CHAPTER 4

RESULTS

Experiment 1: Effects of defoliation and girdling on panicle position of potassium chlorate treated trees.

This study was started on March 2003. All of potassium chlorate treated trees (treatment no. 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 13) were flowering 28 days after application (Table 4) except treatment no. 5, 12 and 13 while the untreated tree did not flowering. Although, treatment no. 5, 12 and 13 did not flowering on 28 days but there were flowering after that. After treatment, the leaves of the trees in treatment 12 and 13 were died back from the shoot tip to the 3rd compound leaves. The first symptom was chlorosis and leaf drying before falling. But in treatment 5, it had new shoot flushing on terminal bud and then flowering later about 1 month after all treated longan trees were flowered. The leaves of longan trees in treatment 12 and 13 were falling down and new shoot flushed from axillary buds.

The first day of flowering was 21 days after potassium chlorate treatments at 10.00 g/pot (15 inches dimension). The percentage of flowering in treatment 3, 7, 9, and 11 were 100% while treatment 2, 8 were 67 % and the other treatments (treatment 4, 6 and 10) were 33% of flowering. However, the percentages of all treatments were 100% later.

All of none girdling treatments that had flowers at the shoot apex and the treatments without defoliation at 1st site had flower at the apex too. Treatments of girdling at 1st site with defoliated at 1st site were flowering at 2nd axillary bud or buds. Treatment of girdling at 2nd site with defoliated at 1st and 2nd site were flowering at 3rd axillary bud or buds.

Table 4 Effect of girdling and defoliation on flowering percentage and site of flowering in longan after treated with potassium chlorate for 28 days.

Treatment	Girdling	Defoliation	Potassium chlorate	Percentage of flowering [a/]	Site of flowering
1	none	None	no	0 b	-
2	none	None	yes	67 ab	terminal bud
3	none	1st site	yes	100 a	terminal bud
4	none	2nd site	yes	33 ab	terminal bud
5	none	1st site and 2nd site	yes	0 b	-
6	1st site	None	yes	33 ab	terminal bud
7	1st site	1st site	yes	100 a	2nd axillary bud
8	1st site and 2nd site	None	yes	67 ab	terminal bud, 2nd and 3rd axillary bud
9	1st site and 2nd site	1st site	yes	100 a	2nd and 3rd axillary bud
10	1st site and 2nd site	2nd site	yes	33 ab	terminal bud
11	1st site and 2nd site	1st site and 2nd site	yes	100 a	3rd axillary bud
12	1st site	2nd site	yes	0 b	-
13	1st site	1st site and 2nd site	yes	0 b	-
% CV	-	-	-	8.32	

[a/] : The Means in the same column followed by different letters were significant difference at p=0.05 by DMRT

The positions of panicles were depended on the sites of girdling and defoliation. If the segments of the shoot which created by girdling contained with the leaves (without defoliated), the terminal bud or axillary buds in those segments were changed to flower buds after treated with potassium chlorate but if the segments did not contained the leaves (with defoliated), the terminal bud or axillary buds in those segments were not changed to flower buds after treated with potassium chlorate.

Figure 10 The flowering of longan tree at terminal bud.

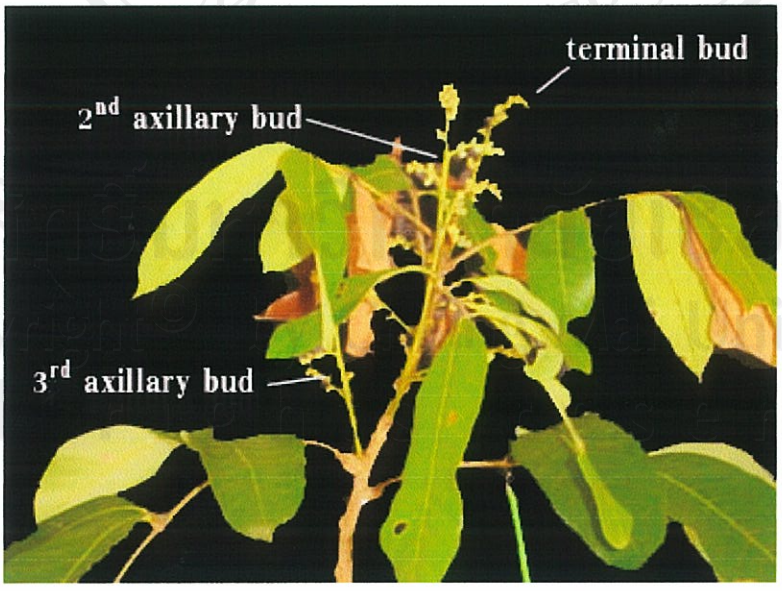

Figure 11 The flowering of longan tree at terminal, 2nd and 3rd axillary bud.

Figure 12 The flowering of treated with 1st site girdling, 1st site defoliation and potassium chlorate after the experiments had been finished.

Figure 13 The flowering of treated with 1st site girdling, 1st, 2nd site defoliation and potassium chlorate after the experiments had been finished.

Experiment 2 Effects of leaves maturity on some isozymes changes in potassium chlorate treated longan trees.

2.1 Effect of leaf maturation on flowering at 28 days after treatment.

This experiment was started in November 2003 which was near the natural flowering season of longan. It was found that treatments of 15 days leaf age with and without potassium chlorate treatments did not flowering at 28 days after treatment. Treatment of 30 days leaf age without potassium chlorate treatment did not flowering as those of 15 days leaf age treatments but treatment of 30 days leaf age which treated with potassium chlorate were flowered at 28 days after treatment. The treatments of 45 days leaf age with and without potassium chlorate treatment were flowered at 28 days after treatment (Table 5).

Table 5 The flowering of treated and untreated longan trees on difference leaf ages.

Treatment	Compound leaf age (days after flushing)	Treated with potassium chlorate	Flowering at 28 days after treatment
1	15	no	no
2	15	yes	no
3	30	no	no
4	30	yes	yes
5	45	no	yes
6	45	yes	yes

2.2 The pattern of isozymes

2.2.1 The pattern of peroxidase isozymes

It was found that the peroxidase isozyme patterns and zymograms of 15 days old leaves from treated and untreated longan trees were not differences after potassium chlorate application. The different of peroxidase isozyme patterns showed up by the maturity of the leaves. The treated and untreated 15 days old leaves from 0 and 1 days after potassium chlorate treatment showed only one band of peroxidase isozymes which those of 3 to 28 days after treatment showed two bands of peroxidase isozymes (Figure 14).

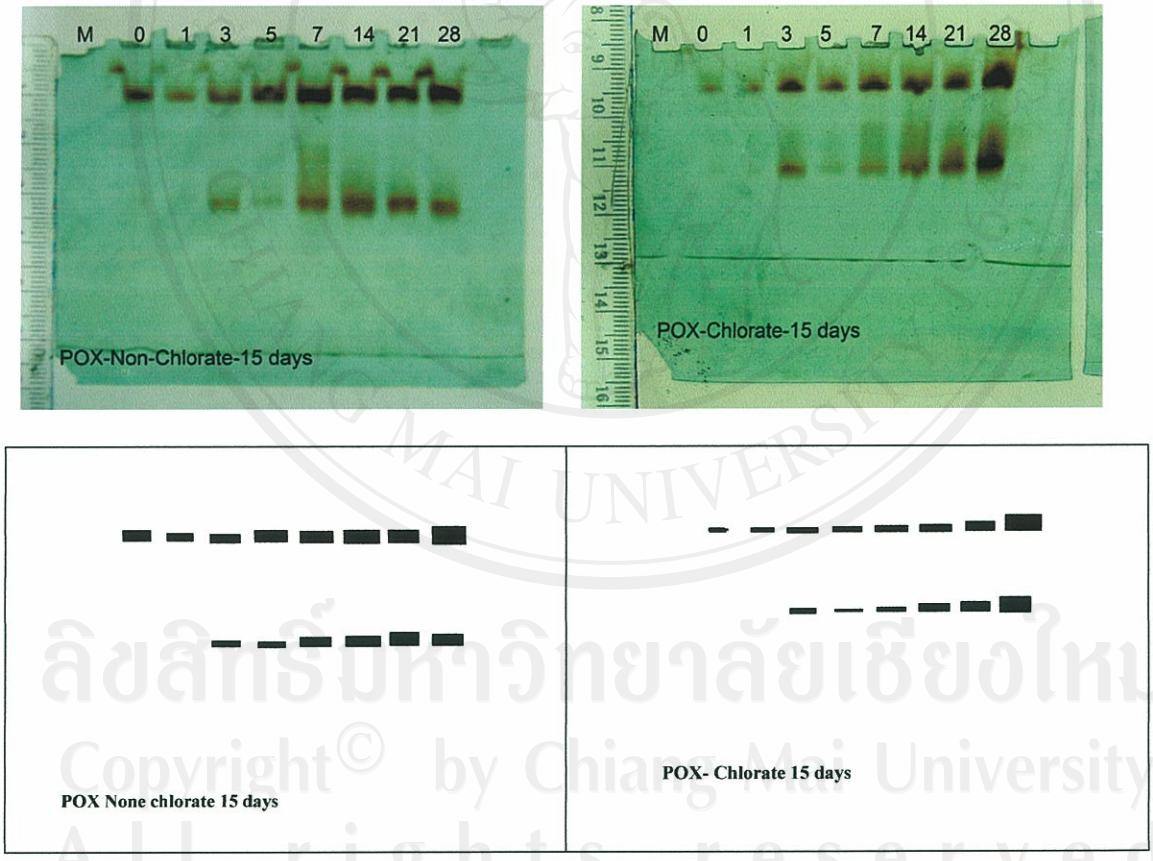

Figure 14 Peroxidase isozyme patterns and zymograms of 15 days old leaves of longan which treated and untreated with potassium chlorate.

It was found that the peroxidase isozyme patterns and zymograms of 30 days old leaves from treated and untreated longan tree were differences after potassium chlorate application. The pattern of treated longan trees had darker and wider bands of peroxidase isozymes than untreated longan trees (Figure 15).

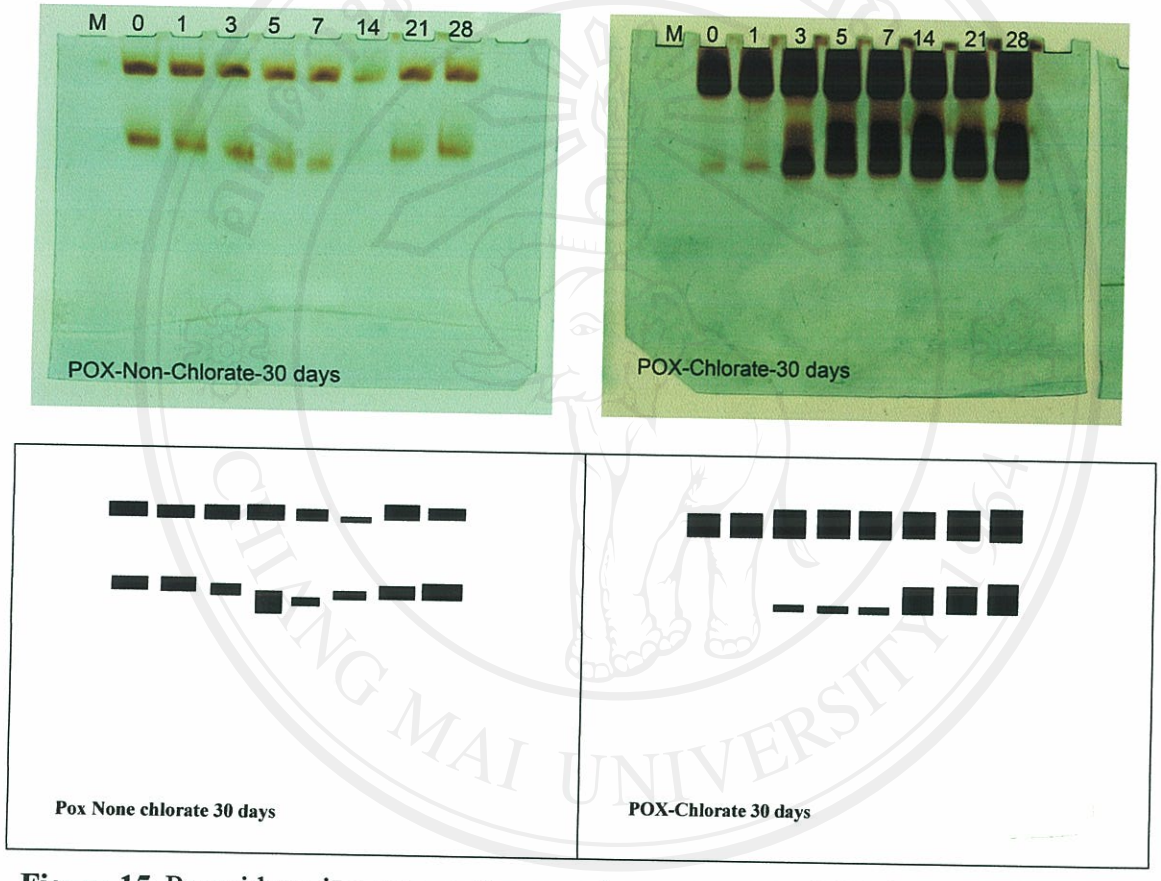

Figure 15 Peroxidase isozyme patterns and zymograms of 30 days old leaves of longan which treated and untreated with potassium chlorate.

It was found that the peroxidase isozyme patterns and zymograms of 45 days old leaves from treated and untreated longan tree were not differences after treated with potassium chlorate but the bands of peroxidase isozymes 3 to 28 days after treatment were darken than those of 15 and 30 days old leaves (Figure 16).

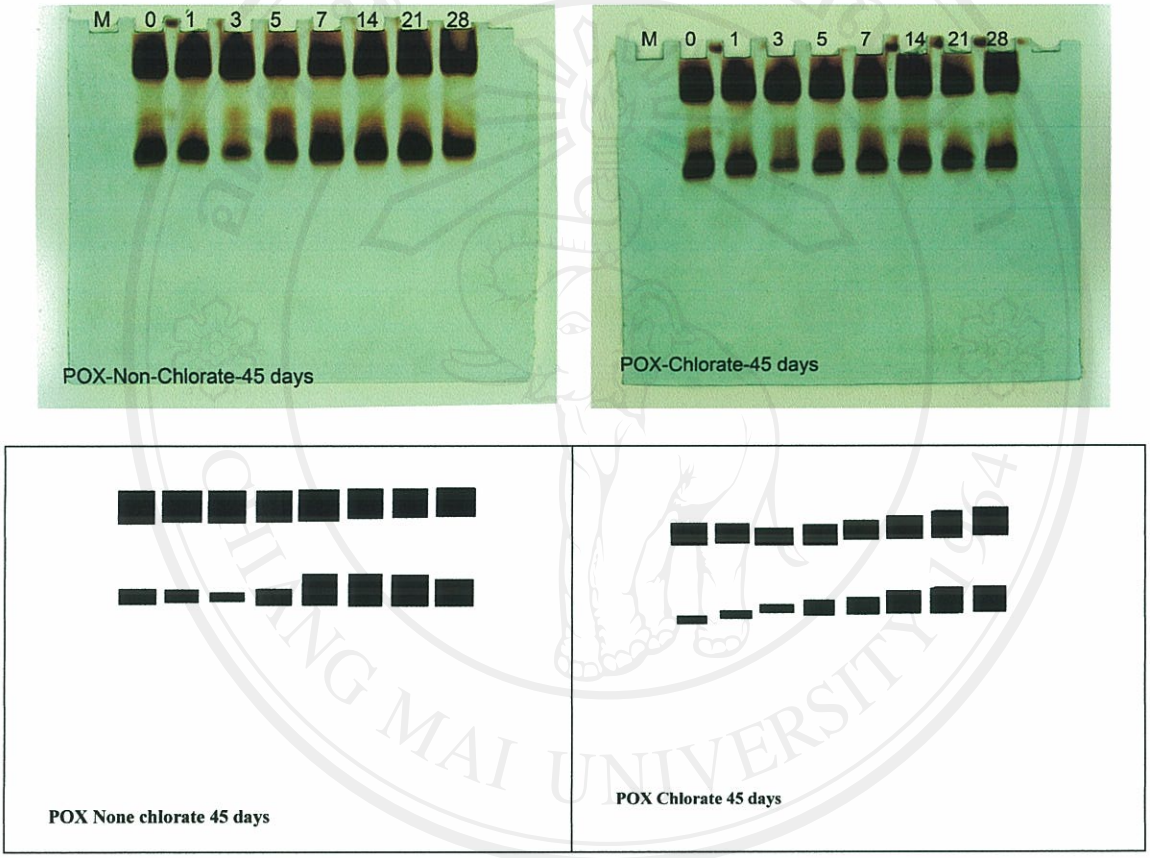

Figure 16 Peroxidase isozyme patterns and zymograms of 45 days old leaves of longan which treated and untreated with potassium chlorate.

2.3 The pattern of esterase enzyme

It was found that the esterase isozyme patterns and zymograms of 15 days old leaves from untreated longan trees were differences from treated longan trees. A number of bands in untreated longan trees were 2 bands but treated longan trees were 3 bands of esterase isozymes 7 to 28 days after treatment (Figure 17).

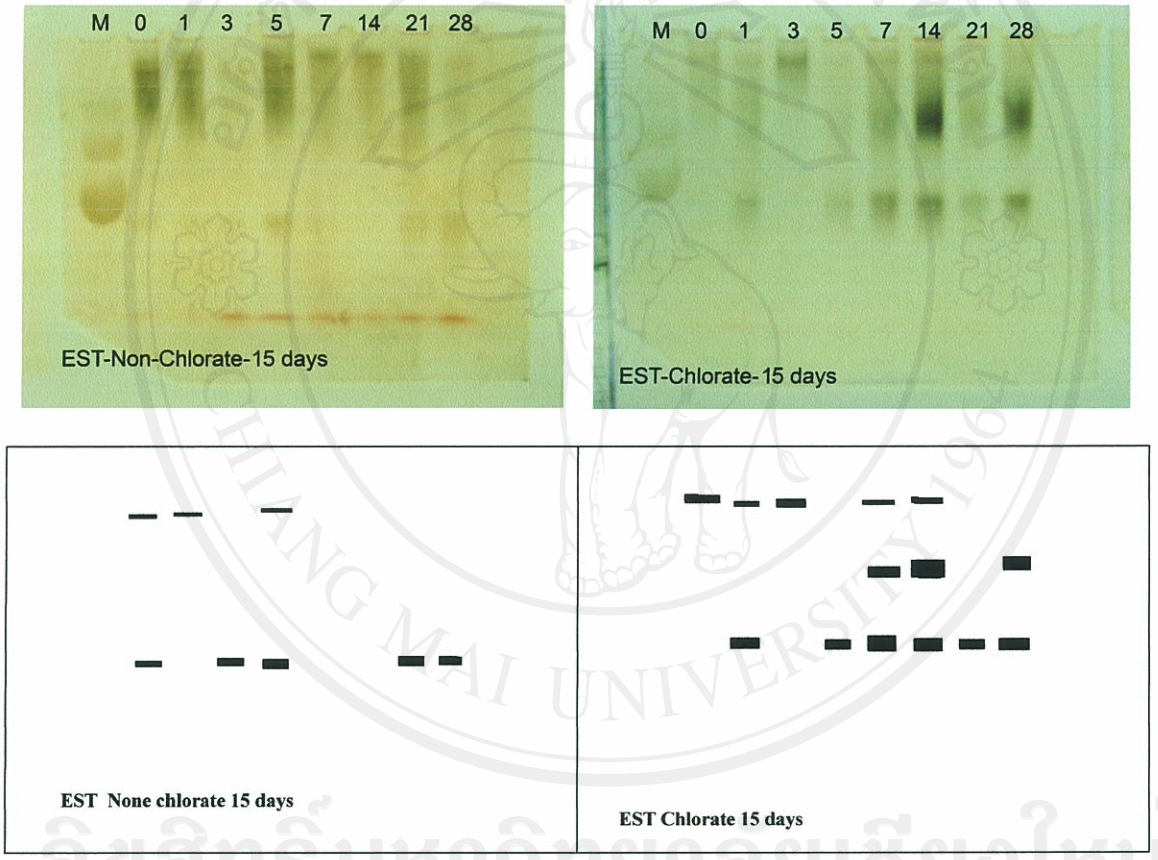

Figure 17 Esterase isozyme patterns and zymograms of 15 days old leaves of longan which treated and untreated with potassium chlorate.

It was found that the esterase isozyme patterns and zymograms of 30 days old leaves from untreated longan trees were differences from the treated longan trees. A number of esterase isozyme bands in untreated longan trees were 2 bands but treated longan trees were 4 bands of esterase isozyme 3 to 28 days after treatment (Figure 18).

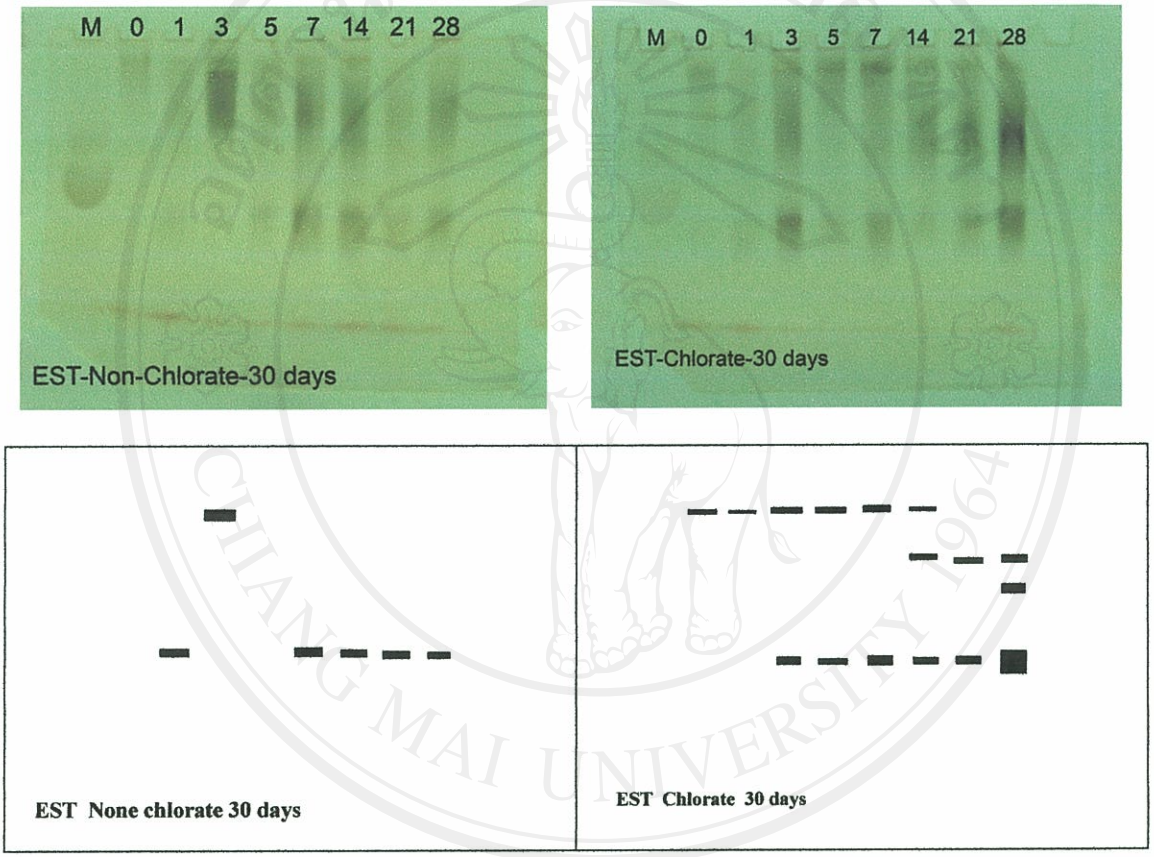

Figure 18 Esterase isozyme patterns and zymograms of 30 days old leaves of longan which treated and untreated with potassium chlorate.

It was found that the esterase isozyme patterns and zymograms of 45 days old leaves from untreated longan trees were differences from the treated longan trees. The esterase isozyme bands of untreated longan trees were 3 bands 0 to 7 days after treatment while treated longan trees were 4 bands of the isozymes. The one new band had been seen on 14 to 28 days after treatment and another new band had been seen on 28 days after treatment (Figure 19).

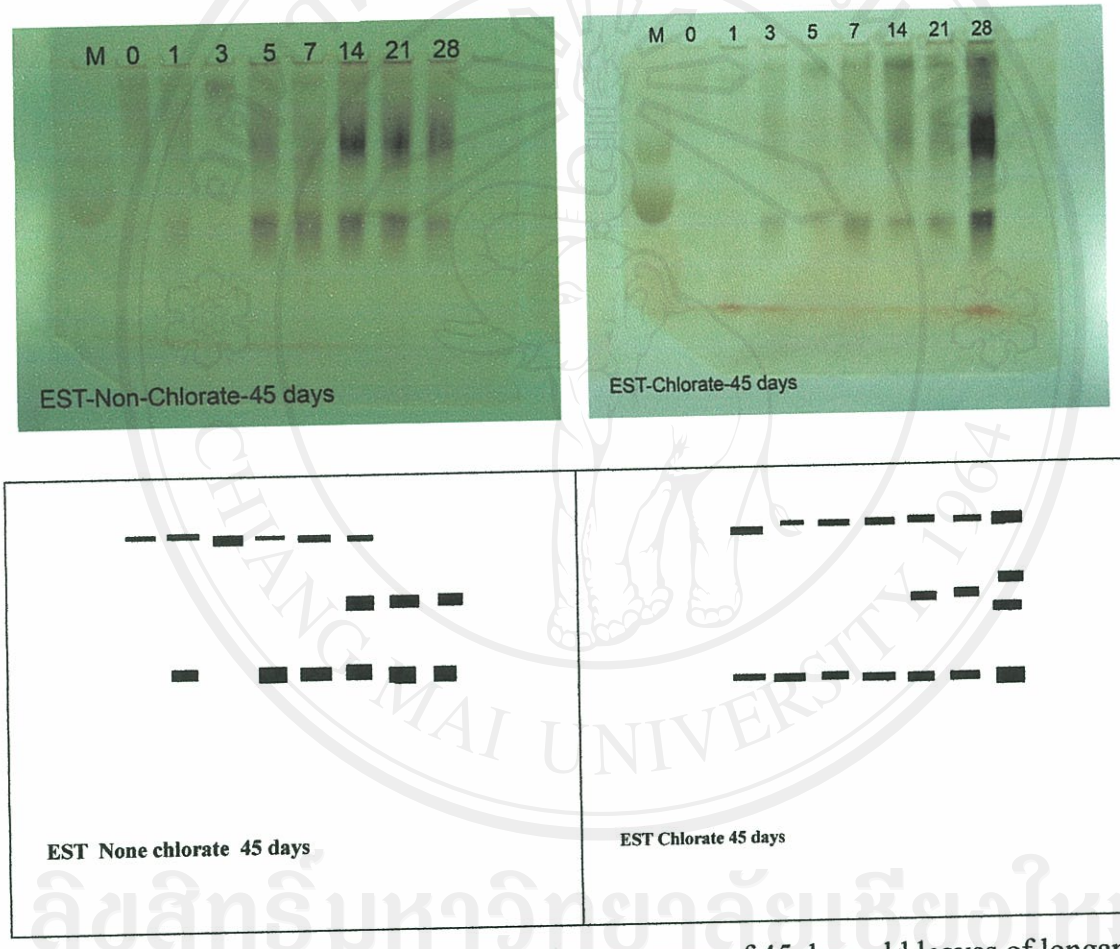

Figure 19 Esterase isozyme patterns and zymograms of 45 days old leaves of longan which treated and untreated with potassium chlorate.

2.4 The pattern of shikimic dehydrogenase, malate dehydrogenase, superoxidase dismutase, glucose-6-phosphate dehydrogenase isozymes.

In this study, shikimic dehydrogenase, malate dehydrogenase, superoxidase dismutase, glucose-6-phosphate dehydrogenase isozyme patern could not investigate. The isozyme bands of all leaf maturation stages, 15, 30 and 45 days old leaves, of these isozymes from treated and untreated trees did not show on the PAGE (Figure 20).

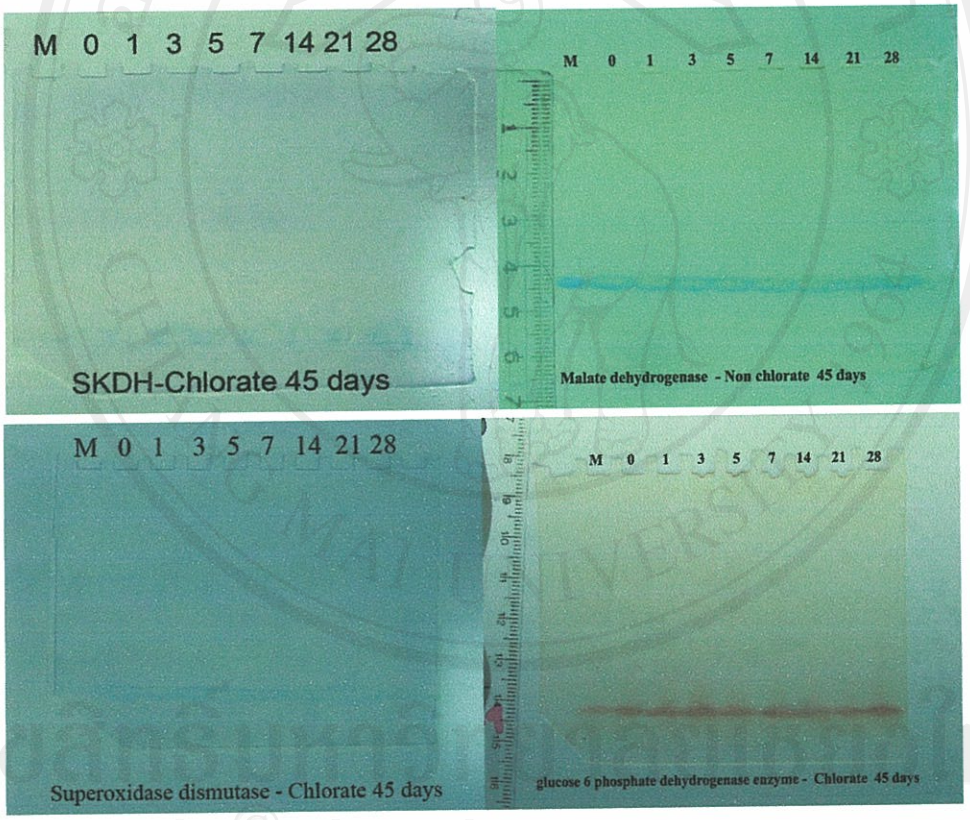

Figure 20 Shikimic dehydrogenase, malate dehydrogenase, superoxidase dismutase, glucose-6-phosphate dehydrogenase isozyme patterns of 45 days old leaves of longan which treated and untreated with potassium chlorate.

Experiment 3: Effects of potassium chlorate on changes of proteins in longan leaves during flowering period.

Experiment 3.1: Effect of leaf age on protein contents in longan leaves during flowering period.

This experiment was started in November 2003. That time was near the natural flowering season of longan. Therefore, some of untreated longan trees were flowering too.

The protein contents of from, the none-flowering trees (Tr.1, 2 and 3) were lower than those of the flowering trees. The protein contents of longan leaves on the day 0 in each treatment were 0.23, 0.23, 0.26, 0.26, 0.23 and 0.24 mg/ml respectively and on day 28th after treatment, the protein contents were 0.30, 0.31, 0.31, 0.40, 0.43 and 0.46 mg/ml respectively (Figure 21 and Table 6).

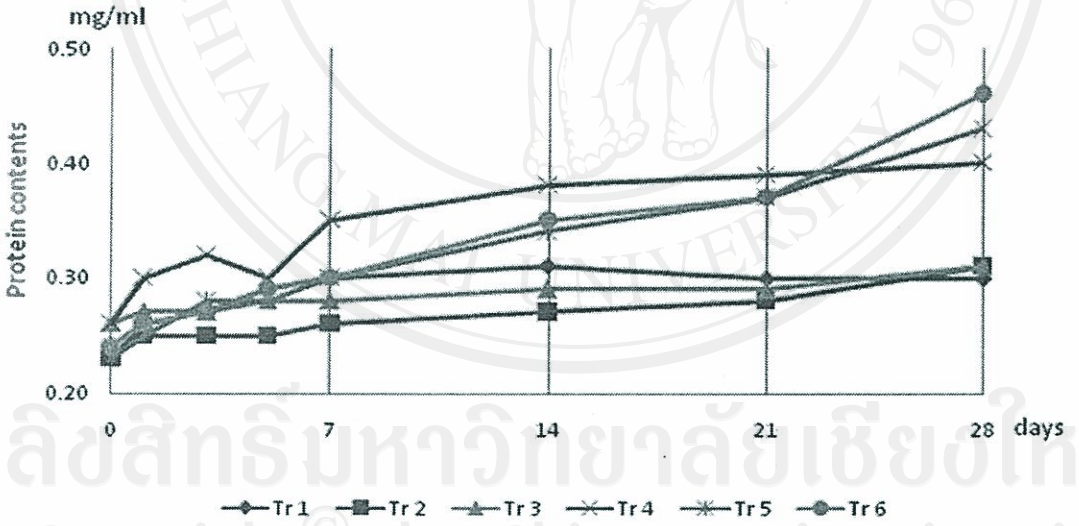

Figure 21 Graph of the protein contents of longan leaves in 0 – 28 days after treatment.

Table 6 Total protein contents of longan leaves after flowering induction by potassium chlorate at 10 g/tree.

Treatments	Total of protein contents in leaves (mg/ml)[a/]								Flowering at 28 day after treated
	Days after treatment								
	0	1	3	5	7	14	21	28	
1	0.23 b	0.26 b	0.27 b	0.28 b	0.30 b	0.31 c	0.30 b	0.30 d	no
2	0.23 b	0.25 b	0.25 c	0.26 c	0.26 d	0.27 e	0.28 c	0.31 d	no
3	0.26 a	0.27 b	0.27 b	0.28 b	0.28 c	0.29 d	0.29bc	0.31 d	no
4	0.26 a	0.30 a	0.32 a	0.30 a	0.35 a	0.38 a	0.39 a	0.40 c	yes
5	0.23 b	0.25 b	0.28 b	0.28 b	0.30 b	0.34 b	0.37 a	0.43 b	yes
6	0.24 b	0.26 b	0.27 b	0.29ab	0.30 b	0.35 b	0.37 a	0.46 a	yes
% CV	3.78	4.87	3.30	3.24	3.05	2.82	3.87	2.49	

[a/] : The Means in the same column followed by different letters were significant difference at p=0.05 by DMRT

Experiment 3.2: Effects of potassium chlorate on changes of proteins in longan mature leaves during flowering period.

3.2.1 Protein contents in longan mature leaves (45 days old leaves) during flowering period.

This experiment was started in April 2004. It was found that the treated longan trees were flowering about 21 days after treated with potassium chlorate. The leaf samples were taken in the morning before 9 o'clock. The total protein contents in leaves of untreated longan were almost stable from 0 to 28 days, it was 0.24, 0.26, 0.24, 0.27, 0.26, 0.29, 0.28 and 0.27 mg/ml in 0, 1, 3, 5, 7, 14, 21 and 28 days after treatment respectively. The total protein contents in leaves of potassium chlorate treated trees tended to increases, 0.25, 0.27, 0.29, 0.27, 0.28, 0.38, 0.39 and 0.44 mg/ml in 0, 1, 3, 5, 7, 14, 21 and 28 days after treatment respectively (Table 7). The total protein contents of treated trees were higher than the untreated trees at 14 to 28 days after treatment.

Table 7 The total protein contents in mature leaves of potassium chlorate treated longan trees.

Treatment	Total of protein contents in leaves (mg/ml)							
	Days after treatment							
	0	1	3	5	7	14	21	28
Treated chlorate	0.25	0.27	0.29	0.27	0.28	0.38	0.39	0.44
Non-treated chlorate	0.24	0.26	0.24	0.27	0.26	0.29	0.28	0.27
t-distribution	ns	ns	*	ns	ns	*	**	**

3.2.2 Protein patterns in longan mature leaves (45 days old leaves) during flowering period.

Total protein patterns of the leaves from potassium chlorate treated and untreated trees did not difference for 0, 3, 5 and 7 days after treatment (Figure 22 and 23). At 14 days after treatment, there were two new protein bands appeared in the total protein pattern of the treated tree, but these two new protein bands did not appear in those of the untreated trees (Figure 23).

The two new bands of proteins had the molecular weights of 34.88 and 17.18 Kilo-Dalton (kDa). These two new protein bands appeared during the flowering period which induced by potassium chlorate.

3.3 Some property of new protein

The new proteins with the molecular weights of 17.18 and 33.88 kDa were transferred to PVDF by western blotting and took the PVDF to determine N-terminal protein sequencing. It was found that, HT profiles of the first residual of proteins (17.18 kDa of molecular weight) had shown the amino acid sequence in monocodes were D-N-S-T-G-H-A-R-Y-P-M-V-DPTU-W-F-T-K (Figure 24). The residual of the second protein (33.88 kDa of molecular weight) had shown the amino acids sequence in monocodes were D-N-S-H-A-R-Y-P-V-DPTU-I (Figure 25). This showed that there was more than one type of protein which contained in both bands of proteins.

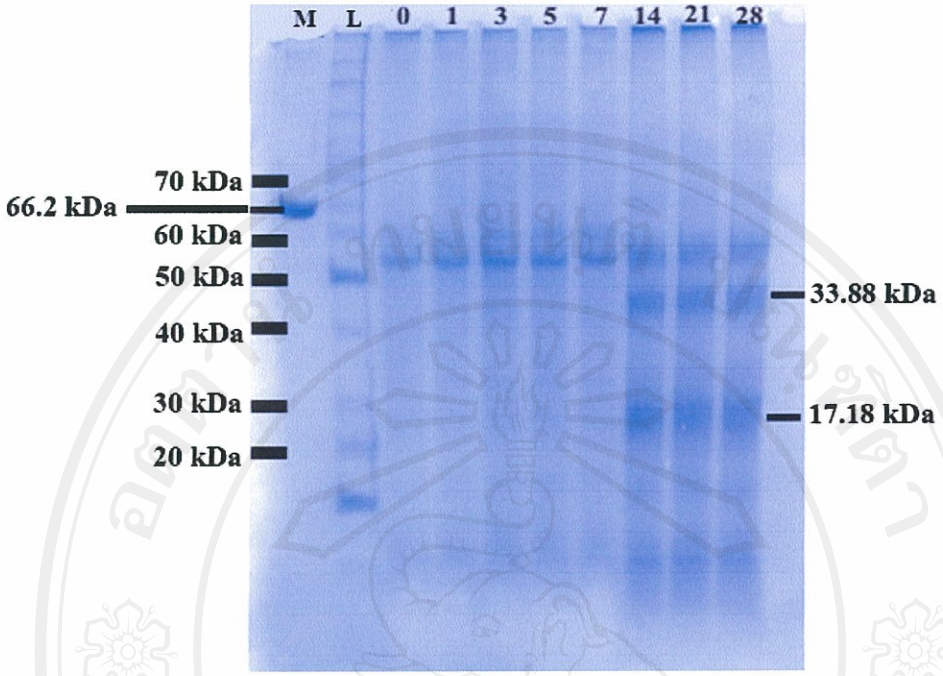

Figure 22 The protein patterns of total proteins of longan leaves in treated longan trees. M= marker (BSA), L= ladder (standard protein), 0, 1, 3, 5, 7, 14, 21, 28 = 0, 1, 3, 5, 7, 14, 21, 28 days after treatment respectively.

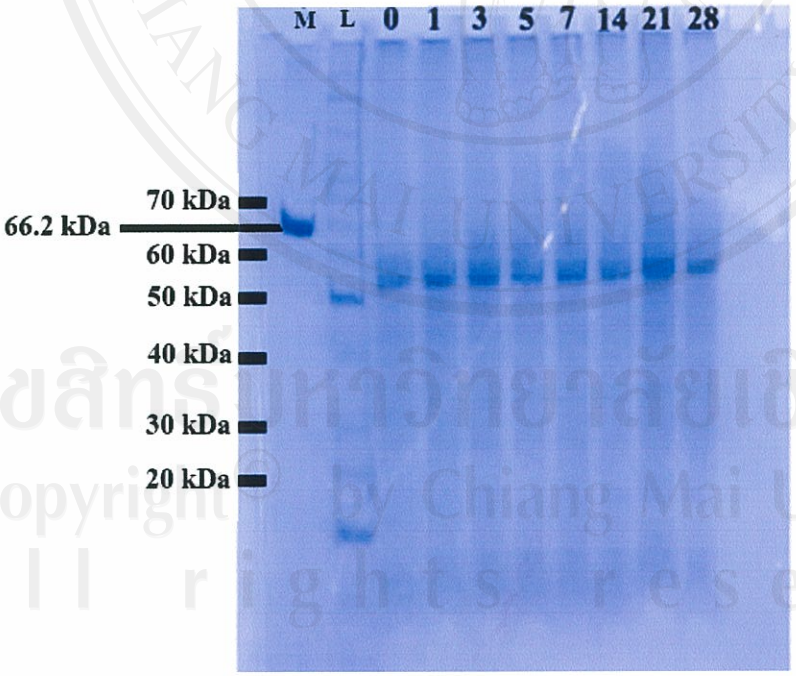

Figure 23 The protein patterns of total proteins of longan leaves in untreated longan trees. M= marker (BSA), L= ladder (standard protein), 0, 1, 3, 5, 7, 14, 21, 28 = 0, 1, 3, 5, 7, 14, 21, 28 days after treatment respectively.

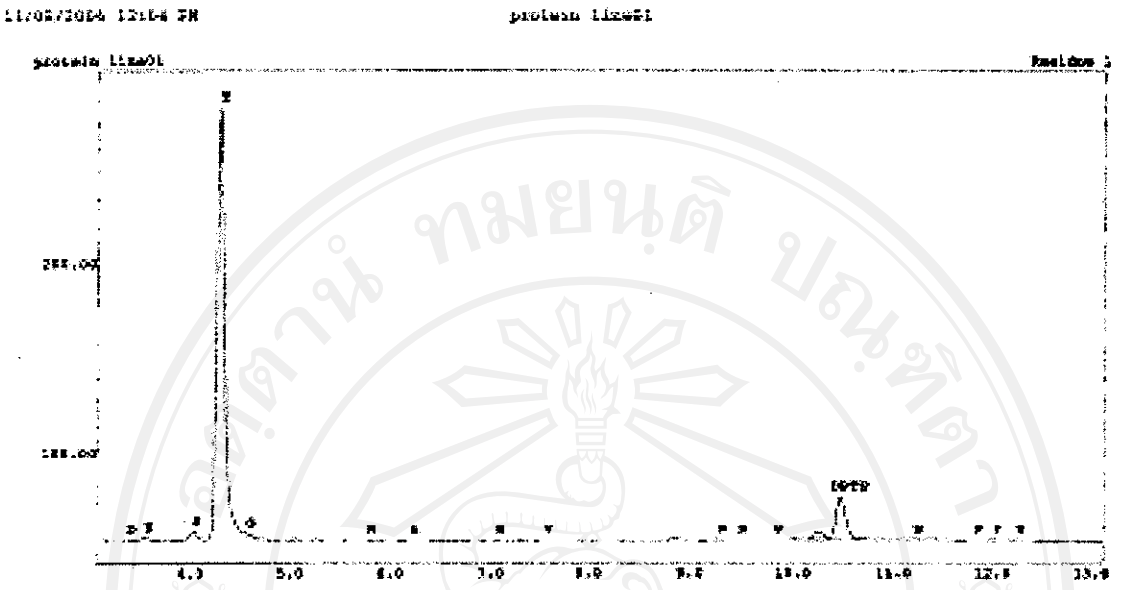

Figure 24 HT profiles of the 17.18 kDa protein sample (residual 1).
When D=asp, N= asn, S= ser, T= thr, G= gly, H= his, A= ala, R= arg, Y= tyr, P= pro, M = met, V = val, DPTU =N,N′-diphenylthiourea , W= trp, F = phe, T= thr, K= lys

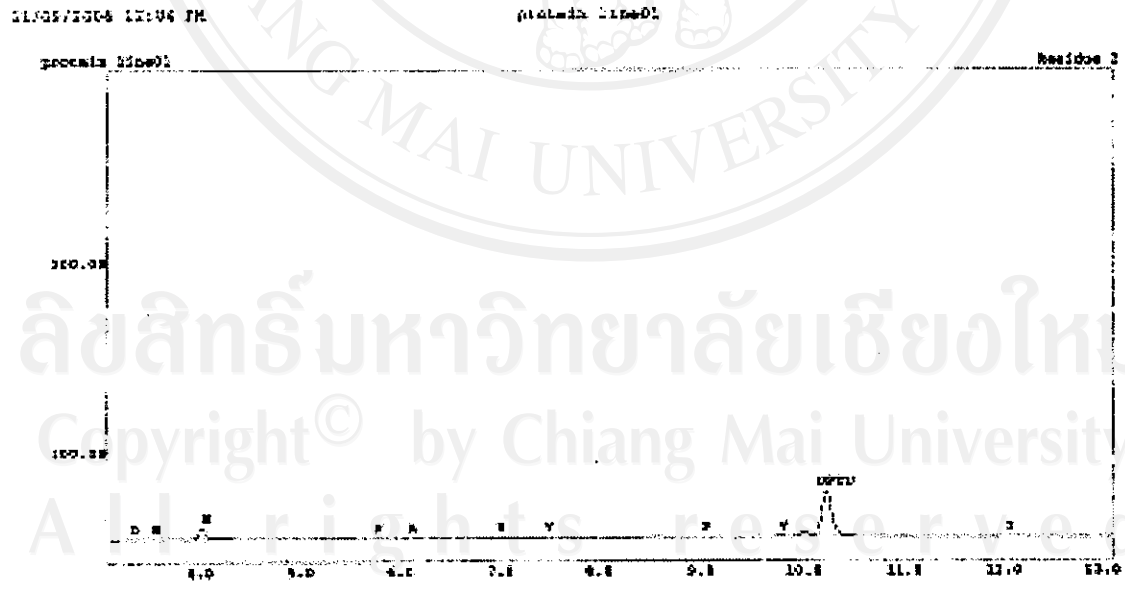

Figure 25 HT profiles of the 33.88 kDa protein sample (residual 2).
When D=asp, N= asn, S= ser, H= his, A= ala, R= arg, Y= tyr, P= pro, M = met, V = val, DPTU = N,N′-diphenylthiourea , I= Ile