CHAPTER 5

DISCUSSION

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

The results confirmed that potassium chlorate could induce flowering in longan effectively. All of the potassium chlorate treated trees were flowering mostly within 28 days after treatment. Some were flowered about 75 days after treatment due to the chlorate toxicity, chlorosis and leaf fall (Thaping-Kae, 1999). This result agreed with Wangsin and Pankasemsuk (2005) which reported that potassium chlorate could induce flowering in longan all year round. By girdling, it revealed that potassium chlorate was transported from the root to the shoot of the treated trees via xylem. Therefore, the leaves located above the girdling line still could show the effect of potassium chlorate which applied by soil drenching. By girdling with defoliation, they revealed that leaves played an important role in flower induction process by potassium chlorate. From the flowering position, the buds without leaves in the same segment which created by girdling did not develop to flower buds. These results revealed that there should be some substances and/or signals which were synthesized from the leaves and transported to the buds via phloem transport. These substances play some part of the flowering induction process. Therefore, the segments which phloem transportation were blocked by girdling and did not contain some leaves were not flowering while the segments which contained some leaves were flowered. However, chlorate ion had toxic to plant so that when it was absorbed, plant trees were reduced it to chloride and final to chlorine. In this processes free oxygen was produced and it interrupted DNA methylation in gibberellins synthesis. Therefore, the synthesis of gibberellins had decreased (La Brie *et al.,* 1991). The flowering process of fruit trees was combination processes, the main process were phytohormones. For longan tree Lin *et al.* (2001) found that Indo-3-acetic acid, cytokinin and abscisic in shoot were accumulated at high

levels but gibberellins were decreased. Therefore, potassium chlorate should have some role in deviated the hormonal balances of the treated trees.

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

The isozyme patterns in leaf, there were found only peroxidase and esterase isozymes. The patterns of peroxidase isozymes in leaves were found in flowering trees and did not find flowering trees. The peroxidase isozymes were enzymes used to reduce active oxygen in photorespiration. The active oxygen was causing membrane damages. So, the peroxidase isozymes had been produced to eradicate it. The result also showed that peroxidase isozymes tended to increase with leaf maturation which agreed with Tarayre *et al.* (1997) who reported that the produced of peroxidase isozymes had been belong to age of leaf. However, the amount of peroxidase isozymes contents in leaves of flowering trees tended to higher than none flowering trees.

The esterase isozyme patterns changes were the same as the peroxidase isozymes. Esterase isozymes (EC3.1) are hydrolase enzymes that splited esters into an acid and alcohol in a chemical reaction with water called hydrolysis. (Moss, 2006). The esterase isozymes were fundamental enzymes like the peroxidase isozymes. The pattern of esterase isozymes of flowering trees were darker and clearer than none flowering trees.

Peroxidase and esterase isozymes should be involved in some process of flower induction but the evidence to support this hypothesis is still unclear.

The patterns of other isozymes, shikimic dehydrogenase (SKDH, EC 1.1.1.25), malate dehydrogenase (MDH; EC 1.1.1.37), superoxide dismutase (SOD, EC 1.15.1.1) and glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49) were not found in this study. The results revealed that the isozymes had a little of volume and the method of detection (PAGE) could not detected them. Therefore, another techniques should be employed to detect these isozymes in longan leaves.

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.

It was found that total protein contents in the leaves increased during flower induction. Leaf maturity also played an important role in the flowering process. The flowered longan trees had higher total protein contents than the none flowering. The none flowering longan trees were Treatment 1 (leaf age 15 days and without potassium chlorate treatment), 2 (leaf age 15 days and with potassium chlorate treatment) and 3 (leaf age 30 days and without potassium chlorate treatment). It should be due to their leaves did not mature enough to response to potassium chlorate for the flower induction. In the flowering trees, they were consisted with Treatment 4 (leaf age 30 days and with potassium chlorate treatment), 5 (leaf age 45 days and without potassium chlorate treatment) and 6 (leaf age 45 days and with potassium chlorate treatment). It could be concluded that the flowering of Treatment 4 were caused by potassium chlorate because Treatment 3 (without potassium chlorate treatment) which shared the same leaf maturity (30 days leaf age) as Treatment 4 did not flower. For Treatment 5 and 6 (45 days of leaf age without and with potassium chlorate treatment) were flowering. It should be due to the leaves were mature enough for involving in the flowering process. Manochai *et al.* (1999 a) reported that the suitable leaf age of longan for flowering induction by potassium chlorate was 45 days. In this study, although longan leaf age was only 30 days, it had a potential for flowering.

The total protein contents in leaves of potassium chlorate treated trees were higher than the untreated trees. It should be caused by chlorate ion had increased physiology activities in longan. Therefore the isozymes were increased for activating the physiology process that symbolized by increasing of the total protein contents.

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

It was found that two groups of new proteins were synthesized during the flower inducing period. The new groups of proteins molecular weights were 17.18 and 33.88 kDa. These groups of proteins were composed of some difference types of proteins which had the same molecular weight but difference in isoelectric charges. These two groups of protein could be the floringens. The synthesis of new proteins was agreed with many reports. Matsumoto (2006) found that the processes of flowering in longan had express at least 65 genes, that related with tissue system and differentiation of tissue and it seemed to occur in *Arabidopsis* spp. Dennis *et al.* (1996) reported that *Asparagus officinalis* had synthesis a new protein, early flowering protein; EFP; 17 KDa in molecular weight, in the flowering period. Abe *et al.* (1999) also found the EFP protein as reported by Dennis *et.al.* (1996). Yumiko *et al.* (2001) found that rice synthesized many proteins when it flowering and 33 KDa protein was one of these proteins and that the proteins of 33 kDa were interaction with Photosystem II (Carola and Bricker, 1996).

In a Western blot, proteins that were separated on polyacrylamide gels on the basis of size were transferred to a membrane (polyvinylidene fluoride; PVDF) for peptide sequence analysis. It was found that the two bands of separated proteins could not analysis because the contamination of other type proteins in both bands. Therefore, the seperated proteins that took from this study should not be a single protein. These proteins should be group of protein which had the same molecular weight but they had difference isoelectric points. Therefore, the 2D eletrophorysis should be employed to separate these groups of proteins to obtain the single protein before amino acid sequencing of the peptides.