer with or without treatments.

Table 5.5 Effects of fertilizer rates and AMF mixed with or without EDB treatments on inflorescence fresh weight of *C. alismatifolia* at 12 WAP**.**

Factors		Fresh weight (g)					
		Rhizome	Storage roots	Fibrous roots	New rhizome	Leaves	Inflorescence
Fertilizer rates	0.0 g/pot	3.15 b	16.62	34.02 a	4.19 b	19.17 b	32.66 b
	7.5 g/pot	3.36 a	17.63	22.59 b	6.61 a	28.84 a	44.09 a
F-test		*	ns	*	*	*	*
LSD0.05		0.16	1.43	2.21	0.55	1.61	2.89
AMF+EDB	Non-EDB	3.06 b	15.98	28.06	5.43 a	20.42 b	37.74
	ECS202	3.36 a	17.75	27.95	5.74 a	26.41 a	38.27
	ECS203	3.39 a	18.10	30.64	5.78 a	27.06 a	40.56
	ECS202+203	3.22 ab	16.68	26.57	4.65 b	22.13 b	35.93
F-test		*	ns	ns	*	*	ns
$LSD_{0.05}$		0.22	2.03	3.12	0.78	2.28	4.10

Means within the factor in the same columns followed by different characters showed significant difference between treatments by LSD test at *P* <0.05.

The symbol “ns” is : not significantly different and “*” is significantly different at the *P*<0.05

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Table 5.6 The combination of fertilizer rates and AMF mixed with or without EDB treatments on fresh weight of *C. alismatifolia* at 12 WAP.

Fertilizer	EDB	Fresh weight (g)					
		Rhizome	Storage roots	Fibrous roots	New rhizome	Leaves	Inflorescence
0.0 g/pot	AMF+Non	3.04	15.68	34.47	3.78 c	18.08 d	30.75
0.0 g/pot	AMF+ECS202	3.25	17.34	32.95	4.35 bc	19.13 d	32.41
0.0 g/pot	AMF+ECS203	3.14	16.41	35.81	4.54 bc	19.93 cd	33.82
0.0 g/pot	AMF+ECS202+203	3.16	17.06	32.82	4.10 c	19.55 cd	33.67
7.5 g/pot	AMF+Non	3.07	16.27	21.64	7.09 a	22.76 bc	44.73
7.5 g/pot	AMF+ECS202	3.47	18.16	22.45	7.12 a	33.69 a	44.13
7.5 g/pot	AMF+ECS203	3.63	19.80	25.46	7.02 a	34.18 a	47.3
7.5 g/pot	AMF+ECS202+203	3.28	16.30	20.32	5.20 b	24.71 b	40.19
F-test		ns	ns	ns	*	*	ns
$LSD_{0.05}$		0.31	2.86	4.41	1.10	1.61	5.79

Means within the factor in the same columns followed by different characters showed significant difference between treatments by LSD test at P <0.05.

The symbol "ns" is : not significantly different and "*" is significantly different at the P<0.05

5.3.4 Dry weight of *C. alismatifolia* at 12 WAP

Main effects

The fertilizer rate at 7.5 g/pot significantly effected in dry weight of rhizome, new rhizome, leaves and inflorescence when compared with fertilizer rate at 0 g/pot (Table 5.7).

Application of AMF plus EDB gave the significant differences in leaf DW and inflorescence DW, as shown in Table 5.7. The dry weight in all organs of AMF+ECS203 gave the best result compared with the others, followed by AMF+ECS202.

Interaction between factors

The interaction between fertilizer rates and AMF+EDB treatments had effects on leaf and inflorescence dry weight (Table 5.8). Treatment of fertilizer mixed with

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

AMF, AMF+ESC202, AMF+ECS203 and AMF+ECS202+ECS203 gave the highest average of inflorescence DW than non-fertilizer with or without EDB treatments.

Table 5.7 Effects of fertilizer rates and AMF mixed with or without EDB treatments on dry weight of *C. alismatifolia* at 12 WAP**.**

Factors		Dry weight (g)					
		Rhizome	Storage roots	Fibrous roots	New rhizome	Leaves	Inflorescence
Fertilizer rate	0.0 g/pot	0.50 b	1.66	2.06 a	1.78 b	2.55 b	2.42 b
	7.5 g/pot	0.72 a	1.75	1.32 b	1.99 a	3.26 a	3.18 a
F-test		*	ns	*	*	*	*
$LSD_{0.05}$		0.05	0.15	0.17	0.10	1.16	0.21
AMF+EDB	Non-EDB	0.59	1.56	1.65	1.88	2.60 b	2.84 a
	ECS202	0.62	1.73	1.68	1.94	3.09 a	2.84 a
	ECS203	0.63	1.81	1.78	1.92	3.24 a	3.05 a
	ECS202+203	0.60	1.72	1.65	1.80	2.70 b	2.46 b
F-test		ns	ns	ns	ns	*	*
$LSD_{0.05}$		0.07	0.21	0.24	0.14	0.23	0.30

Means within the factor in the same columns followed by different characters showed significant difference between treatments by LSD test at P <0.05.

The symbol "ns" is : not significantly different and "*" is significantly different at the P<0.05

Table 5.8 The combination of fertilizer rates and AMF mixed with or without EDB treatments on dry weight of *C. alismatifolia* at 12 WAP.

Fertilizer	EDB	Dry weight (g)					
		Rhizome	Storage roots	Fibrous roots	New rhizome	Leaves	Inflorescence
0.0 g/pot	AMF+Non	0.47	1.47	2.18	1.67	2.38 c	2.24 b
0.0 g/pot	AMF+ECS202	0.50	1.73	2.06	1.84	2.61 bc	2.37 b
0.0 g/pot	AMF+ECS203	0.50	1.70	2.12	1.86	2.67 bc	2.61 b
0.0 g/pot	AMF+ECS202+203	0.54	1.74	1.88	1.76	2.54 bc	2.45 b
7.5 g/pot	AMF+Non	0.70	1.64	1.12	2.09	2.82 b	3.44 a
7.5 g/pot	AMF+ECS202	0.75	1.73	1.30	2.05	3.57 a	3.32 a
7.5 g/pot	AMF+ECS203	0.76	1.92	1.45	1.97	3.81 a	3.49 a
7.5 g/pot	AMF+ECS202+203	0.67	1.70	1.42	1.84	2.85 b	2.48 b
F-test		ns	ns	ns	ns	*	*
$LSD_{0.05}$		0.10	0.30	0.35	0.20	0.32	0.43

Means within the factor in the same columns followed by different characters showed significant difference between treatments by LSD test at P <0.05.

The symbol "ns" is : not significantly different and "*" is significantly different at the P<0.05

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

5.3.5 Nitrogen (N) concentration in each organ

Main effects

Fertilizer application at 7.5 g/pot gave the higher N concentration than non-fertilizer application (Table 5.9). N concentrations (mg/g DW) were 33.55, 15.64, 37.65. 6.07, 18.37 and 8.18 in old rhizome, old storage roots, new rhizome, new fibrous roots, leaves and inflorescence, respectively, when plants were supplied with 7.5 g/pot of fertilizer. There concentrations became very low when plant were not supplied with the fertilizer, ranging about 3.13-6.14 mg/g DW, as shown in Table 5.9.

The AMF mixed with or without EDB treatment had affected N concentrations in all organs. Adding only AMF without EDB gave the lower N concentrations in all organs and adding with AMF+ECS203 gave the highest N concentration in all organs (Table 5.9).

Interaction between factors

The interactions between two main factors were found in new rhizome and leaves of *C. alismatifolia* at flowering stage, while the old rhizome, old storage roots, new fibrous roots and inflorescence were not affected. The combination between fertilizer application at 7.5 g/pot adding with AMF+ECS203 or AMF+ECS202 gave the highest N concentrations in new rhizome and leaves (Table 5.10).

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Table 5.9 Effects of fertilizer rates and AMF mixed with or without EDB treatments on nitrogen concentration of *C. alismatifolia* at 12 WAP**.**

Factors		Nitrogen concentration (mg/g DW)					
		Old rhizome	Old storage roots	New rhizome	New fibrous roots	Leaves	Inflorescence
Fertilizer rates	0.0 g/pot	6.14 b	5.12 b	5.60 b	3.13 b	6.75 b	4.49 b
	7.5 g/pot	33.55 a	15.64 a	37.65 a	6.07 a	18.37 a	8.18 a
F-test		*	*	*	*	*	*
$LSD_{0.05}$		0.66	0.38	0.93	0.29	0.59	0.59
AMF+EDB	Non-EDB	19.02 b	9.97 b	20.45 c	4.44 b	11.59 b	5.60 c
	ECS202	19.94 ab	10.46 ab	22.26 ab	4.72 ab	12.61 a	6.15 bc
	ECS203	20.30 a	10.77 a	22.72 a	4.86 a	13.28 a	7.84 a
	ECS202+203	20.12 a	10.31 ab	21.08 bc	4.41 b	12.76 a	6.73 b
F-test		*	*	*	*	*	*
$LSD_{0.05}$		0.93	0.54	1.31	0.41	0.83	0.83

Means within the factor in the same columns followed by different characters showed significant difference between treatments by LSD test at *P* <0.05.

The symbol "ns" is : not significantly different and "*" is significantly different at the *P*<0.05

Table 5.10 The combination of fertilizer rates and AMF mixed with or without EDB treatments on nitrogen concentration of *C. alismatifolia* at 12 WAP.

Fertilizer	EDB	Nitrogen concentration (mg/g DW)					
		Old rhizome	Old Storage roots	New rhizome	New fibrous roots	Leaves	Inflorescence
0.0 g/pot	AMF+Non	5.29	4.66	4.63 c	2.81	5.10 d	3.86
0.0 g/pot	AMF+ECS202	6.22	5.28	5.98 c	3.28	6.90 c	4.67
0.0 g/pot	AMF+ECS203	6.11	5.24	5.72 c	3.24	7.40 c	6.04
0.0 g/pot	AMF+ECS202+203	6.95	5.28	6.07 c	3.22	7.61 c	5.38
7.5 g/pot	AMF+Non	32.76	15.28	36.26 b	6.06	18.08 ab	7.35
7.5 g/pot	AMF+ECS202	33.66	15.63	38.53 a	6.17	18.33 ab	7.63
7.5 g/pot	AMF+ECS203	34.50	16.30	39.73 a	6.48	19.16 a	9.65
7.5 g/pot	AMF+ECS202+203	33.29	15.34	36.08 b	5.59	17.92 b	8.08
F-test		ns	ns	*	ns	*	ns
$LSD_{0.05}$		1.31	0.76	1.86	0.58	1.17	1.18

Means within the factor in the same columns followed by different characters showed significantly different between treatments by LSD test at *P* <0.05.

The symbol "ns" is : not significantly different and "*" is significantly different at the *P*<0.05

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

5.3.6 Phosphorus (P) concentration in each organ

Main effects

The result suggested that fertilizer rate at 7.5 g/pot significantly increased P concentration of all organs, i.e., old rhizome, old storage roots, fibrous roots, new rhizome, leaves and inflorescence compared with 0 g/pot fertilizer application (Table 5.11).

Application of AMF plus EDB gave the significant difference in old rhizome, old storage roots, fibrous roots, new rhizome, leaves and inflorescence as shown in Table 5.9. Adding AMF+ECS202 and AMF+ECS203 gave higher P concentration in new rhizome than the others. The values were 6.19 and 6.32 mg/g DW, respectively.

Interaction between factors

The interaction between fertilizer rates and EDB treatments were significantly different in old rhizome, new rhizome and leaves as shown in Table 5.12. The combination between fertilizer application at 7.5 g/pot adding with AMF+ECS203 or AMF+ECS202 gave the best of P concentration in old rhizome, old storage roots, new rhizome, fibrous roots, leaves and inflorescence.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Table 5.11 Effects of fertilizer rates and AMF mixed with or without EDB treatments on phosphorus concentration of *C. alismatifolia* at 12 WAP**.**

Factors		Phosphorus concentration (mg/g DW)					
		Old rhizome	Old storage roots	New rhizome	New fibrous roots	Leaves	Inflorescence
Fertilizer rates	0.0 g/pot	4.76 b	4.84 b	5.25 b	2.94 b	3.08 b	2.85 b
	7.5 g/pot	6.10 a	5.71 a	6.62 a	3.35 a	3.61 a	3.48 a
F-test		*	*	*	*	*	*
$LSD_{0.05}$		0.29	0.45	0.34	0.25	0.31	0.26
AMF+EDB	Non-EDB	4.71 c	5.00	5.18 b	2.78 b	2.87 b	2.66 b
	ECS202	5.60 ab	5.14	6.19 a	3.31 a	3.49 a	3.29 a
	ECS203	5.93 a	5.80	6.32 a	3.32 a	3.55 a	3.41 a
	ECS202+203	5.47 b	5.16	6.04 a	3.17 a	3.47 a	3.29 a
F-test		*	ns	*	*	*	*
$LSD_{0.05}$		0.40	0.63	0.49	0.35	0.44	0.37

Means within the factor in the same columns followed by different characters showed significant difference between treatments by LSD test at *P* <0.05.

The symbol “ns” is : not significantly different and “*” is significantly different at the *P*<0.05

Table 5.12 The combination of fertilizer rates and AMF mixed with or without EDB treatments on phosphorus concentration of *C. alismatifolia* at 12 WAP.

Fertilizer	EDB	Phosphorus concentration (mg/g DW)					
		Old rhizome	Old storage roots	New rhizome	New fibrous roots	Leaves	Inflorescence
0.0 g/pot	AMF+Non	3.65 e	4.21	4.05 d	2.50	2.19 b	2.09
0.0 g/pot	AMF+ECS202	4.96 d	4.71	5.61 c	3.12	3.38 a	3.01
0.0 g/pot	AMF+ECS203	5.43 cd	5.76	5.88 bc	3.13	3.42 a	3.21
0.0 g/pot	AMF+ECS202+203	4.97 d	4.68	5.46 c	3.00	3.34 a	3.11
7.5 g/pot	AMF+Non	5.77 bc	5.79	6.32 ab	3.07	3.55 a	3.24
7.5 g/pot	AMF+ECS202	6.23 ab	5.57	6.76 a	3.50	3.60 a	3.57
7.5 g/pot	AMF+ECS203	6.43 a	5.85	6.76 a	3.50	3.68 a	3.61
7.5 g/pot	AMF+ECS202+203	5.97 abc	5.54	6.63 a	3.34	3.60 a	3.48
F-test		*	ns	*	ns	*	ns
$LSD_{0.05}$		0.57	0.90	0.70	0.50	0.63	0.52

Means within the factor in the same columns followed by different characters showed significant difference between treatments by LSD test at *P* <0.05.

The symbol “ns” is : not significantly different and “*” is significantly different at the *P*<0.05

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

5.3.7 Potassium (K) concentration in each organ

Main effects

Fertilizer application at 7.5 g/pot gave significant increase in K concentration when compared with non-fertilizer application, except in new rhizome (Table 5.13). When plants were supplied with fertilizer at 7.5 g/pot, the K concentration were 24.45, 56.64, 89.14, 181.31, 159.96 and 3.14 mg/g DW in old rhizome, old storage roots, new rhizome, new fibrous roots, leaves and inflorescence, respectively.

The AMF mixed with or without EDB treatment had affected K concentrations in all organs. Adding with AMF+ECS203 gave the highest K concentration in all organs, except new fibrous roots and leaves while adding with AMF+ECS202 and AMF+ECS202+ECS203 gave the highest K concentrations in leaves and new fibrous root, respectively. Adding only AMF without EDB gave lower K concentrations in all organs (Table 5.13).

Interaction between factors

The combination of fertilizer rates and EDB treatments on K concentration was found to affect old rhizome and fibrous roots as shown in Table 5.14. Treatments of fertilizer mixed with AMF+ESC202, AMF+ECS203 and AMF+ECS202+ECS203 gave the highest averages than non-fertilizer with or without EDB treatments.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Table 5.13 Effects of fertilizer rates and AMF mixed with or without EDB treatments on potassium concentration of *C. alismatifolia* at 12 WAP**.**

Factors		Potassium concentration (mg/g DW)					
		Old rhizome	Old storage roots	New rhizome	New fibrous root	Leaves	Inflorescence
Fertilizer rates	0.0 g/pot	20.89 b	43.63 b	88.14	107.25 b	128.38 b	2.99 b
	7.5 g/pot	24.45 a	56.64 a	89.14	181.31 a	159.96 a	3.14 a
F-test		*	*	ns	*	*	*
$LSD_{0.05}$		1.31	2.07	3.01	7.55	8.83	0.16
AMF+EDB	Non-EDB	20.80 b	44.98 c	84.27 c	123.62 c	127.51 b	2.79 b
	ECS202	22.75 a	48.22 b	88.03 bc	140.13 b	153.98 a	3.26 a
	ECS203	23.49 a	56.97 a	92.53 a	144.91 b	143.43 a	3.20 a
	ECS202+203	23.02 a	50.37 b	89.74 ab	168.46 a	151.77 a	3.20 a
F-test		*	*	*	*	*	*
$LSD_{0.05}$		1.85	2.93	4.26	10.68	12.48	0.22

Means within the factor in the same columns followed by different characters showed significant difference between treatments by LSD test at $P < 0.05$.

The symbol “ns” is : not significantly different and “*” is significantly different at the $P<0.05$

Table 5.14 The combination of fertilizer rates and AMF mixed with or without EDB treatments on potassium concentration of *C. alismatifolia* at 12 WAP.

Fertilizer	EDB	Potassium concentration (mg/g DW)					
		Old rhizome	Old storage roots	New rhizome	New fibrous roots	Leaves	Inflorescence
0.0 g/pot	AMF+Non	18.51	34.69 e	83.18	83.18 e	107.8	2.82
0.0 g/pot	AMF+ECS202	21.47	41.05 d	88.55	107.92 d	140.03	3.00
0.0 g/pot	AMF+ECS203	21.93	52.71 b	91.78	100.05 d	128.9	3.07
0.0 g/pot	AMF+ECS202+203	21.63	46.09 c	89.07	137.83 c	136.8	3.09
7.5 g/pot	AMF+Non	23.09	55.28 b	85.35	164.05 b	147.22	2.78
7.5 g/pot	AMF+ECS202	24.02	55.38 b	87.52	172.34 b	167.93	3.53
7.5 g/pot	AMF+ECS203	25.06	61.23 a	93.29	189.77 a	157.95	3.33
7.5 g/pot	AMF+ECS202+203	24.42	54.65 b	90.41	199.09 a	166.74	3.32
F-test		ns	*	ns	*	ns	ns
$LSD_{0.05}$		2.61	4.15	6.03	15.10	17.65	0.31

Means within the factor in the same columns followed by different characters showed significant difference between treatments by LSD test at $P < 0.05$.

The symbol “ns” is : not significantly different and “*” is significantly different at the $P<0.05$

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

5.3.8 Quality of rhizome at harvest stage

Main effects

This research indicated that fertilizer rate significantly influenced the diameter of storage roots and number of rhizomes. Fertilizer rate at 7.5 g/pot gave greater diameter of storage roots and number of rhizomes than those in the fertilizer rate at 0 g/pot. However, the diameter of rhizome, numbers of storage roots and length of storage roots were not different between the fertilizer rate treatments (Figure 5.3 and Table 5.15).

The result showed that EDB treatments had affected diameter of storage roots, number of rhizome, number of storage roots and length of storage roots, except diameter of rhizome (Figure 5.3, Table 5.15). AMF+ECS203 gave the best result compared with the other treatments.

Interaction between factors

The interaction between fertilizer rates and EDB treatments was significantly different in diameter of storage roots and number of storage roots, while it had no effected on diameter of rhizome, number of rhizome and length of storage roots (Table 5.16). The combination between fertilizer application and AMF adding gave the highest diameter of storage roots and it was not significantly different from the combination between fertilizer application and AMF+ECS203 and AMF+ECS202+ECS203 treatments (Table 5.16). However, the number of storage

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

roots were the lowest in the concentration of non-fertilizer application plus AMF adding and fertilizer application plus AMF+ECS202+ECS203 treatments (Table 5.16).

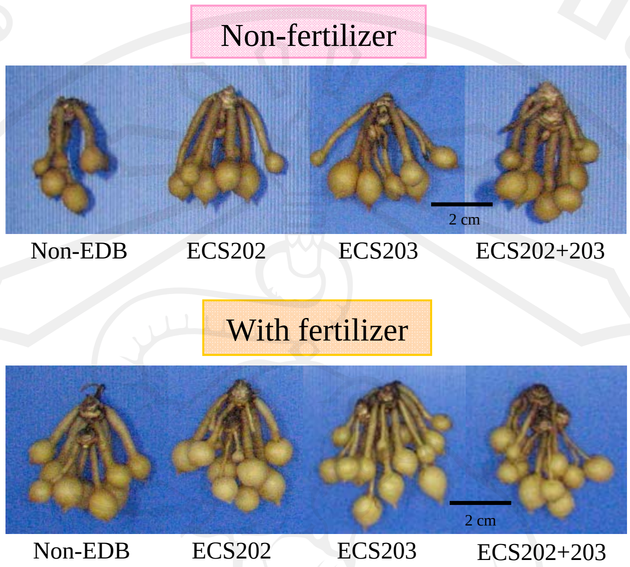

Figure 5.4 Effects of fertilizer rates and AMF mixed with or without EDB treatments on quality of rhizome of *C. alismatifolia* at harvest stage.

Table 5.15 Effects of fertilizer rates and AMF mixed with or without EDB treatments on quality of rhizome of *C. alismatifolia* at harvest stage.

Factors		Quality of rhizome at harvest stage				
		Diameter of rhizome	Diameter of storage roots	Number of rhizome	Number of storage roots	Length of storage roots
Fertilizer rates	0.0 g/pot	2.05	2.06 b	2.21 b	6.23	7.03
	7.5 g/pot	2.15	2.18 a	2.81 a	6.28	7.06
F-test		ns	*	*	ns	ns
LSD0.05		0.11	0.08	0.27	0.64	0.28
AMF+EDB	Non-EDB	2.04	2.06 b	2.15 c	5.55 b	7.14 a
	ECS202	2.08	2.06 b	2.35 bc	6.35 b	6.86 b
	ECS203	2.14	2.22 a	2.80 a	7.65 a	7.34 a
	ECS202+203	2.15	2.14 ab	2.73 ab	5.48 b	6.86 b
F-test		ns	*	*	*	*
$LSD_{0.05}$		0.16	0.12	0.38	0.91	0.40

Means within the factor in the same columns followed by different characters showed significant difference between treatments by LSD test at P <0.05.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright by Chiang Mai University
All rights reserved

The symbol “ns” is : not significantly different and “*” is significantly different at the P<0.05

Table 5.16 The combination of fertilizer rates and AMF mixed with or without EDB treatments on quality of rhizome of *C. alismatifolia* at harvest stage.

Fertilizer	EDB	Quality of rhizome at harvest stage				
		Diameter of rhizome	Diameter of storage roots	Number of rhizome	Number of storage roots	Length of storage roots
0.0 g/pot	AMF+Non	1.98	1.82 d	2.0	4.7 c	6.94
0.0 g/pot	AMF+ECS202	1.99	2.00 c	2.0	6.9 b	6.76
0.0 g/pot	AMF+ECS203	2.05	2.25 ab	2.5	7.0 b	7.38
0.0 g/pot	AMF+ECS202+203	2.19	2.18 ab	2.3	6.3 b	7.05
7.5 g/pot	AMF+Non	2.10	2.29 a	2.3	6.4 b	7.34
7.5 g/pot	AMF+ECS202	2.16	2.13 abc	2.7	5.8 bc	6.95
7.5 g/pot	AMF+ECS203	2.24	2.19 ab	3.1	8.3 a	7.29
7.5 g/pot	AMF+ECS202+203	2.11	2.10 bc	3.1	4.6 c	6.67
F-test		ns	*	ns	*	ns
$LSD_{0.05}$		0.23	0.17	0.54	1.28	0.57

Means within the factor in the same columns followed by different characters showed significant difference between treatments by LSD test at P <0.05.

The symbol “ns” is : not significantly different and “*” is significantly different at the P<0.05

5.3.9 Fresh and dry weight of rhizome at harvest

Main effects

Fertilizer rate at 7.5 g/pot generally produced better fresh and dry weight as compared with the non-fertilizer treatment (Table 5.17).

The result demonstrated that AMF+EDB each isolate were significantly different with AMF+non-EDB treatment on fresh and dry weight of rhizome and storage roots. Adding with AMF+ECS203 gave 10.7 g of rhizome FW, 67.09 g of storage roots FW, 2.43 g of rhizome DW and 9.71 g of storage roots DW (Table 5.17).

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Interaction between factors

There was an interaction between fertilizer rates and AMF+EDB treatments on fresh and dry weight of storage roots. AMF+ECS203 gave the best fresh and dry weight of rhizome compared with the other treatments (Table 5.18).

Table 5.17 Effects of fertilizer rates and AMF mixed with or without EDB treatments on fresh and dry weight of rhizome and storage roots of *C. alismatifolia* at harvest stage.

Factors		Fresh weight (g)		Dry weight (g)	
		Rhizome	Storage roots	Rhizome	Storage roots
Fertilizer rates	0.0 g/pot	7.88 b	45.76 b	1.64 b	6.54
	7.5 g/pot	11.16 a	62.06 a	2.58 a	7.91
F-test		*	*	*	ns
$LSD_{0.05}$		1.11	5.78	0.35	1.46
AMF+EDB	Non-EDB	7.65 b	51.25 b	1.52 b	6.16 b
	ECS202	9.59 a	51.04 b	2.11 a	6.59 b
	ECS203	10.70 a	67.09 a	2.43 a	9.71 a
	ECS202+203	10.13 a	46.27 b	2.38 a	6.42 b
F-test		*	*	*	*
$LSD_{0.05}$		1.57	8.18	0.49	2.06

Means within the factor in the same columns followed by different characters showed significant difference between treatments by LSD test at *P* <0.05.

The symbol “ns” is : not significantly different and “*” is significantly different at the *P*<0.05

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Table 5.18 The combination of fertilizer rates and AMF mixed with or without EDB treatments on fresh and dry weight of rhizome and storage roots of *C. alismatifolia* at harvest stage.

Fertilizer	EDB	Fresh weight (g)		Dry weight (g)	
		Rhizome	Storage roots	Rhizome	Storage roots
0.0 g/pot	AMF+Non	6.15	35.94 e	1.08	4.73 c
0.0 g/pot	AMF+ECS202	7.76	42.19 de	1.67	6.41 bc
0.0 g/pot	AMF+ECS203	8.84	56.26 bc	1.71	7.72 b
0.0 g/pot	AMF+ECS202+203	8.78	48.66 cd	2.10	7.28 bc
7.5 g/pot	AMF+Non	9.15	66.56 ab	1.96	7.60 bc
7.5 g/pot	AMF+ECS202	11.42	59.88 bc	2.55	6.78 bc
7.5 g/pot	AMF+ECS203	12.56	77.92 a	3.15	11.69 a
7.5 g/pot	AMF+ECS202+203	11.49	43.87 de	2.66	5.56 bc
F-test		ns	*	ns	*
$LSD_{0.05}$		2.22	11.56	0.69	2.92

Means within the factor in the same columns followed by different characters showed significant difference between treatments by LSD test at $P<0.05$.

The symbol “ns” is : not significantly different and “*” is significantly different at the $P<0.05$

5.3.10 Nitrogen (N) concentration at harvest

Main effects

The result showed that fertilizer rate at 7.5 g/pot significantly increased N concentration in all organs. This indicated that the fertilizer rate at 7.5 g/pot was the major factor affecting all organs compared with non-fertilizer (Table 5.19).

AMF+EDB treatments significantly increased N concentration in all organs compared with AMF+non-EDB treatment (Table 5.19). AMF+ECS203 treatment gave the best N concentration in new rhizome. N concentration was 35.14 mg/g DW while N concentration in new storage roots of AMF+ECS202 treatment was 7.70 mg/g DW.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Interaction between factors

The interactions between fertilizer application and AMF+EDB treatments were found in new rhizome of N concentration. The fertilizer application and AMF+EDB treatments gave higher result when compared with non fertilizer application and with or without EDB treatments. Fertilizer+AMF+ECS203 had high N concentration in new rhizome (38.61 mg/g DW) while fertilizer+AMF+ECS202 had high N concentration in new storage roots (9.48 mg/g DW) as shown in Table 5.20.

5.3.11 Phosphorus (P) concentration at harvest

Main effects

Fertilizer application at 7.5 g/pot gave higher P concentration than non-fertilizer application (Table 5.19). P concentrations were 8.37 and 9.92 mg/g DW in new rhizome and new storage roots, respectively, when plants were supplied with 7.5 g/pot of fertilizer.

The AMF mixed with or without EDB treatment had affected P concentrations in new rhizome and new storage roots. Adding only AMF without EDB gave the lower P concentrations in new rhizome and new storage roots and adding with AMF+ECS203 gave the highest P concentrations in new rhizome and new storage roots (8.30 and 9.75 mg/g DW, respectively) (Table 5.19).

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright © by Chiang Mai University
All rights reserved

Interaction between factors

The interactions between two main factors were found in new rhizome and new storage roots of *C. alismatifolia* at harvest stage. The combination between fertilizer application at 7.5 g/pot adding with AMF+ECS203 gave the highest P concentrations in new rhizome and new storage roots. P concentrations were 8.42 and 11.04 mg/mg DW, respectively (Table 5.20).

5.3.12 Potassium (K) concentration at harvest

Main effects

Fertilizer application at 7.5 g/pot significantly increased K concentration compared with non fertilizer application in new rhizome and new storage roots (Table 5.19). When plants were supplied with fertilizer at 7.5 g/pot, the K concentrations were 115.33 and 163.01 mg/mg DW in new rhizome and new storage roots, respectively

The AMF mixed with or without EDB treatment had affected K concentrations in new rhizome and new storage roots. Adding with AMF+ECS203 or AMF+ECS202+ECS203 gave the highest K concentrations in new rhizome and new storage roots. The K concentration of AMF+ECS203 was highest in new rhizome (107.75 mg/g DW), while that of AMF+ECS202+ECS203 was highest in new storage roots (136.12 mg/g DW) (Table 5.19).

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Interaction between factors

The combinations of fertilizer application and EDB treatments on K concentration were found to affect new rhizome and new storage roots as shown in Table 5.20. Treatment of fertilizer mixed with AMF+ESC202, AMF+ECS203 and AMF+ECS202+ECS203 gave the highest K concentrations in new rhizome and new storage roots compared with non-fertilizer with or without EDB treatments.

Table 5.19 Effects of fertilizer rates and AMF mixed with or without EDB treatments on nitrogen, phosphorus and potassium concentrations of *C. alismatifolia* at harvest stage.

Factors		Nitrogen concentration (mg/g DW)		Phosphorus concentration (mg/g DW)		Potassium concentration (mg/g DW)	
		New rhizome	New storage roots	New rhizome	New storage roots	New rhizome	New storage roots
Fertilizer rates	0.0 g/pot	29.75 b	5.65 b	7.80 b	8.21 b	91.26 b	84.65 b
	7.5 g/pot	37.51 a	9.02 a	8.37 a	9.92 a	115.33 a	163.01 a
F-test		*	*	*	*	*	*
$LSD_{0.05}$		1.59	0.45	0.29	0.34	2.38	4.26
AMF+EDB	Non-EDB	30.14 b	6.67 b	7.49 b	8.26 c	92.80 b	103.17 c
	ECS202	34.75 a	7.70 a	8.28 a	9.02 b	105.13 a	124.64 b
	ECS203	35.14 a	7.52 a	8.30 a	9.75 a	107.75 a	131.38 a
	ECS202+203	34.50 a	7.43 a	8.28 a	9.23 b	107.51 a	136.12 a
F-test		*	*	*	*	*	*
$LSD_{0.05}$		2.25	0.63	0.42	0.48	3.37	6.03

Means within the factor in the same columns followed by different characters showed significant difference between treatments by LSD test at P <0.05.

The symbol "ns" is : not significantly different and "*" is significantly different at the P<0.05

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Table 5.20 The combination of fertilizer rates and AMF mixed with or without EDB treatments on nitrogen, phosphorus and potassium concentrations of *C. alismatifolia* at harvest stage.

Fertilizer	EDB	Nitrogen concentration (mg/g DW)		Phosphorus concentration (mg/g DW)		Potassium concentration (mg/g DW)	
		New rhizome	New storage roots	New rhizome	New storage roots	New rhizome	New storage roots
0.0 g/pot	AMF+Non	23.61 c	4.69	6.72 b	7.51 e	87.58 d	61.60 e
0.0 g/pot	AMF+ECS202	32.10 b	5.92	8.16 a	8.43 d	91.22 cd	88.37 d
0.0 g/pot	AMF+ECS203	31.66 b	6.04	8.17 a	8.46 d	93.76 bc	94.94 d
0.0 g/pot	AMF+ECS202+203	31.63 b	5.94	8.17 a	8.45 d	92.50 c	93.69 d
7.5 g/pot	AMF+Non	36.66 a	8.65	8.26 a	9.01 cd	98.02 b	144.75 c
7.5 g/pot	AMF+ECS202	37.41 a	9.48	8.40 a	9.62 bc	119.04 a	160.90 b
7.5 g/pot	AMF+ECS203	38.61 a	9.00	8.42 a	11.04 a	121.74 a	167.82 b
7.5 g/pot	AMF+ECS202+203	37.37 a	8.93	8.40 a	10.00 b	122.53 a	178.55 a
F-test		*	ns	*	*	*	*
$LSD_{0.05}$		3.18	0.89	0.59	0.68	4.76	8.52

Means within the factor in the same columns followed by different characters showed significant difference between treatments by LSD test at P <0.05.

The symbol "ns" is : not significantly different and "*" is significantly different at the P<0.05

5.4 Discussion

Fertilizer effects

Ruamrungsri *et al*. (2006) reported that growers supplied N-P-K [15-0-0] or [21-7-14] fertilizer grade once a month from the two-leaf fully-expanded stage until the flowering stage, and then changed the fertilizer ratio to 13-13-21. In this experiment, it was found that the application of fertilizer rate at 7.5 g/pot was satisfactory for *Curcuma* production. Plant height, chlorophyll content, total leaf area, fresh and dry weight were significantly increased by fertilizer rate at 7.5 g/pot, except fresh and dry weight of storage roots. The similar results also were found by Siritrakulsak (2010) that the fertilizer application at 7.5 g/pot should be recommended

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

for *C. alismatifolia* production. Furthermore, there were differences in leaf diffusible IAA levels of fertilizer rates in this experiment, suggesting that fertilizer application treatment was related to IAA synthesis in plant (Table 5.1). Fertilizer is important for plant growth and development. So, fertilizer can increase IAA in plant because N is a basic element of IAA structure. Zahir *et al*. (2007) reported that IAA blended N enriched compost plus 60 kg/ha N increased plant height and tillers more than using urea fertilizer only (120 kg/ha N) in wheat. However, IAA-treated N-enriched compost plus 60 kg/ha N fertilizer gave fresh biomass (9.5 t/ha) lower than kinetin-blended N enriched compost plus 60 kg/ha N (10.0 t/ha). Similarly, Zahir *et al*. (2000) reported up to 50% increase in fresh biomass of soybean by the application of L-tryptophan (precursor of IAA).

Moreover, fertilizer rate at 7.5 g/pot significantly increased inflorescence length, inflorescence width, number of green bracts and pink bracts compared with non-fertilizer rate, while peduncle length was not different. Lee *et al*. (2008) also found that application of liquid fertilizer at 6.6 g/l could promote inflorescence stem length of this plant.

Fresh and dry weight of some organs derived from fertilizer rate at 7.5 g/pot treatment were significantly different from non-fertilizer in rhizome, fibrous roots, new rhizome, leaves and flower, except in storage roots. Similarly, Ohtake *et al*. (2006) showed that the number and dry weight of new rhizomes, organs for the new generation, increased with the N-feeding level. In contrast, the dry weight of the storage roots decreased with N supply.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

The fertilizer application treatment had the high effect in diameter of storage roots, number of rhizome, fresh weight of rhizome and storage roots and dry weight of rhizome (Tables 5.15 and 5.17). Lee *et al*. (2008) reported that application of liquid fertilizer at 2.7 g/l gave the best result on rhizome yield of *C. alismatifolia,* compared with 0, 1.3, 4.0, 5.3 and 6.6 g/l, and the different species of *Curcuma* showed the different response to fertilizer rate. Fertilizer application at 7.5 g/pot could increase quantity and quality attributes of *C. alismatifolia*, it was due to net photosynthesis. Marschner (1986) revealed that mineral nutrition was required for many process to the formation and function of chloroplasts, mainly as enzyme protein. Non-fertilizer-application treatment brought about the deficiency of mineral nutrient in plant tissue in each organ as shown in Tables 5.9, 5.11, 5.13 and 5.19. The deficiency of mineral nutrient is directly involved in the electron transport chain and in photophosphorylation, leading to a decrease in photosynthetic activity (Marschner, 1986). It caused the decrease of growth and development of *C. alismatifolia* in this research. Tapun (2006) reported that the nitrogen status in *C. alismatifolia* at flowering stage averages (mg/g DW) 6.6 in fibrous roots, 17.7 in leaves, 7.2 in inflorescence and 24.8 in new rhizome. The concentrations were close to those in this experiment, indicating that these concentrations were in the adequate range for optimum growth of this plant.

Microorganism effects

Kuklinsky-Sobral *et al*. (2004) found that some EDB of soybean had potential for promoting plant growth by the production of IAA and nitrogen fixation. Similar to this experiment, the inoculated plant with AMF+ECS203 gave the best value in plant

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

height and total leaf area. However, the inoculated plant with AMF+ECS202 gave higher IAA concentration than the others, followed by AMF+ECS202+ECS203 treatment. Ruamrungsri *et al*. (2009) also found that ECS202 isolated from leaves of *C. alismatifolia* could produce IAA concentration more than the other isolates. Similar to rice, four strains of *Pseudomonas fluorescens*, *Pseudomonas tolaasii*, *Pseudomonas veronii* and *Sphingomonas trueperi* significantly enhanced plant growth in the absence of pathogens, as evidenced by increases in plant height and dry weight (Adhikari *et al*., 2001).

AMF+EDB treatment increased peduncle length, inflorescence length, inflorescence width, number of green bracts and pink bracts. Chang *et al*. (2008) reported that bio-fertilizer composition consisting of *Rhizoctonia* sp. (BCRC930076) and *Rhizoctonia* sp. (BCRC930077) increased the flowering rate and flowering quality, decreased the occurrence of root disease to prevent the usage of pesticide and increased the beneficial components toward human health of medicinal orchids.

Fresh weights of each organ from AMF+EDB treatments were significantly higher than the others in rhizome, new rhizome and leaves but dry weight was significantly higher in leaves and flower only. Nassar and El-Tarabily (2005) reported that plant inoculated with *Williopsis saturnus* significantly enhanced the growth of maize plants as evidenced by increase in the dry weight and lengths of roots and shoots compared with control plant. It was also found that *Pseudomonas* inoculants significantly increased root dry weight in spring wheat (Walley and Germida, 1997). *Sphingomonas* sp. isolated from potato also increased 66% of shoot fresh weight and 55% of root fresh weight (Sturz *et al*., 1998).

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

AMF+ECS203 gave highest in diameter of storage roots, number of rhizome, number of storage roots, length of storage roots, fresh weight and dry weight of rhizome and storage root. Zahir *et al*. (1997) studied the effect of inoculation with auxin-producing *Azotobacter* on potato yield. The result showed that application of *Azotobacter* increased the tuber and straw yield. The *Rhizobium* was also able to colonise the interior of the rice roots. It could increase shoot and root growth, grain yield and agronomic N-fertilizer efficiency significantly (Biswas *et al*., 2000).

Field experimental results in India showed that application of *Acetobacter diazotrophicus* by incoculating increased sugercane yield for varieties significantly when applied in association with AMF (Muthukumarasamy *et a*l., 1999). These results of fertilizer and AMF+EDB treatments were significant in diameter of storage roots, number of storage roots, fresh weight and dry weight of storage roots (Tables 5.16 and 5.18).

Biswas *et al*. (2000) reported that *Rhizobium* in rice roots could increase the uptake of N, P and K significantly. Similar to this experiment, AMF+ECS203 treatment significantly increased N, P and K concentration in each organ when compared to AMF adding with non-EDB. Boddey *et al*. (1991) studied activity of *Acetobacter diazotrophicus* by ^{15}N dilution/N balance. The result confirmed that up to 60 - 80% of sugarcane plant N (equivalent to over 200 kg N/ha/yr) was derived from biological nitrogen fixation (BNF). The study of N_2 fixation in soybean nodules by tracing experiment with $^{15}N_2$ gas also revealed that 60 - 75% of N assimilation in soybean was derived from N_2 fixation (Ohyama *et al*., 2010). The best P-solubilizing isolates were two *Bacillus* strains. The P-solubilizers were tested for their effects on

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

growth and P-uptake of canola plants in a P-deficient soil amended with rock phosphate. Some of the P-solubilizing rhizobacteria significantly increased plant height or pod yield and increased P-uptake. The most effective inoculant was a *B. thuringiensis* isolate which significantly increased the number and weight of pods and seed yield without rock phosphate (Freitas *et al*., 1996).

The increase of K concentration, especially when adding with EDB, may be caused by an indirect effect of ion uptake by plant. The role of K^+ in the cation-anion balance is reflected in nitrate metabolism. It is a counter-ion for NO_3^- transport in xylem and also for storage in vacuole (Marschner, 1986).

Interaction effects

It was interesting that there was an interaction between fertilizer application and AMF+EDB adding, especially on plant growth and quality attributes of inflorescence in *Curcuma*. The relation of fertilizer application and symbiotic N_2 fixation was reported in legume plant. Application of P affected nodulation and nodule growth of soybean. When legumes dependent on symbiotic nitrogen receive an inadequate supply of P, they will suffer from nitrogen deficiency. Furthermore, N application can stimulate or depress N_2 fixation in legume (Marschner, 1986). In this experiment, fertilizer application combined with AMF+EDB treatment could stimulate N and P uptake, especially in new rhizome and leaves, while K concentration was affected in old storage roots and new fibrous roots. However, non-fertilizer application and added with AMF+EDB gave the better of plant height, chlorophyll content, diameter of storage roots and the number of storage roots than

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

adding with only AMF. It may be due to the role of EDB on N_2 fixation and IAA synthesis in this plant as described in Chapter 4.

The interaction among fertilizer treatments and AMF+EDB treatments of N, P and K concentration at 12 WAP or flowering stage and harvest stage showed that fertilizer rate at 7.5 g/pot with AMF+EDB treatment had higher result than non-fertilizer with AMF+EDB treatments. This indicated that fertilizers had influence more than AMF mixed with EDB. The AMF and EDB had beneficial effects, such as provide nutrients (N and P) to plant (Paraskevopoulou-Paroussi *et al.*, 1997). Andrews *et al.* (2003) reported that when endophytic N_2 fixing bacteria, i.e., *Azotobacter* and *Azospirillum* were inoculated on non-legume plant, i.e., sugarcane, maize, wheat and barley, plant growth and yield were increased when grown in soil N deficiency. It was concluded that EDB could reduce the need for fertilizer N. Mia *et al.* (2010) showed that inoculating plant with plant growth promoting rhizobacteria (PGPR) also increased the plant growth, fruit quality, N yield and fixed N_2 in association with banana. Application of PGPR results in minimal or reduced level of fertilizer-N. It was suggested that PGPR interactions within the rhizosphere might have played an important role in restricting expression of growth promotion. Inoculation with PGPR increased sugar beet root weight, leaf, root and sugar yield by 2.8 - 46.7%, 15 - 20.8%, 12.3 - 16.1% and 9.8 - 14.7%, respectively, when compared without PGPR and mineral fertilizer (control). PGPR inoculations and fertilizer application also affected quality parameters investigated. Plant response to PGPR could be associated with other mechanisms rather than by direct N-fertilizer and P-solubilization. Plant growth parameters were generally enhanced by PGPR inoculation (Çakmakçi *et al.*, 2006). Furthermore, Paraskevopoulou-Paroussi *et al.*

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

(1997) studied AMF and fertilizer of strawberry cv. Selva. The result showed that shoot dry weight, root dry weight, leaf area, number of leaves, stolons and crowns of AMF-inoculated plant were greater than those of non-AMF plant which received N, P and K fertilizer. Inoculation significantly increased P concentration and uptake in the leaves of AMF plants, similar to AMF plants which significantly increased P concentration, P-uptake, %P derived from fertilizer and P-availability in vetiver. At 60 kgP_2O_5/ha level of application, the highest percentage of P derived from fertilizer (0.545%) were obtained from *Acaulospora scrobiculata* (Techapinyawat *et al*., 2002).

5.5 Conclusion

The effect of fertilizer rates and AMF mixed with EDB treatments on physiological responses of *C. alismatifolia* revealed that growth, inflorescence and rhizomes quality were increased with fertilizer application combined with AMF+EDB treatment. Fertilizer application promotes growth, led to the increases in general plant growth, IAA concentration and inflorescence quality of plant. In addition, AMF+EDB treatments could increase growth of this plant. The best treatment for *C. alismatifolia* plant in this experiment should be the use of fertilizer rate at 7.5 g/pot mixed with AMF+ECS203. However, further experiments should be carried out to reduce the application rate of chemical fertilizer.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved