รายงานวิจัยฉบับสมบูรณ์

การรวบรวม ศึกษา และปรับปรุงพันธุ์สบู่ดำต้นเตี้ยเหมาะกับการผลิตเป็นการค้า

Jatropha Germplasm Collection and varietal Development For Dwarfism Suitable for Commercial Cultivation

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งานวิจัยนี้ได้รับทุนอุดหนุนจากงบประมาณแผ่นดินประจำปีงบประมาณ พ.ศ. 2550-2553

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เรื่อง

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ประจำปีงบประมาณ พ.ศ. 2550-2553

Executive Summary

Jatropha (*Jatropha curcas*) had recently been identified as an additional, sustainable source of biodiesel, due to its wider adaptation and lower water requirement, making it a useful crop on poor agricultural land, providing a major, positive impact on rural communities; the price of Jatropha oil is likely to be more stable as it is not related to alternative uses as food or cooking oil. However, certain agronomic and biological issues currently limit the use of Jatropha seed oil as a biofuel including **i**) tall stature, flowering pattern and carcinogen content, unstable and wide variation in seed and oil yield, and lack of germplasm centers, and very limited breeding programs; **ii**) limited understanding of the genetic and biochemical pathways for plant growth, development, seeds and oil production; and **iii**) lack of understanding of optimal agronomic management practices suitable for industrial scale production; and compatibility with fossil diesel fuel. Limited R&D work has been done on Jatropha, compared with that on rapeseed, soybean, and palm oils, made it not ready for commercial production. This basic information would be necessary for varietal improvement, which could lead to a Jatropha plant with high oil yield and good biodiesel properties, suitable for commercial biodiesel production.

Research Objectives

1. To collect and study *J. curcas* germplasms
2. To study genetic diversity of the collected germplasm
3. To preliminary investigate mechanism for dwarfism using apical dominance hormone inhibitors (ADHI)
4. To investigate possibility of mutagenesis using Nitroso Methyl Urea (NMU) to increase genetic variation of *J. curcas*.

Scope of works

1. *J. curcas* germplasms collection
2. Establishing DNA fingerprints of at least 20 accessions in the germplasm using

Amplified Fragment Length Polymorphism (AFLP) and karyology to identify genetic diversity

3. Investigating plant response, in terms of height, to plant-height-related hormones inhibitors
4. Investigate possibility of mutagenesis using NMU to increase genetic variation by testing optimum dose, LD_{50} and techniques to treat seeds with NMU, generating M_1 and M_2 population, and investigating genetic diversity

Conclusion of results and suggestions

- More than 60 accessions of *J. curcas* were collected.
- Although yield of all varieties were still low and genetic improvement is needed, managing environmental condition or cultural practices might be able to increase *J. curcas* yield.
- DNA fingerprints in terms of AFLP and karyology of 22 *J. curcas* accessions were successfully done and analyzed for their genetic diversity. AFLP analysis using 64 pairs of primers could be able to differentiate plant from different specie *J. podariga* from all the other *J. curcas* accessions, as well as successfully grouped the *J. curcas* accessions according to their geographical distribution and their toxicity. High similarity index and percent polymorphism suggested that there is low genetic variation/ diversity among Thai *J. curcas* accessions, especially on plant height and seed production.
- Biotechnology techniques such as induce mutagenesis to create population genetic diversity, interspecific hybridization or various types of genetically modification techniques would be necessary to introduce new desirable traits in to *J. curcas* gene pools, which is necessary for varietal improvement. Work on induced mutagenesis is now on-going.
- GA inhibitors at the concentrations of 250 and 500 µM could inhibit the plant height without reducing the total dry matter production The height reducion caused by internode length reduction, not the node numbers and branching, and should not effect flowering possibility as the plant will flowering at the branch. Therefore, genetically manipulating the involving genes could be useful for manipulating plant height. This finding might lead to the biochemical pathways

important for genetically manipulating height of *J. curcas* plant, as genes related to GA biosynthesis have been studied/known. Confirming effect of GA inhibitor and investigating yield of the field grown plants is now on-going.

- The significant correlation between CO_2 assimilation and carbon sequestration suggests that measuring photosynthesis might be more suitable as a non-destructive estimation of the carbon sequestration of *J. curcas* plant than the measurement of the dry matter production which is more destructive.
- Optimum techniques for NMU seed treatments were explored, and LD_{50} was found at 0.2%. No significant abnormality found in all the treated plants and none of them flowering at the time of this report so no M2 population could be produced. In conclusion, mutagenesis of *J .curcas* with NMU is unsuccessful.

Other knowledge generation

- Students' thesis/special problems, publications in the International –full refereed- conferences, and an international technical workshop with 50 international participants represented researchers, community, policy makers from academics industrial and business sectors, with published proceedings.
- Interest from an industrial partner to establish collaborative research project based on the findings of this project.

Future/on-going research

- Using AFLP technique to investigate genetic diversity of new and unknown accession, as well as improve specific markers.
- Investigating possibility for silencing genes involved in gibberellins biosynthesis as a technique to produce dwarf *J. curcas* (New NRCT funding project)
- Investigating yield potential of the existing accessions in a big plantation with high input cultural systems with optimum water and fertilizer management, as a basis for considering Jatropha in an industrial scale. (Collaborative project with a industry).

List of Thesis, Senior Projects and Publications from this Project

Thesis and Senior Projects

1. Hadiwijaya, B. 2009. Effect of Gibberelin Inhibitors on Growth and Carbon Dioxide Assimilation of *Jatropha curcas* L. Master thesis in Biotechnology., School of Science, Mae Fah Luang University.
2. Sengkeaw, P. 2007. Effect of apical dominant hormones on Jatropha plant height, growth & development. Senior Project Report. School of Science, Mae Fah Luang University.
3. Lertsakulchinda, K and Sangsuriyaroj, A. 2008. Genetic Diversity analysis of some Jatropha curcas L. accession by AFLP technique and karyology. Senior Project Report. School of Science, Mae Fah Luang University.
4. Saninjuk, K and Wongsaroj, L. 2010. Investigating the genetic diversity of different accession of *J. curcas* L. using AFLP and SCAR markers. Senior Project Report. School of Science, Mae Fah Luang University.

Publications in Refereed International Conference

1. Thongbai, P, A. G. O'Donnell, D. Wood, and J. K. Syers. 2006. Biofuels Research and Development at Mae Fah Luang University. Proceeding of the 2nd Joint International Conference on "Sustainable Energy and Environment (SEE 2006)" 21-23 November 2006, Bangkok, Thailand.
2. Thongbai, P, P. Eungwanichayapant, S. Dechathai, S. Popluechai, S. Unto, R. Kalapa and P. Sengkeaw. 2007. Improving Jatropha Plant to be more Suitable Biodiesel Crop. Proceedings of the 6th Asian Crop Science Association Conference and BioAsia 2007. Queen Sirikit National Convention Center, 5-9 November 2007, Bangkok, Thailand.
3. Thongbai, P., A. G. O'Donnell , D. Wood, J. K. Syers, S. Popluechai, S. Unto, P. Damrongkool, M. Jaisai, and W. Songsee. 2007. Jatropha Research and Development at Mae Fah Luang University. Thailand Research Expo 2007, organized by Thailand National Research Council 6-8 September, 2007, Bangkok, Thailand.
4. Hadiwijaya, B and P. Thongbai. 2008. Relationships between CO_2 Assimilation, Dry Matter and Carbon Sequestration of *Jatropha curcas* L. Proceedings of the International Conference Sustainable Development to Save the Earth ; Technologies and Strategies Vision 2050 (SDSE2008).
5. Thongbai, P. and B. Hadiwijaya. 2009. Effects of Gibberellin Inhibitors on Growth and Photosynthesis of *Jatropha curcas* L. Proceedings of the International Conference Agricultural Biotechnology International Conference on ABIC 2009: Agricultural Biotechnology for Better Living and a Clean Environment, 22 – 25 September, 2009, Queen Sirikit National Convention Center, Bangkok, Thailand.
6. Thongbai, P, B. Hadiwijaya, and P. Sengkeaw. 2010. Estimating carbon sequestration of J. curcas L. from plant CO_2 assimilation and dry matter accumulation. Proceedings of the International Conference 14h Annual Symposium on Computational Science and Engineering (ANSCSE 14). 23-26 March 2010, Mae Fah Luang University. Chiang Rai, Thailand.

ABSTRACT

Jatropha (*Jatropha curcas*) had recently been interested as an additional, sustainable source of non-edible biodiesel, but certain agronomic characteristics of non-domesticated plant limited basic information necessary for varietal improvement and R&D work has been done on *J. curcas*, compared to rapeseed, soybean, and palm oils, made it not ready for commercial production. This study aimed to gain these basic informations by 1) collecting and studying *J. curcas* germplasms; 2) studying genetic diversity of the collected germplasm using Amplified Fragment Length Polymorphism (AFLP) technique; 3) investigating mechanism for dwarfism using apical dominance hormone inhibitors (ADHI); and 4) investigating possibility of mutagenesis using Nitroso Methyl Urea (NMU) to increase genetic variation of *J. curcas*. In conclusion, more than 60 accessions of *J. curcas* from Thailand and elsewhere were collected. AFLP analysis using 64 pairs of primers could be able to differentiate *J. podariga* from all the other *J. curcas* accessions, and grouped all *J. curcas* according to their geographical distribution and their toxicity. However, high similarity index and percent polymorphism suggested that there is low genetic variation/ diversity among Thai *J. curcas* accessions, especially on plant height and seed production, thus made conventional breeding unsuitable. Biotechnology techniques such as induce mutagenesis to create population genetic diversity and/or various types of genetically modification techniques would be necessary to introduce new desirable traits in to *J. curcas* gene pools, is necessary for varietal improvement. However, mutagenesis using gamma ray radiation and chemical mutagent Nitroso Methyl Urea (NMU) was proved unsuccessful to create genetic diversity within the gene pool in this study.

GA inhibitor at the concentrations of 250 and 500 µM could inhibit the plant height and GA content without reducing total dry matter production and CO_2 sequestration. The lower plant height caused by shorter internode length, not less node numbers and branching, and should not affect flowering possibility as the plant will flowering at the branch. This finding might lead to the biochemical pathways important for genetically manipulating height of *J. curcas* plant, and genetically manipulating the involving genes could be useful for manipulating plant height. Confirming effect of GA inhibitor on gene expression and yield evaluation of the field grown plants is objectives of the new on-going project.

บทคัดย่อ

สบู่ดำ (*Jatropha curcas*) ได้รับความสนใจอย่างมากในการใช้เป็นพืชพลังงานทดแทนที่ไม่ใช่อาหารเพื่อใช้ในการผลิตไบโอดีเซล แต่ยังไม่สามารถผลิตเป็นการค้าได้เนื่องจากเป็นพืชป่าที่ยังไม่ได้รับการปรับปรุงพันธุ์ทำให้มีลักษณะไม่เหมาะสมเช่น ผลผลิตต่ำ ออกดอกไม่พร้อมกันและต้นสูง ทั้งยังขาดการรวบรวมพันธุ์และข้อมูลพื้นฐานที่จำเป็นสำหรับการปรับปรุงพันธุ์ด้วย งานวิจัยนี้จึงมุ่งเป้าศึกษาข้อมูลพื้นฐานเหล่านี้ โดยมีวัตถุประสงค์คือ 1) รวบรวมและศึกษาพันธุ์สบู่ดำทั้งในและต่างประเทศ 2) ศึกษาและวิเคราะห์ความหลากหลายทางพันธุกรรมจากลายพิมพ์ DNA โดยเทคนิค Amplified Fragment Length Polymorphism (AFLP) 3) ศึกษากลไกการเกิดต้นเตี้ยโดยการยั้บยั้งฮอร์โมนที่ข่มตาข้าง และ 4) ศึกษาความเป็นไปได้ในการใช้สารก่อกลายพันธุ์ Nitroso Methyl Urea (NMU) เพื่อเพิ่มความหลากหลายทางพันธุกรรมให้มากขึ้น ผลการทดลองโดยสรุปคือสามารถรวบรวมสายพันธุ์ทั้งในและต่างประเทศได้ กว่า 60 สายพันธุ์ การวิเคราะห์ AFLP โดยใช้คู่ไพรเมอร์ 64 คู่ สามารถจำแนกกลุ่ม *J. curcas* ออกจาก *J. podariga* และกลุ่ม สบู่ดำที่มีพิษและไม่มีพิษออกจากกันได้ แต่เนื่องจากค่า similarity index and percent polymorphism สูงมาก จึงสรุปได้ว่าไม่มีความแตกต่างทางพันธุกรรมของสายพันธุ์ทั้งหมด โดยเฉพาะในแง่ของความสูงและการให้ผลผลิต จึงไม่เหมาะกับการปรับปรุงพันธุ์โดยวิธีธรรมดา และจะเป็นต้องใช้เทคนิคทางวิศวพันธุกรรมและการศึกษาทางสรีรวิทยาเพื่อศึกษาลักษณะที่ต้องการปรับปรุง การก่อกลายพันธุ์ด้วยสารก่อกลายพันธุ์ NMU ในการศึกษานี้ ไม่สามารถก่อให้เกิดต้นพืชที่มีลักษณะแตกต่างอย่างชัดเจนจึงไม่สามารถสร้างความแปรปรวนทางพันธุกรรมให้เกิดขึ้นตามที่ต้องการได้

สารยับยั้งฮอร์โมนจิบเบอริลิน (GA) ที่ความเข้มข้น 250 และ 500 µM สามารถลดความสูงของและปริมาณ GA ในต้นสบู่ดำได้อย่างมีนัยยะสำคัญ โดยน้ำหนักแห้งและการใช้ CO_2 ของพืช การลดความสูงเป็นผลจากการลดความยาวปล้องแต่ไม่ลดจำนวนข้อและจำนวนกิ่ง จึงไม่น่าจะมีผลต่อการลดจำนวนดอกเนื่องจากพืชจะออกดอกที่กิ่ง ผลการทดลองนี้นำไปสู่ความเข้าใจในขบวนการและยีนที่เกี่ยวข้องกับสร้างฮอร์โมน GA อันจะเป็นประโยชน์ต่อการดัดแปลงพันธุกรรมเพื่อควบคุมความสูงของต้นสบู่ดำได้ การยืนยันผลของสารยับยั้ง GA ต่อการแสดงออกของยีนที่เกี่ยวข้องกับการสังเคราะห์ GA รวมทั้งผลต่อการให้ผลผลิตของสบู่ดำในแปลงขนาดใหญ่ จึงเป็นเป้าหมายต่อไปของโครงการใหม่ที่กำลังดำเนินการอยู่

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Chapter 1

Introduction

1.1 Statement and significance of the problem

The increasing global demand for energy and recognition of limitations in the availability of the crude oil supply have resulted in a large increase in crude oil prices in the past three years, and will continue to do so. The International Energy Agency forecasts that the world will require 50 percent more energy over present consumption levels by 2020 (IEA, 2004). This highlights the need to develop alternative energy sources which are sustainable and environmentally friendly (less carbon dioxide emissions). Biofuels such as ethanol or biodiesel are the most convenient renewable energy sources that can be used to partially replace crude oil-based transportation fuels for the next ten years, especially for a country like Thailand which imports about 90% of its crude oil requirements mainly for transportation. In the next ten years, it seems that biofuel is the only renewable energy source that can be used conveniently in the transportation sector, which takes up about 60% of the global crude oil consumption (IEA, 2004; Minns, 2005).

There is a growing interest in biodiesel (methyl esters of vegetable oils), which may be used to partially replace petroleum-based diesel as a transportation fuel. Biodiesel has mainly straight chain structures and shorter carbon chain profiles and a narrower boiling point ranges than petroleum diesel. These properties, combined with the presence of oxygen in biodiesel, result in higher engine combustion temperatures and a "cleaner burn", compared to petroleum diesel (Wood, 2006). Biodiesel has successfully been introduced in Germany (based on rape seed oil) and the USA (based on soybean oil and spent cooking oil), and plans have been announced for Malaysia (based on palm oil). The future demand for biofuels requires cultivation of dedicated bioenergy crops. The availability of sufficient land and efficient crop production systems are the most critical factors in developing a biofuels industry. Exploitation of biomass in a non-sustainable way, such as the clearing of forests or the reduction of soil carbon stock could, however, causes major net releases of carbon to the atmosphere with its consequent negative impact on climate change.

Jatropha (*Jatropha curcas*) (Box.1) had recently been identified as an additional, sustainable source of biodiesel, and being investigated worldwide such as in India, Central America and South Africa. Jatropha may be a more suitable crop for sub-tropical countries, especially in Thailand, due to its wider adaptation and lower water requirement, making it a useful crop on poor agricultural land, providing a major, positive impact on rural communities. The methyl ester qualities of Jatropha oil are up to EDIN51606 standards (Table 1), but the price of Jatropha oil is likely to be more stable as it is not related to alternative uses for food or as cooking oil. Moreover, pure Jatropha oil could be used either directly or blends with diesel in a direct-injection single-cylinder diesel engine (Forson et al, 2004).

Regardless of technical possibilities mentioned above, there are certain agronomic and biological issues which could currently limit the widespread use of Jatropha seed oil as a biofuel. These include (Francis et. Al, 2001):

Jatropha (*Jatropha curcas*) has recently been identified as an additional, sustainable source of biodiesel. It may be a more suitable crop for sub-tropical countries, due to its wider adaptation and lower water requirement, making it a useful crop on poor agricultural land, providing a major, positive impact on rural communities; the price of Jatropha oil is likely to be more stable as it is not related to alternative uses as food or cooking oil. However, limited R&D work has been done on Jatropha, compared with that on rapeseed, soybean, and palm oils, made it not ready for commercial production. Certain agronomic and biological issues currently limit the use of Jatropha seed oil as a biofuel including **i)** tall stature, indeterminate flowering pattern and carcinogen content, unstable and wide variation in seed and oil yield, and lack of germplasm centers and very limited breeding programs; **ii**) limited understanding of the genetic and biochemical pathways for plant growth, development, seeds and oil production; and **iii**) lack of understanding of optimal agronomic management practices suitable for industrial scale production; **iii**) lack of understanding of optimal agronomic management practices suitable for industrial scale production.

This basic information would be necessary for varietal improvement, which could lead to a Jatropha plant with high oil yield and good biodiesel properties, suitable for commercial biodiesel production.

1.2 Objectives

1. To collect and study Jatropha germplasms
2. To study genetic diversity of the collected germplasm
3. To preliminary investigate mechanism for dwarfism using apical dominance hormone inhibitors (ADHI)
4. To investigate the possibility of induced mutation technique using Nitroso Methyl Urea (NMU) to increase genetic variation of *Jatropha curcas*.

1.3 Scope of study

1. Germplasms collection
2. Establishing DNA fingerprints of at least 21 accessions in the existing germplasm using Amplified Fragment Length Polymorphism (AFLP) and karyology to identify genetic diversity
3. Investigating plant response, in terms of height, to plant-height-related hormones inhibitors
   3.1. Testing effect of apical dominance hormone inhibitors on plant height: Cytokines vs. IAA, Paclobutrazol vs. GA
   3.2. Testing effect of GA inhibitors on plant height, growth, photosynthesis, and C sequestration: Paclobutrazol vs. Mapiquep chloride
4. Investigate the possibility of induced mutation technique using Nitroso Methyl Urea (NMU) to increase genetic variation of *Jatropha curcas*
   4.1 Preliminary dose response experiments to identify optimum NMU concentration and suitable soaking treatments
   4.2 Creating mutant population M1 of *J. curcas* by treating the seeds with dose and technique from 4.1,
   4.3 Choosing seeds from M1 mutant plants with different characters to grow M2 population, and investigate characteristics and genetic of M1 and corresponding M2 plants.

1.4 Expected benefits from this research

1. Collection of *J. curcus* and other *Jatropha* spp. for genetic stocks and other basic information as a basis for further varietal improvement
2. DNA Fingerprints and Dendrogram of at least 20 lines of *J. Curcus* & other *Jatropha* spp.
3. Mechanism(s) to control plant height
4. Mutagenesis technique and mutants with diverse characters

Chapter 2

Review of Related Literature

2.1. General Information of Jatropha (*Jatropha curcas* L)

Taxonomy and nomenclature

Jatropha plant or physic nut (*Jatropha curcus* L.) is an oil seed plant belongs to the family Euphorbiaceae which contains approximately 170 known species. The scientific classification of *Jatropha curcas* is:

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Malpighiales
Family	:	Euphorbiaceae
Subfamily	:	Crotonoideae
Tribe	:	Jatrophaeae
Genus	:	*Jatropha*
Species	:	*curcas*

Characteristic of *Jatropha curcus* L.

Jatropha has been believed to be native to Central and South America and distributes in Latin America, Caribbean, Asian, Africa and grows on good and degraded soil, in low and high rain fall (Heller, 1996). Normally, five roots are formed from seedlings, one central and four peripheral. A tap root is not usually formed by vegetative propagated plants. The plants are 2-8 meter height perennial shrub with diameter up to 20 cm; trunk is straight, branching low above the ground, very long and contains latex; bark is thin and yellowish. The leaves are 5-7 shallow loped leaves about 6 cm. long and 15 cm. wide and alternately arranged. The trees are deciduous, shedding the leaves in the dry season. Early growth is fast and with good rainfall conditions, nursery plants may bear fruits after the first rainy season; direct sown plants after the second rainy season. Flowering occurs during the wet season and two flowering peaks are often seen. In permanently humid regions, flowering occurs throughout the year. Inflorescences are indeterminate cymes formed terminally on branches and are complex, possessing main and co-florescences with paracladia contains more unisexual than

hermaphrodite flowers which pollens and stigmas receptive at different time so they need to be pollinated by insects, especially honey bees. Fruit is trilocular ellipsoidal with green exocarp that turns yellow when ripe and contain 3 black oil bearing seeds, about two cm long and one cm thick. There are 2000-2400 seeds per kg (Figure 2.1).

Figure 2.1 Part of the *Jatropha*: (a) flowering branch, (b) bark, (c) leaf veinature, (d) pistillate flower, (e) staminate flower, (f) cross-cut of immature fruit, (g) fruits and (h) longitudinal cut of fruits (Heller, 1996).

The seeds mature about 1-3 months after flowering, the higher temperature the faster maturity. The seeds are orthodox and should be dried to low moisture content (5-7%) and stored in air-tight containers. At room temperature the seeds can retain high viability for at least one year. However, because of the high oil content the seeds cannot be stored for as long as most orthodox species. Freshly harvested seeds show dormancy after ripening and pre-treatment is necessary before the seeds can germinate. Dry seed will normally germinate readily without pre-treatment. If this is the case, it is not recommended to remove the seedcoat before sowing. Although it speeds up germination there is a risk of getting abnormal seedlings. Germination is fast, under good conditions it is complete in 10 days. Germination is epigean (cotyledons emerge above ground). Soon after the first leaves have formed the cotyledons wither and fall off. In the nursery, seeds can be sown in germination beds or in containers. Although the seedling grows very fast they should stay in the nursery for 3 months until they are 30-40 cm tall. By then the plants have developed their repellent smell and will not be browsed by animals (Heller, 1996).

Although the whole plant contains many toxins namely curcin, curcasin, hydrocyanin, saponin and phobolesters, the oil from the seed could be used directly in the two strokes diesel engines, or used to produce biodiesel (Heller, 1996) (Table 1.1). Thus, they are interesting plant for economic purpose (Ackom and Ertel, 2005).

Table 2.1 Some important parameters of raw and transesterified jatropha oil from India. (From Francis and Becker, 2001[4])

Parameter	Raw Jatropha Oil	Jatropha Oil Transesterified	EDIN 51606 standard
Density (g cm^{-3} at 20°C)	0,920	0,879	0.875 - 0.890
Flash Point (°C)	236	191	> 110
Cetane no. (ISO 5165)	23-41	51	> 49
Viscosity (mm^2/s at 30°C)	52	4.84	3.5 - 5 (40°C)
eutralisation no. (mg KOH/g)	0.92	0.24	< 0.50
Total glycerine (%)	-	0.088	< 0.250
Free glycerine (%)	-	0.015	< 0.02
Phosphorus (ppm)	290 (17 in degummed oil)	17.5*	<10
Sulphated ash (%)	-	0.014	< 0.03
Methanol (%)	-	0.06	< 0.3

* negligible when de-gummed oil is used

However, morphology of Jatropha plant is still not suitable for the large scale production because it is too high to harvest fruits and seeds. Moreover, the long branching characteristic requires wide plant spacing which lowers number of plant and seed production per unit area (Heller, 1996). Therefore, it is important to inhibit plant height for reducing area per plant, increasing number of plants and yields per unit area. At the present, the only method to control plant height and increase seed yield is pruning. However, with all the toxins in the whole plant, large scale pruning should increase a chance for the farmers to take toxins in to their bodies, as well as increases production cost on labour and machinery. Inhibiting apical dominance hormones might be another way to reduce Jatropha plant height, increase lateral bud development and solve all the problems previously mentioned.

Study of genetic diversity

For succession of breeding program, the diversity of parental population is needed to produce variation in daughter generation. Thus, the study on genetic diversity can be useful as the basic information for genetic improvement of the crops (Kumar, 1999).

Karyology is one of the genetic methods that can be used to identify genetic variation of plants (Paterson *et al*, 2000). Karyology is the study of chromosomes characteristics which are usually observed and compared sizes of chromosomes, the position of centromeres, and basic number of chromosomes (Tolliver and Robbins, 1991). Karyology could be used to study the genetic diversity since the difference in number and size of chromosomes usually related to difference in morphology of plant (Stebbins, 1966). Chromosome is composed of 2 sister chromatids which connected at centromere. The chromosome is characterized by the position of centromere into 4 types: metacentric, submetacentric, acrocentric, and telocentric (Kumpiranon, 1997). The morphology and number of chromosomes can be used to determine the species of an organism which identification is questionable, construct phylogenies when karyotypes are compared across taxa, and diagnose diseases associated with chromosomal aberrations (Tolliver and Robbins, 1991). Normally phase of cell division that easy to study the chromosome characteristics is Metaphase, because the chromosomes are contracted and thick. Oliveira (2004) found that *Stevia rebaudiana* with more chromosomes had bigger pollens and the lesser dense stomata and triploid strain produced the shorter plants and less inflorescence, whereas the tetraploid strain had the largest leaves. Peterson *et al* (2000) studied about comparative genomics of plant chromosomes, which they found the plant in the Brassicaceae has different chromosome number and at lest 15 molecular genetic maps have been constructed for *Brassica* that included all of the major cultivated species. The karyology of genus *Jatropha* has been studied by Soonthornchainakseang and Jenjitikul (2003) using the pollen. They found that most of genus *Jatropha* in Thailand is diploid ($2n = 22$), and the tetraploid was found only *J. curcas* L. In Thailand, there is no study about somatic chromosome number in the cells of difference accessions of *J. curcas* L.

Recently, many DNA fingerprinting techniques are also used for studying genetic diversity and relationship of many organisms such as, Restriction Fragment Length Polymorphism (RFLP), Randomly Amplified Polymorphic DNAs (RAPD) and Amplified Fragment Length Polymorphism (AFLP) (Henry, 2001). Among these techniques, AFLP is the most robust and reliable technique, because of its reproducible result and any prior knowledge about the genetic background of the population is not needed. The AFLP technique is based on the detection of DNA restriction fragments by PCR amplification, and can be used for DNAs of any origin or complexity (Vos and Kuiper, 1997). AFLP markers were successfully employed to detect diversity and genetic differentiation of many plants such as *Oryza* species (Aggarwal et al., 1998;), barley (Lindhout, 1996) and *Caladium bicolor* (Loh et al, 1999;).

So far, most of the study about genetic diversity of *J. curcas* was recently done in Indian accessions (Ganesh Ram *et al*, 2007; Basha and Sujatha, 2007; Sudheer Pamidiamarri *et al*, 2008). RAPD was employed in the study of genetic diversity among *Jatropha* species (Ram *et al*, 2007). Basha and Sujatha (2007) used RAPD and ISSR markers to characterize the variability of *J. curcas* accessions in India. There is still no report about the genetic diversity of *J. curcas* in Thailand.

2.2 Hormones controlling plant height

The central philosophy in the study of the regulation of plant shoot architecture is the concept of apical dominance, whereby the growing apical meristem suppresses the growth of axillary meristems, lying in the axils of leaves below it. Auxin and gibberellin are known as apical dominance hormones promoting apical bud growth and inhibit the growth of lateral buds (Hopkins, 1995; Mohr & Schopfer, 1995; Shani, 2006). By decapitating plants and substituting various compounds for the apex, Thimann and Skoog (1934) demonstrated that apical dominance could be mediated by the plant hormone auxin. This led to the direct inhibition hypothesis, which proposed that auxin, synthesized at the shoot apex, would be transported basipetally down the stem to the bud, which it enters to mediate growth inhibition (Booker *et al.*, 2003). The

apex of a shoot inhibits the outgrowth of secondary, or lateral, shoots via decapitation (i.e. shoot tip removal), which leads to the development of lateral shoots. Lateral shoots do not randomly materialize, but rather emerge from tiny buds that are located along the main stem in the axis of the leaves. This region where the leaf and bud are attached to the stem is called the node (Hopkins, 1995; Mohr & Schopfer, 1995).

Auxin, gibberellin and cytokinin are hormones that have main role on shoot apical meristem. The shoot apical meristem (SAM) is located at the shoot apex and leaves, stems and axillary meristems are produced from its derivative cells (Hopkins, 1995; Mohr & Schopfer, 1995; Shani *et al.,* 2006). Auxin, which in plant is predominantly indole-3-acetic acid (IAA), is produced at the growing shoot apex, has been demonstrated for a number of plant species, although the precise contribution of the meristem, leaf, and young stem tissues to the production of auxin is not known (Booker *et al.,* 2003). Gibberellin (GA) is found can promote the plant growth (Shani *et al.,* 2006). Cytokinin can promote shoot development, inhibit root development, and stimulate cell enlargement and branching (Brault & Madiney, 1999).

The previous study found that IAA and GA had different mechanism on plant height regulation. IAA increases the plant height with increasing the number of nodes, but GA_3 through internodes elongation. Therefore, BA (synthetic cytokinin) inhibiting IAA that increase plant height through node number could express by reducing number of nodes. In the other hand, GA inhibitor had effect on GA which is hormone that increases internodes length. The previous result showed that GA inhibitor could inhibit plant height by reducing internodes length but number of nodes is still equal to the normal plant (Saengow, 2007).

The primary auxin in plants is indole-3-acetic acid (IAA) although other compounds with auxin activity, such as indole-3-butyric acid, phenyl acetic acid, and 4-chloro-IAA, are also present in plants (Normanly *et al.,* 1995). Although it promotes shoot growth, auxin inhibits root growth. Many studies on auxin, both physiological and genetic, report root length. Although easy to assay, root length as a parameter hides considerable complexity because the length of the root is

specified by both cell expansion and division (Beemster & Baskin, 1998; Green, 1976).

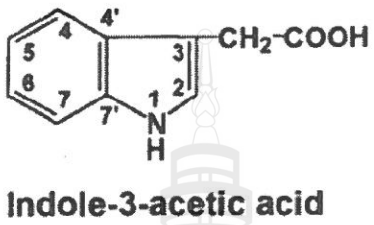

Figure 2.2 Molecular structure of IAA (Kende & Zeevaart, 1997).

West and Phinney (1956) found that Gibberellins are plant hormones produced in higher plants. Since 1957 gibberellins was widely used in agriculture for increase size of plant. Gibberellins (GAs) are large family of tetracyclic, diterpenoid plant hormones that control plant growth and development (Lange, 1997; Matsuoka *et al.,* 2008). GAs are signaling molecules that regulate shoot elongation, root development and the expansion of the leaf (Lange *et al.,* 2005; Fleet & Sun, 2005). GA_3 is the end product of the gibberellins pathway and regulating many different growth processes (Bearder, 1983). GA_3 could stimulate parenchyma and epidermis elongation (Ono *et al.,* 2000). GA biosynthetic genes are expressed in specific cell- and tissue-types during the development, and their transcript levels are often elevated in the rib meristem of shoot apex, elongating internodes, developing anthers and embryo axes (Fleet & Sun, 2005).

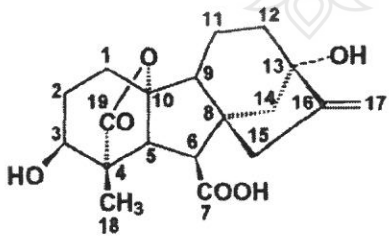

Figure 2.3 Molecular structure of GA (Kende & Zeevaart, 1997).

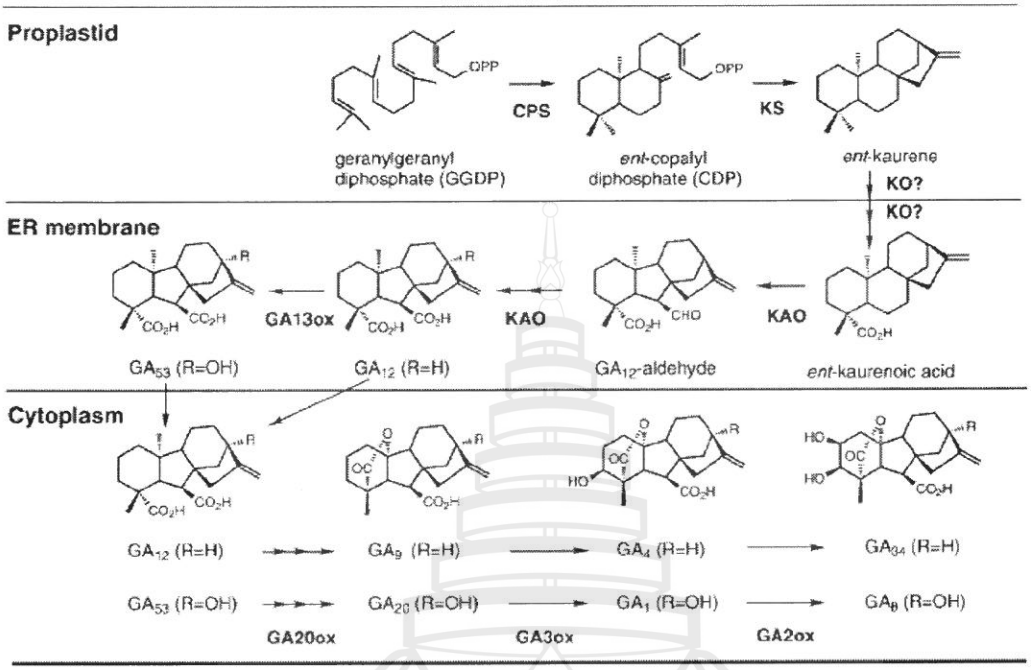

Figure 2.4 Major GA Biosynthetic and Catabolic Pathways in Higher Plants. The enzyme names are shown in boldface below or to the right of each arrow. GA_9 and GA_{20} also can be converted to GA_{51} and GA_{29} by GA2ox. GA_4 and GA_1 are the bioactive GAs, and GA_{34} and GA_8 are their inactive catabolites (Olszewski *et al.*, 2002)

The biosynthesis of GAs can be divided into three main stages: (1) the formation of *ent*-kaurene in plastids, (2) conversion of *ent*-kaurene to GA12 by membranebound cytochrome P450 monooxygenases and (3) formation and deactivation of bioactive C19-GA in the cytoplasm (Olszewski *et al.*, 2002).

The conversion of GGPP to *ent*-kaurene is catalyzed by the enzymes *ent*copalyl diphosphate synthease (CPS) and *ent*-kaurene synthease (KS). In *Arabidopsis*, high activity of the *AtCPS* promoter has been detected in rapidly growing tissues, including shoot apices, root tips, developing anthers and seeds (Silverstone *et al.*, 1997). Vascular tissue of some non-growing organs like expanded leaves also exhibit *AtCPS* promoter activity, suggesting that CPS present in leaves may initiate GA biosynthesis destined for transport to other organs. In

contrast to the tissue- and developmental stage- specific expression pattern of *CPS*, *KS* transcript has been found in all organs analyzed (Smith *et al.,* 1998; Yamaguchi *et al.,* 1998), and at least in *Arabidopsis* is present at much higher levels than that of *CPS* (Yamaguchi *et al.,* 1998).

The oxidation steps from the non-polar *ent*-kaurene to the soluble GA_{12} are catalyzed by the generally membrane-bound *ent*-kaurene oxidase (KO) and *ent*kaurenoic acid oxidase (KAO) (Helliwell *et al.,* 2002). Moreover, similar fusions using N-terminal regions of KO and KAO show that KO is targeted to the outer envelope of the chloroplast, while KAO is targeted to the endoplasmic reticulum (ER). These findings suggest that KO may link the two spatially separated parts of the GA biosynthesis pathway, but the mechanism whereby the non-soluble *ent*-kaurene is transported to the ER-bound enzymes remains to be elucidated. At a tissue specificity level, at least the two KAO present in *Arabidopsis* are expressed in all tissues thus far examined (Helliwell *et al.,* 2001).

Once GA_{12} has been formed it can be 13-hydroxylated into GA_{53}, marking the starting points of two parallel pathways in the last stage of GA formation: the early non-hydroxylation pathway and the early 13-hydroxylation pathway. The relative predominance of the two pathways is species-dependent, for instance in pea, rice and lettuce more GA_1 is formed, whereas higher levels of GA_4 are formed in *Arabidopsis* and cucumber (Kamiya & Garcia-Martinez, 1999). The penultimate step in both pathways is carried out by the multifunctional enzyme GA 20-oxidase (GA20ox) and involves a stepwise oxidation of the C-20 in GA_{12} and GA_{53}, giving rise to the C19-compounds GA_9 and GA_{20}, respectively. All bioactive GAs are C19-metabolites, but to obtain bioactivity GA 3-oxidase (GA3ox) must convert the inactive GA_9 and GA_{20} into GA_4 and GA_1, respectively. The level of bioactive GA is thus governed by the rate of biosynthesis, but the rate of turnover or deactivation catalyzed by the enzyme GA 2-oxidase (GA2ox) also plays a role. In this process, GA_4 and GA_1 are catabolized into the inactive forms GA_{34} and GA_8, respectively. In addition, the products of the enzyme GA20ox may be diverted away from the route towards bioactive GA by 2-hydroxylative degradation. A novel class of GA2ox has recently been identified in *Arabidopsis* that possesses the ability to 2-hydroxylate C20-GAs and not C19-GAs, thereby further diversifying GA

catabolism (Schomburg *et al.,* 2003). Moreover, the GA levels may be affected by conjugation, as both bioactive GAs and precursors can exist in conjugated, inactive forms (Schneider & Schliemann, 1994). GA20ox, GA3ox and GA2ox are soluble dioxygenases that use 2-oxoglutarate as a co-substrate and are dependent on Fe2+ and ascorbate for high activity (Hedden *et al.,* 2001; Schomburg *et al.,* 2003).

2.3 Gibberellins inhibitors

2.3.1 Mepiquat chloride (PIX)

Mepiquat chloride is onium compounds growth retardants which have quaternary ammonium group that block the cyclases copalyl-diphosphate synthase and *ent*-kaurene synthase involved in the early steps of GA metabolism on plants (Rademacher, 2000). Mepiquat chloride (PIX) was used as plant growth regulator as a management tool in controlling vegetative growth of cotton plant. Mepiquat chloride is a gibberellic acid suppressant that is absorbed by the green portions of the plant and serves to reduce cell elongation, thus offering the potential of decreasing leaf area and restricting additional plant height increases (Reddy *et al.,* 2004). An analysis of plant growth parameters at the time of application revealed that all positive yield responses occurred when the height to node ratio was above the baseline levels and fruit retention was usually above baselines. (Norton & Silvertooth, 2000).

Some plants have a low response to mepiquat chloride, but cotton plant is highly responsive to PIX application. Mepiquat chloride could decrease production of GA and resulting in shorter cotton. Mepiquat chloride has been shown to increase the number of sympodial nodes and reproductive branches, decrease internode length, increase maturity rate, and decrease boll rot. The effects on maturity and the number of reproductive branches have also been linked to the enhanced retention of early buds and bolls (Nichols, 2003). Moreover, mepiquat chloride application also could increase the single leaf CO_2 exchange rate of cotton plant. The increasing of leaf CO_2 exchange rate because mepiquat chloride could increase stomatal conductance and specific leaf weight (SLW) of cotton plants (Zhao and Oosterhuis, 2000). The similarity of growth pattern between cotton and

Jatropha plant suggest PIX also could be used as growth regulator to reduce *Jatropha* plant height.

2.3.2 Paclobutrazol

Paclobutrazol is a growth retardant with a Nitrogen-containing heterocycle which block cytochrome P450-dependent monooxygenases, thereby inhibiting oxidation of *ent*-kaurene into *ent*-kaurenoic acid (Rademacher, 2000). Gibberellin-inhibiting tree-growth regulators such as Paclobutrazol (PCB) have been shown to reduce shoot elongation, leaf expansion, and stem diameter growth of many tree species. PCB treatment did result in a significant increase in fine root density on chlorotictrees compared to untreated controls, but not on trees with normal foliage color. Rapidly growing plants may be more effectively growth-regulated by PCB. Fine root density, starch content of the woody roots, and percentage of mycorrhizal root tips were unaffected by PCB treatment. PCB produced no reduction in leaf size or twig growth of the white oaks at any time during the 7 years. Greener leaves, especially on chlorotic trees, were evidence that PCB was affecting the crowns. Most studies on growth regulation with PCB are on moderate-sized, heavily pruned trees with rapid sprouting (utility and orchard), or small vigorous plants. Annual twig growth on these large mature trees was typically only 5 to 8 cm (2 to 3.2 in) per year. Growth regulation by PCB may be less effective on slowly growing plants (Watson, 2006).

Results from these previous studies suggested that it might be possible to control *Jatropha* plant height using the apical dominance hormones inhibitors. The preliminary study of Saengow (2007) on inhibitors of IAA and GA apical dominance hormones in *Jatropha curcas* showed that inhibiting gibberellic acid (GA) is most effective to reduce *Jatropha* plant height in the vegetative growth stage without reducing total dry matter production. Gibberellins inhibitor could inhibit plant height by reduced internode length but number of nodes was still similar to the normal plant, but there is no report on grain yield. Further study on the effect of type, concentration and application time of GA inhibitors on plant growth and yield is needed for the most effective way to reduce *Jatropha* plant height.

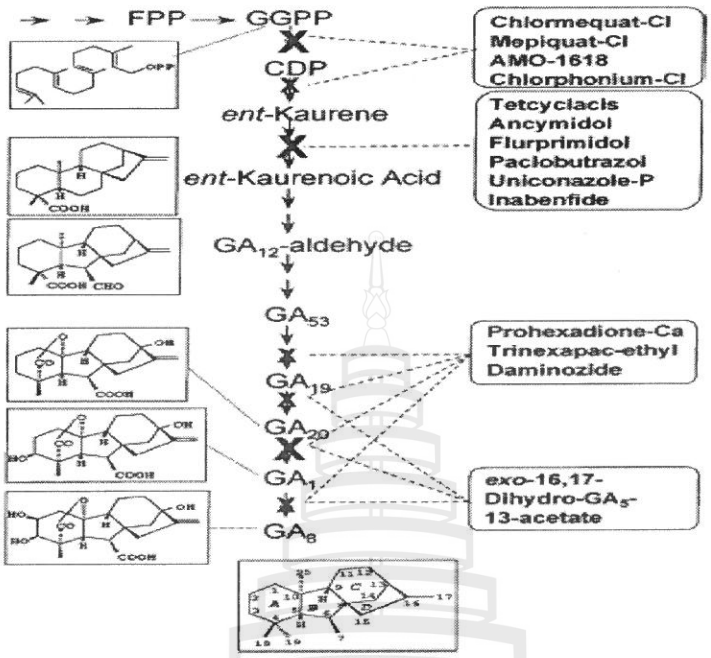

Figure 2.5 Simplified scheme of biosynthetic steps involved in GA biosynthesis and points of inhibition by plant growth retardants (**X**, x = major and minor activity, respectively) (Rademacher, 2000).

2.3.3 Mutation

Mutation means changes in genetic material of the cells that alters the sequence of the nucleotide bases in the genetic material (DNA) of an organism or cell, which are not by normal recombination or segregation, and could be transferred to the next generation. Alterations of the DNA base pairs occur either a) genetic mutation by displacement, addition, deletion, cross-linking, of the base pair; b) structural mutation by physical destruction or abnormal configuration of he base pair; and 3) polyploidy or change chromosome number of the cell. The mutation alteration to the DNA sequence would alter its meaning and its ability to produce the normal amount or normal kind of protein, so the (organism or cell) is itself altered. Such an altered organism is called a mutant, and can be a useful tool

to generate genetic diversity for a breeding program of the plant population with very low genetic diversity.

Mutation is the process naturally happening, but spontaneous mutation by natural causes takes time. Induce mutation or mutation caused by human using mutagenic agents, or mutagens, accelerates the mutation processes and provides more new plant varieties and wider ranges of populations in crops such as rice, cotton, castor beans and cassava (Koornneef et al., 1985; IAEA Database, 2001) Types of induced mutations are Knight, 1979; Martı´nez-Herrera et al, 2006):

1. *Gene manipulation mutation* refers to the target changes at the exact known gene(s) with known functions, such as antisense or silencing techniques.
2. *Mutagnic induced mutation* refers to the use of mutagents to create non-target changes to the cell. The changes are random and unpredictable, but provide wider range of changes than method 1). Types of popular mutagents are:
   2.1. Ionizing radiation such as X-ray, neutron, gamma ray, radioisotopes, ultraviolet light, to treat on seed, pollen, and callus of the plant;
   2.2. Chemical mutagens which are alkalating agents such as ethyl methane sulfonate (EMS), ethyleneimine nitro ethyl urethane, nitroso methyle urea (NMU), diethyl sulphhate and sodium azide etc. The process is done by soaking plant parts such as seed, bud, or stock in the chemical solution for a certain period. Chemical mutagens are easier and safer to use, and induce more gene mutation with less abnormal chromosome.

To conduct induced mutation, dose response has to be tested for the optimum concentration of the mutagen that creates the best segregation without destroying the plant tissue, and LD_{50} dose with could reduce plant growth 50% without killing the plant. The treated plants M1 will be grown to get the seed which will be regrown and called M2 which will be checked, visually or by DNA fingerprints such as AFL{ , whether the changes in M1 still exist. Successful induced mutation is reported in *Musa* spp., *Arabidopsis thaliana*, *Taxus*

cannadensis Marsh. (12, 13, 19, 23) Effective mutation could be only 10% so the large M1 population at least 1000 plants is needed (37, 38))

As the study on genetic diversity of *J. curcas* suggested that this plant in Thailand is low variation so cannot be used as parents for conventional breeding It cannot use as parents for conventional breeding (Thongbai et al, 2006), mutation is one choice for increasing the genetic diversity. Previous work by Wimolrat et al (1990) found that gamma radiation provided the shorter plant with early flowering, but the seeds were smaller than the untreated plants, with no yield and oil data. As our preliminary attempt using gamma radiation was unsuccessful, the chemical mutation Nitroso Methyl Urea (NMU) will be tested.

Chapter 3

Research Methodology

3.1. Germplasm collection

Seeds of *J. curcas* acquired from different places were germinated in the small plastic bags for 4 months before planting into the field, six plants per accession, with spacing 2x3 m between plant. One kg of compost per plant was applied basal at planting, and 5 gm of 15-15-15 fertilizer were applied about 1 month afterward and each year after pruning, with adequate pest and weed control but no irrigation during dry season.

3.2. Study genetic diversity of the germplasm collection using AFLP and karyology technique for DNA finger printing analysis

3.2.1 Plant materials

The 21 accessions of *Jatropha curcas* L. and *J. podariga*, as an out-group, were collected from germplam collection in Mae Fah Luang University, Chiang Rai. The list of accessions is in the table 3.1.

Table 3.1 List of plant materials of 21 accessions of *J. curcas* and *J. podariga*.

No.	Accession	No.	Accession
1	PC 18	12	PC79
2	PC 20	13	KR
3	PC 21	14	SMK
4	PC 24	15	PCB
5	PC 29	16	CTB
6	PC 31	17	KPS
7	PC 35	18	Stool
8	PC39	19	CR
9	PC43	20	India
10	PC65	21	Mexico
11	PC69	22	J. *podariga*

3.2.2 DNA extraction

Genomic DNA of the total 22 *Jatropha* accessions (as in Table 3.1) was extracted by CTAB method adapted from Agarwal *et al* (1992). Young leaves were collected and cleaned with water. Approximately 1 g of tissue was grinded to fine powder in a sterile mortar and pestle with liquid nitrogen. The powder was transferred into 500 µl of pre-warmed extraction buffer (4% CTAB, 1.8 M NaCl, 40 mM EDTA pH 8.0, 200 mM tris-HCl pH 8.0) and 2 µl 2-mercapto ethanol in 1.5 ml microcentrifuge tube and incubated at 65°C for 60 minutes, before the 500 µl mixture of chloroform: isoamyl alcohol (24: 1) was added and centrifuged at 12,500 rpm, 4 °C for 5 minutes. The clear solution was transferred to new tube and precipitated with 10 µl of linear polyacrylamide and 700 µl of cold isopropanol. The mixture was incubated at -20°C for 30 minutes then centrifuged at 12,500 rpm, 4 °C for 15 minutes. After that, the liquid was discarded and the pellet was dried in air. The pellet was resuspended in 500µl mixture of RNase buffer (10mM Tris-HCl pH 8.0, 15 mM NaCl) and 2 µl of RNaseA was added, and incubated at 37°C for 30 minutes. After that, the 500 µl mixture of phenol: chloroform: isoamyl alcohol (25: 24: 1) was added and centrifuged at 4°C, 12,500 rpm for 5 minutes. The aqueous layer was transferred to a new tube and mixed with 400 µl mixture of chroloform: isoamyl alcohol (24: 1). Then transfered was added and centrifuged at 12,500 rpm, 4°C for 5 minutes. The aqueous layer was collected to new tube and precipitated with 2 volumes of cold absolute ethanol. The tube was incubated at -20°C for 30 minutes. DNA pellet was resuspend in 20 – 50 µl of TE buffer (pH 8.0) and stored at -20°C. Quality and quantity of extracted DNA was determined by agarose gel electrophoresis and staining with ethidium bromide.

3.2.3 AFLP analysis

The AFLP assay was performed as described by Vos et al. (1995). The flow diagram of AFLP procedure is shown in figure 3.1. Genomic DNA(250 ng) was restrictly digested with 2 restriction enzymes, *Mse*I and *Eco*RI and incubated at 37 °C for 2 hr or overnight. Digested DNA was ligated with *Mse*I and *Eco*RI adapters by addition of ligation mixture containing T4 DNA ligase. The solution was incubated at 37°C for 1 hour then 20 °C for 2 hours. Ligated DNA

was first pre-amplified using *Mse*I and *Eco*RI primer with one selective nucleotide at the 3' end each (M-C, E-A). The pre-amplified product was diluted 1:20 with TE buffer to use as template in selective amplification. The selective amplification was performed using *Mse*I and *Eco*RI primer with three selective nucleotides at the 3' end each. The selective primer used in selective amplification was shown in table 3.2.

Table 3.2 List of primer pairs used in selective amplification

Primer	M-CAA	M-CAC	M-CAG	M-CAT	M-CTA	M-CTC	M-CTG	M-CTT
E-AAC	1	2	3	4	5	6	7	8
E-AAG	9	10	11	12	13	14	15	16
E-ACA	17	18	19	20	21	22	23	24
E-ACC	25	26	27	28	29	30	31	32
E-ACG	33	34	35	36	37	38	39	40
E-ACT	41	42	43	44	45	46	47	48
E-AGC	49	50	51	52	53	54	55	56
E-AGG	57	58	59	60	61	62	63	64

3.2.4 Polyacrylamide gel electrophoresis

The sample were prepared by 3.5 µl of DNA sample from 2.3.2 and 2 µ of loading dye were mixed and incubated at 80°C for 4 minutes. Each 4 µl of sample were loaded on 6 % denaturing polyacrylamide gels and run electrophoresis at constant volt (300 V). The gel was washed in fixative solution (10% acetic acid) for 10 minutes with shaking then distilled water for 30 minutes. After that, the gel was stained with 0.2% Silver nitrate for 20 minutes and rinse with water briefly. The gel was developed in developer (2.5% sodium carbonate, 0.02% formaldehyde, 2 µg/ml sodium thiosulfate) until the bands appear. The reaction was stopped by washing with 5% acetic acid.

3.2.5 Data analysis

Data analysis of polymorphic bands was record manually as present or absent. The fingerprint patterns were scored as the presence (1) or absence (0) of each fragment. The similarity index was calculated by using NTSYS version 2.01e, and the dendrogram was constructed by using Untweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis.

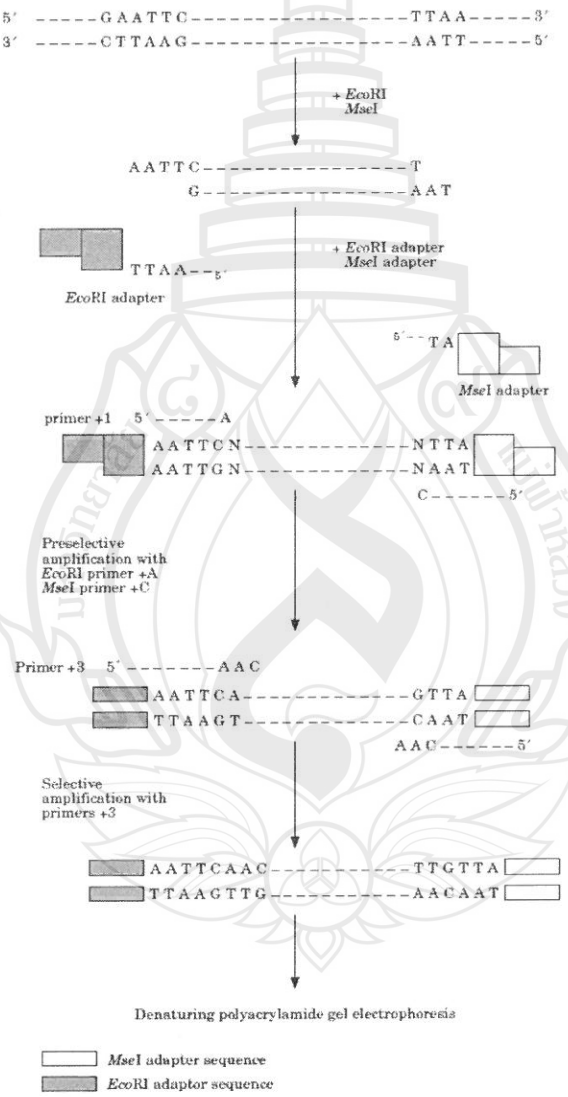

Figure 3.1 Flow diagram of AFLP procedure using one representative primer pair (Loh et al., 1999).

3.3. Chromosome study using karyology technique

3.3.1 Seeds Germination

10 Seeds of *J.curcas* were soak in water over night and were laid on wet tissue paper for 5-7 days to let the seeds germinate, before collecting the root tips to study chromosome.

3.3.2 Pretreatment and fixation

The root tips of each accession of *J.curcas* were collected at 8 to 10 am from germinated seeds. The root tips were cut and clean with water, then pretreated in 0.002 M 8-hydroxyquinoline at 16 °C for 4 hours. After that, the root tips were fixed in Canoy's solution (95% ethanol: pure acetic acid, 3: 1, v/v) at 4 °C for 4 hours, and were preserved in 70% ethanol at 4°C.

3.3.3 Chromosome observation

The root tips from 2.2.2 were hydrolyzed and macerated in 1 M HCl at 60°C for 5 minutes the washed with distilled water. Then the macerated root tips were stained on slide glass with aceto-orcein (orcein 2.2g: acetic acid 100 ml) for 5 minutes, and suspended cell using squash technique. The chromosomes were observed under light microscope at 1000x.

3.4. Investigating plant response, in terms of height, to plant-height-related hormones inhibitors

3.4.1. Effect of apical dominant hormones inhibitors on growth of *J. Curcus*

To test the effect of apical dominance hormone inhibitors on height and early growth of *Jatropha*, paclobutazol (GA. inhibitor) and BA (IAA inhibitor) was chosen to treat on *Jatropha* seedlings and observed growth at vegetative stage. Concentrations of hormones in the plant tissue were then analyzed to confirm effect of the inhibitors.

In this experiment, two months old *Jatropha* seeding were divided into 5 groups according to the treatments as shown in table 3.3.

Table 3.3 Treatments of apical dominance hormones and inhibitors used.

Treatments	Hormones	Concentration (μM/L)	References
1	Untreated control	-	-
2	IAA (Auxin)	100.0	Fernandez-falcon et. al. (2003)
3	BA (Cytokinin, counteract IAA activity)	50.0	Leeson et. al. (2006)
4	IAA/BA	100.0/50.0	Radin and Loomis (1973)
5	Paclobutazol (GA inhibitor)	500.0	Commercial recommended rate

The design for this experiment was 5x7 factorials in RCB with 3 replications, total plot of 105 pots.

1) Preparation of *J. Curcus* seedings

J. Curcus (c.v. Chiang Rai) seedlings were prepared from the seeds until 4 leaves-stage (about 2 months) and randomly arranged according to the experimental design. Each seedling was then transplanted into to a 5 kg plastic pot contained soil mixed with husk ratio 3:1, watered daily and managed pest control when necessary.

2) Preparation of hormones and treating

The hormones and inhibitors solutions were prepared according to the treatments as in table 2.1 (Appendix 1) and 50 ml of each was sprayed on to each plant at the start (week 0) and the 2nd week of the experiment. Plants in untreated control treatments were sprayed with 50 ml. deionized water.

3) Plant Growth Data Record

Number of leaves, plant height, and number of branches were measured weekly. One plant from each treatment was then randomly sampling and fresh weight and dry weight of leaves, stem and root were observed at 0, 1, 2, 4, 6, and 8 weeks after treated.

Leaf area were measured by randomly sampling three leaves and cut those in 10 pieces of 1 cm^2 leaf section, measured their fresh and dry weigh and calculated by:-

Total Leaf Area (cm^2/plant) = $\frac{10\ cm^2 \times \text{total leaf weight per plant (g.)}}{\text{Weight of 10 leaf sections (g.)}}$

4) Growth analysis

The growth data were then used for calculating Crop Growth Rate (CGR) or rate of total dry weight increase and Net Assimilation Rate (NAR) or ability of one unit leaf area to produce total dry weight over a period of time, according to the following equations:

1. Crop Growth Rate (CGR) = $\frac{W2-W1}{T2-T1}$

Where: - T1 = starting time, T2 = time after T1

W1 = total dry weight at T1, W2 = total dry weight at T2

2. Net Assimilation Rate (NAR): = $\frac{(W2-W1)(\ln A2-\ln A1)}{(A2-A1)(T2-T1)}$

Where: - T1 = starting time, T2 = time after T1

W1 = total dry weight at T1, W2 = total dry weight at T2

A1 = leaf area at T1, A2 = leaf area at T2

5) Bioassay GA and IAA concentration in the plant tissues

Thirteen plant samples from 5 groups (1 from control, 3 from IAA, 3 from BA, 3 from IAA/BA and 3 from paclobutazol treated group) were used for GA bioassay using High Performance Liquid Chromatography (HPLC) according to Kelen et. al. (2004)

A 5 g. sample of dry leaf tissue from each treatment was homogenized with 70% (v/v) methanol and stirred overnight at 4 °C. The extraction was filtered through a Whatman filter No. 0.2. The methanol was evaporated under vacuum, adjusted pH of the solution with 0.1 M phosphate buffer to pH 8.5 and separated 3 times with ethyl acetate using separation funnel, adjusted pH to 2.5 with 1 N HCL, separated another 3 times with diethyl ether and anhydrous sodium sulfate. The remain diethyl ether was then evaporated from the solution

under vacumm and the recovered dry residue was dissolved in 2.0 mL of methanol and stored at 4 ºC for further injection.

IAA and GA concentration in the prepared sample solution was then assayed using HPLC (Water® 2695 Separation Module and Tactor Water® 2996 Photodiode Array Detector, the column C_{18} 250x4.6 nm Spherisorb C_{18} 206). Two mobile phases used were acetonitrile-water (26:74; 30:70; v/v), and 30 mM phosphoric acid adjusted to pH 4.0 with sodium hydroxide. The Luna C_{18} column was equilibrated 30 min for each mobile phase condition. The column temperature was maintained at constant 25±0.1 ºC. The separation was carried out by isocratic elution with a flow rate of 0.8 mL/min, and an injection volume of 10 µL was use for each analysis.

Standard solution of the individual hormone was prepared in the mobile phase and chromatographed separately to determine the retention time for each acid. The signal of the compounds were monitored at 208 and 280 mm for GA and IAA, respectively. Capacity factors were calculated from $k= (t_R-t_0)/t_0$, where t_0 was the hold up time, and t_R was the retention time of each hormone for each mobile phase. In this equation, the hold up time, t_0, was established for every mobile phase composition using potassium bromide solution. The retention times and capacity factors of the solutes were determined from 2 different injections which at 5.0-5.7 min retention time. Peak identification was based on retention time and spiking of the sample.

6) Statistical Analysis

All data were analyzed for Analysis of Variance (ANOVA) using SPSS software (version 16.0)

3.4.2. Testing effect of GA inhibitors on plant height, growth, photosynthesis, and C sequestration: Paclobutrazol vs. Mapiquep chloride

1) Plant materials

The experiment was conducted at the experimental field of Mae Fah Luang University, Chiang Rai, Thailand. Seeds of *J. curcas* cv. Korat were soaked in water for 24 hours in dark and then germinated on the wet tissue paper tray until four little peripheral roots expanded subsequently, the seedlings were transplanted to 1 kg black plastic bags filled with rice husk ash for about 3 weeks or until the first foliates appeared. The plants were transplanted to a 5 kg plastic pot containing the mixture of soil, husk and manure in the ratio of 3:1:1. Five grams of 15:15:15 N:P:K fertilizer were applied to each plant after 2-months-old. The plants were watered daily, and the pesticide was applied as necessary. The plants were kept growing until they had about three expanded leaves before starting the experiment.

2) Experimental design

The trial was set up in a 2 x 4 factorials in randomized complete block design with three replications. The first factor was two types of GA inhibitors: paclobutrazol and mepiquat chloride (PIX), and the second factor was four concentrations of each inhibitor: 0, 250, 500 and 750 μM per pot.

3) Preparation of gibberllin inhibitors and treatment

Gibberellin inhibitors were prepared according to the treatments each experiment. Fifty mL of freshly-prepared aqueous solutions of the retardants or the same volume of water were applied to each plant by foliar spray one time a week for three consecutive weeks after plants have five true leaves for the first experiment. The untreated control plants were sprayed with 50 ml deionized water.

4) Plant growth measurements

Plant height, number of branches, number of nodes, internode length, stem diameter, and number of leaves were measured at 0, 1, 2, 3, 4, 6, 9, 12, 15, 20, 26 and 32 weeks after applying. One plant from each treatment was randomly sampled and partitioned into leaf, stem and root tissue. The tissues were dried at 62°C for 48 h in a hot air oven. The dry weight of each tissue were recorded.

Leaf area (LA) was measured by randomly sampling 10 leaves and cut those leaves into 10 pieces of 1 cm^2. They were dried in hot air oven for 48 h at 62°C before weighting. The weight of leaf sections were used to calculate LA of the whole plant using equation:

$$\text{Leaf Area } (cm^2/\text{plant}) = \frac{10\ cm^2 \times \text{total leaf dry weight (g/plant)}}{\text{Weight of 10 leaf sections (g)}}$$

The photosynthetic rate (the rate of CO_2 exchange in the leaf chamber of measurement instrument) of each plant as CO_2 assimilation/m^2/s was detected on the youngest fully expanded leaf (4th leaf from the top), during 08.00–11.00 hrs. at 0, 1, 2, 3, 4, 6, 9, 12, 15, 20, 26 and 32 weeks after inhibitor application. The open system Infrared Gas Analyzer (IRGA: LCi portable Photosynthesis System, ADC BioScientific Ltd., England) with broad type chambers of full leaf area 625 mm^2 was used, with the following set up parameters: boundary layer resistance to water vapour 0.17 m^2 s mol^{-1}, PAR at the window 1500 µmol m^{-2} s^{-1}, air flow rate 200 µmol s^{-1} and the chamber window transmission factor 0.88. The atmospheric pressure and leaf chamber temperature were set at ambient. CO_2 assimilation rate per plant or the rate of CO_2 exchange from all leaves of the whole plant was calculated from photosynthetic rate using equation:

CO_2 assimilation = Photosynthetic rate x Leaf Area

The total CO_2 assimilation during the whole experimental period in the form of accumulative CO_2 uptake was calculated from the summation of the area under the curve of the CO_2 assimilation over time.

Plant carbon sequestration was estimated from 50% of the aboveground dry matter and 25% of the aboveground for belowground part (Albrecth & Kandji, 2003).

5) Plant growth analysis

1. Crop Growth Rate (CGR), using equation:

$$\text{Crop Growth Rate (CGR)} = \frac{W_2 - W_1}{T_2 - T_1}$$

Where: T_1 = starting time, T_2 = time after T_1

W_1 = total dry weight at T_1, W_2 = total dry weight at T_2

2. Net Assimilation Rate (NAR), using equation:

$$\text{Net Assimilation Rate (NAR)} = \frac{(W_2 - W_1)(\ln A_2 - \ln A_1)}{(A_2 - A_1)(T_2 - T_1)}$$

Where: T_1 = starting time, T_2 = time after T_1

W_1 = total dry weight at T_1, W_2 = total dry weight at T_2

A_1 = leaf area at T_1, A_2 = leaf area at T_2

3. Leaf Area Ratio (LAR), using equation:

$$\text{Leaf Area Ratio (LAR)} = \frac{(LA_2 - LA_1)}{(W_2 - W_1)}$$

Where: LA_1 = Leaf Area at T_1, LA_2 = Leaf Area at T_2

4. Relative Growth Rate (RGR), using equation:

$$\text{Relative Growth Rate (RGR)} = \frac{\ln W_2 - \ln W_1}{T_2 - T_1}$$

5. Leaf Area Duration (LAD), using equation:

$$\text{Leaf Area Duration (LAD)} = \frac{(LA_2 + LA_1)(T_2 - T_1)}{2}$$

6. Correlation between CO_2 assimilation, dry matter accumulation, and carbon sequestration

6) Bioassay GA concentration in the plant

All plant samples were used for GA bioassay using High Performance Liquid Chromatography (HPLC) according to Kelen *et. al.* (2004), as previously mentioned in 3.4.1.5.

7) Statistical analysis

Statistical analysis of all the data was performed by Repeated Measures ANOVA using SPSS (ver. 16.0 for Windows). Linear regression between two subjects and plotting were established using SigmaPlot (ver. 11).

3.5. Induced mutation using Nitroso Methyl Urea (NMU)

3.5.1. Study effect of seed coat on NMU treating and seed germination

Jatropha seed has thick seed coat hat might be barrier for NMU absorption, an experiment was conducted to compare the effect of NMU treating with normal and naked seeds.

1) Plant materials

Seeds of the local accession of *J. curcus* , 50 seeds per treatments total of 2000 seeds.

2) Experimental design

The trial was set up in 3 replicated randomized complete block design with 12 treatments as follows: 1) naked seed 4hr 0.03%NMU; 2) naked seed 4hr 0.1%NMU; 3) naked seed 4hr 0.3%NMU; 4) naked seed 12 hr 0.03% NMU; 5) naked seed 12 hr 0.1% NMU; 6) naked seed 12 hr 0.3% NMU; 7) normal seed 4 hr 0.03% NMU; 8) normal seed 4 hr 0.1% NMU; 9) normal seed 4 hr 0.3% NMU; 10) normal seed 12 hr 0.03% NMU; 11) normal seed 12 hr 0.1% NMU; and 12) normal seed

12 hr 0.3% NMU, all compared with the controlled seeds soaked in buffer without NMU.

3) Methods

Prepared the seeds and removed seed coat according to the treatments. Prepared stock solution of NMU 10% by dissolved in EtOH, before mixing with phosphate buffer (2M Na_2HPO_4 and 1M citric acid, pH 5.0) to the concentration of 0.03%, 0.1% and 0.3%. Fifty seeds for each treatment were soaked in 100 ml of the solution for either 4 or 12 hrs according to the treatments, with gentle shacking. After the soaking ended, the seeds were drain with running deionized water for 4 hrs. before testing for germination on the moist germination paper incubated at 25 C. Number of germinated seeds in each treatments were recorded to calculate percent seed germination, and analyzed using ANOVA

3.5.2. Effect of phosphate buffer solution on seed germination

As the result from Experiment 1 showed that buffer reduced seed germination, so a experiment was conducted to compare germination of the seeds soaked in buffer and in water.

1) Plant materials

Seeds of the local accession of *J. curcus* , 100 seeds per treatments total of 600 seeds.

2) Experimental design

The trial was set up in 3 replicated randomized complete block design with 6 treatments of soaking as follows: 1) in water for 4 hrs.; 2) in water for 12hrs.; 3) in 0.5x buffer 4hrs.; 4) in 1.0x buffer 4hrs.; 5) 0.5x buffer 12hrs.; and 6) 1.0x buffer 12 hrs.

3) Methods

Prepared phosphate buffer (2M Na_2HPO_4 and 1M citric acid, pH 5.0) at half (0.5x) and full (1x) according to treatments. One hunred seeds for each treatment were soaked in 100 ml of the solution for

either 4 or 12 hrs according to the treatments, with gentle shacking. After the soaking ended, the seeds were drain with running deionized water for 4 hrs. before testing for germination on the moist germination paper incubated at 25 C. Number of germinated seeds in each treatments were recorded to calculate percent seed germination, and analyzed using ANOVA.

3.5.3 Effect of Clorox surface disinfectant on seed germination

As seeds from the previous 2 experiments were infected by fungi and affected seed germination, so an experiment was conducted to compare germination of the normal seeds and the seeds surface sterile with Clorox.

1) Plant materials

Seeds of the local accession of *J. curcus* , 50 seeds per treatments total of 300 seeds.

2) Experimental design

The trial was set up in 6 replicated in CRD with 2 treatments of non-sterile and sterile with Clorox.

3) Methods

All the seeds used were soaked under running water for 16 hrs. Then one half of the seeds for sterile treatments were treated twice, 10 min each, with Clorox 15% before washed twice, 10 min each, with sterile water; and another half of non-sterile seeds were just washed in sterile water all through the process. All the seeds were then tested for germination on the moist germination paper incubated at 25 C. Number of germinated seeds in all treatments were recorded to calculate percent seed germination, and analyzed using ANOVA.

3.5.4. Effect of pH of the phosphate buffer solution on seed germination

Result from the previous experiments showed the higher % seed germination in the treatment with higher concentration of NMU than from the treatments with pure buffer without NMU, This might due to the fact that NMU, an alkaline mutagent, might increase pH of the acidic buffer to be more neutral, and thus

increase seed germination, so a experiment was conducted to compare pH of different NMU concentration in the buffer and in water.

1) Experimental design

The trial was set up in 3 replicated randomized complete block design with 8 types of solutions as follows: 1) pure buffer ; 2) 0.1%NMU buffer; 3) 0.5%NMU buffer; 4) 1.0%NMU buffer; 5) pure water; 6) 0.1%NMU water; 7) 0.5%NMU water; 8) 1.0% NMU water

2) Methods

Phosphate buffer (2M Na_2HPO_4 and 1M citric acid) was prepared and adjusted pH to 6.0. Then the 100 ml of NMU solution with buffer or with deionized water were prepared according to the treatments. The pH of each solution was monitored hourly for 20 hrs.

3.5.5. Induced mutation of J. curcas with NMU

From the previous 4 experiments, we got the technique and optimum condition for conducting mutagenesis experiment of *J. curcas*.

1) Plant materials

Seeds of the local accession of *J. curcus* , 100 seeds per treatments total of 2000 seeds.

2) Experimental design

The trial was set up in 3 replicated randomized complete block design with 8 concentrations of NMU of 0%, 0.05%, 0.10%, 0.20%, 0.40%, 0.80%, and 1.60%, compare with water.

3) Methods

The seeds were soaked under running water for 2 hrs. Prepared stock solution of NMU 10% by dissolved in EtOH, before mixing with phosphate buffer (2M Na_2HPO_4 and 1M citric acid, pH 5.0) to the concentration of 0, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6 % NMU. One hundred seeds for each treatment were soaked in 100 ml of the solution for 14 hrs according to the treatments, with gentle shacking. After the soaking ended, the seeds were drain with running deionized water for 30 min.

before testing for germination on the moist germination paper incubated at 25 C. Number of germinated seeds in each treatments were recorded, and the germinated seeds were transferred to were transplanted to 1 kg black plastic bags filled with rice husk ash for about 3 weeks or until the first foliates appeared. The plants were transplanted to a 5 kg plastic pot containing the mixture of soil, husk and manure in the ratio of 3:1:1, and changed monthly to get new planting media and 5 grams of 15:15:15 N:P:K fertilizer per pots. The plants were watered daily, and the pesticide was applied as necessary.

4) Plant growth measurements

Number of abnormal seedlings and plant, plant height, number of branches, number of nodes, internode length, stem diameter, number and size (width and length) of leaves were measured at 14, 24, 39 and 47 weeks after planting.

5) Statistical analysis

Statistical analysis of all the data was performed by Repeated Measures ANOVA using SPSS (ver. 16.0 for Windows). Linear regression between two subjects and plotting were established using SigmaPlot (ver. 11).

Chapter 4

Results and Discussion

4.1. Germplasm collection

Sixty four accessions of *J. curcas*, including from Prof. Dr. Sujin Jinahyon, were collected and planted (Figure 4.1). All the seeds had low percent germination (as shown in Table 4.1.). The total plants were 384 and the plot plan was sown in Figure 4.2.

Figure 4.1 Planting 5 months old *J. curcas* seedling into the field.

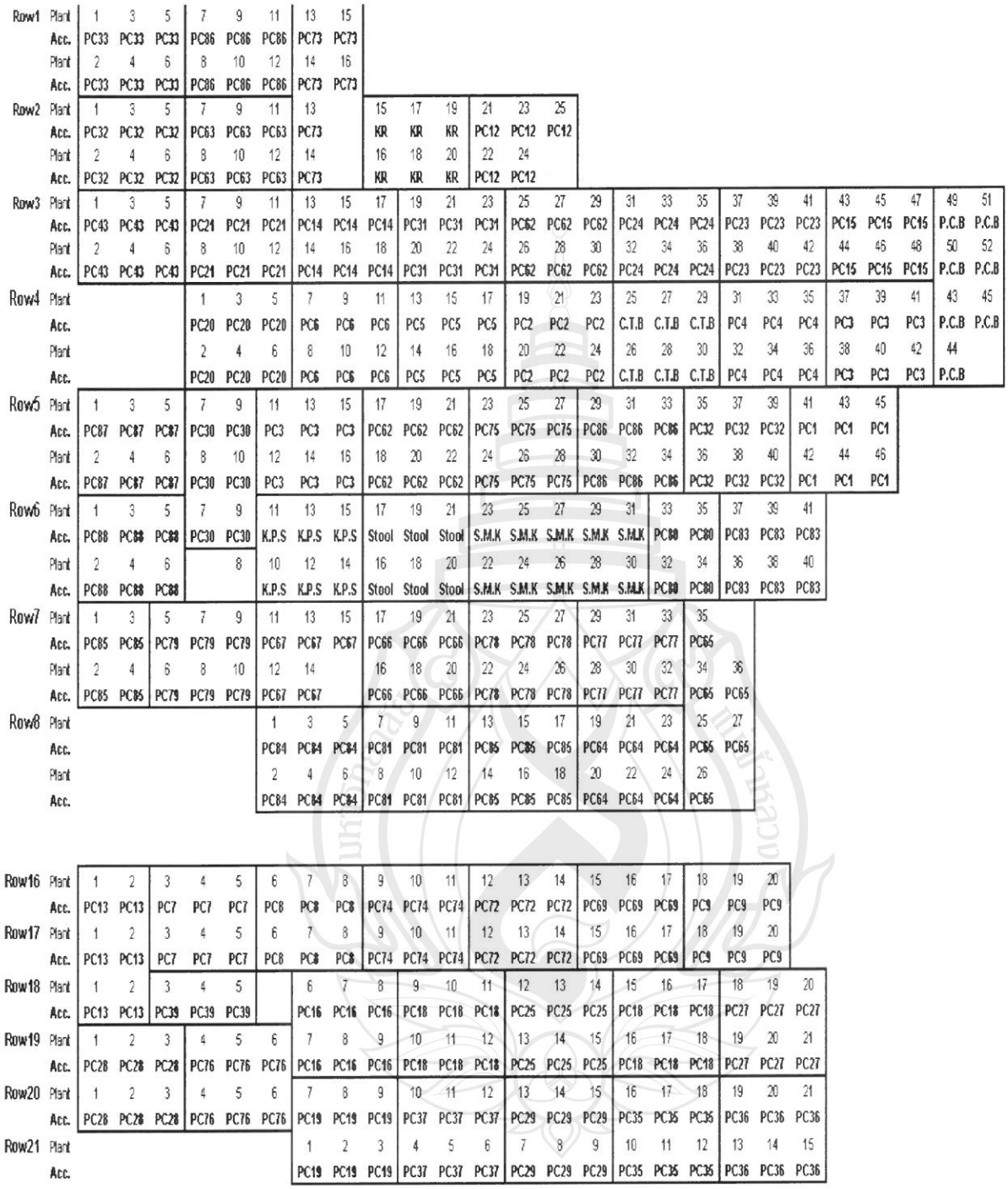

Figure 4.2 Plot plan of J. Curcas collection in Mae Fah Luang University.

Figure 4.3 *J. curcas* plants at 2 weeks after planting

Most of the phenotype namely shape, size and color of leaves, colors of shoot tip and petiole and the appearance of flowers, fruits and seeds were not distinctively different. Plant height were different according to the location in the plot; the plants in the top contour where the soil was more fertile were about 170-210 cm high while plants on the lower contour where the soil was less fertile were 140-170 cm high. The non-uniformity of the area might due to the conture construction, and the waterlogging at the lower part of he slope, and thus affected growth and flowering of the plant (Table 4.2) Plants in the upper contour were flowering at 90-100 days after planting, bared about 20-50 fruits per plant or 3.6-105.7 gm of seeds. The top three accessions were PC 75 (50 fruits and 105.7 gm seeds per plant), Chantaburi (38 fruits and 72.7 gm seeds per plants and PC 1 (32 fruits and 64.6 gm seeds per plant). This result suggested that, while genetic diversity of *J. curcas* was very low and still did not have any good and high yielding vareity, environmental condition and management might play important role in growth, development and yielding ability of *J. curcas*.

Figure 4.4 J. curcas started flowering at 90-100 days after planting

Table 4.1 Percent germination, and plant height of 64 *J. curcas* accessions. (*accession selected for DNA fingerprints)

No	Accession	%germination	Height (cm)
1*	Petchaboon	3	183.8
2*	Chantaburi	18	197.5
3*	KPS	8	153.3
4*	Stool	15	173.3
5	PC 1	90	199.2
6	PC 2	80	178.3
7	PC 3	75	205.8
8	PC 4	80	213.3
9	PC 5	85	180.8
10	PC 6	95	182.5
11	PC 7	75	155.2
12*	PC 8	95	153.3
13	PC 9	90	143.3
14	PC 12	80	188.0
15	PC 13	70	157.5
16	PC 14	85	148.0
17	PC 15	80	200.8
18	PC 16	75	156.7
19*	PC 18	95	156.7
20	PC 19	50	147.5
21	PC 20	30	185.0
22	PC 21	65	165.8
23	PC 23	75	205.8
24	PC 24	75	196.7
25	PC 25	95	162.2
26	PC 27	95	154.2
27	PC 28	80	154.7
28*	PC 29	90	153.8
29	PC 30	75	165.8
30	PC 31	60	180.8
31*	PC 32	100	226.7
32	PC 33	90	190.8
33	PC 34	50	185.8
34	PC 35	75	151.7
35	PC 36	80	143.3
36	PC 37	65	149.2
37	PC 39	20	150.8
38	PC 43	70	173.3
39*	PC 62	95	184.2
40	PC 63	80	186.7
41	PC 64	85	156.8
42	PC 65	85	201.2
43	PC 66	85	147.3
44	PC 67	80	146.8
45*	PC 69	100	155.0
46	PC 71	90	179.2
47	PC 72	90	152.2
48	PC 73	95	171.0
49	PC 74	75	155.0
50	PC 75	85	173.3
51*	PC 76	90	164.2
52	PC 77	95	172.5
53	PC 78	95	137.2
54	PC 79	55	136.7
55	PC 80	85	186.7
56	PC 81	90	175.2
57	PC 83	90	200.0
58	PC 84	100	168.3
59	PC 85	85	166.3
60	PC 86	100	216.7
61	PC 87	90	165.0
62	PC 88	90	154.7
63*	Korat (KR)	46	188.3
64*	SMK	39	193.6
	Maximum	**100**	**226.7**
	Minimum	**3.46**	**136.7**
	Average	**75.45**	**172.0**
	Standard deviation	**23.53**	**21.14**

Table 4.2 Average no. of fruit per plant, seed weight per plant and flowering date of yielding *J. curcas*.

2	Chantaburi	38	72.67	95
3	PC 1	32	64.57	95
4	PC 32	30	61.58	90
5	PC 6	24	47.78	95
6	PC80	24	47.44	95
7	PC 86	24	52.55	95
8	SMK	19	37.90	95
9	PC 33	19	40.30	95
10	Korat (KR)	19	36.28	95
11	PC15	18	28.00	95
12	PC 12	16	25.35	120
13	PC 62	14	20.81	85
14	PC 3	13	24.54	150
15	PC 24	13	18.67	90
16	PC 63	13	23.51	90
17	PC 4	11	20.37	100
18	PC 23	11	15.72	98
19	PC 87	10	20.77	120
20	PC 43	10	18.99	98
21	PC 83	9	17.68	120
22	PC14	8	13.39	120
23	PC 30	8	15.13	90
24	PC 31	7	10.98	90
25	KPS	6	9.83	90
26	Stool	6	6.00	90
27	PC 21	6	8.78	98
28	PC 77	4	5.97	150
29	PC5	3	6.28	150
30	PC 73	2	3.69	85
	Maximum	50.00	105.79	150
	Minimum	2.33	3.69	85
	Average	15.48	29.38	102
	Standard diviation	10.87	23.22	19
	Min	2.33	3.69	
	GEOMEAN	12.14	21.64	

4.2. Karyology Study

Figure 4.5 illustrates chromosome distribution inside the cells of *J. curcas*, and Table 4.3 shows number and size of chromosome for 15 accessions of *J. curcas*. We found that all *J. curcas* accessions have chromosome number 2n = 22, and the mitotic metaphase chromosomes are small with their size ranging from 0.7 to 1.5 µm. The chromosome numbers were similar to what reported by Soonthornchainakseang and Jenjitikul (2003) and Carvalho *et al.*, (2008). Carvalho *et al* (2008) using the monochromatic CCD video camera for capturing chromosome picture and analyzed by the image analysis system, could illustrate that five pairs of chromosome were metacentric and six pairs were submetacentric. In this study, the chromosomes were dark spots dispersed around the nucleus (Figure 4.5), and were too small to identify their shape and detail. This may due to the resolution limit of the microscope used in this study. To identify chromosomal variation in each accession, further cytogenetic technique should be applied for future study.

Figure 4.5 The chromosomes characteristic of *J. curcas* accession Mexico under 1000X microscope.

Table 4.3 The chromosomes number and chromosomes size of 15 accessions of *J. curcas* in this study

accession	chromosome number	Size(μm)
PC 20	22	0.80 -1.20
PC 21	22	0.70 -1.00
PC 24	22	0.80 - 1.20
PC 31	22	1.00 - 1.20
PC 43	22	1.00 -1.20
PC 65	22	0.70 - 1.20
PC 79	22	0.70 - 1.25
KR	22	0.90 -1.20
CTB	22	0.90 - 1.25
SMK	22	0.70 - 1.00
Stool	22	0.75 - 1.30
PCB	22	0.75 - 1.40
KPS	22	0.80 - 1.30
Mexico	22	0.70 - 1.50
India	22	0.70 - 1.00

4.3 AFLP Fingerprinting

Genomic DNA of total 21 accessions of *Jatropha curcas* L. and *J. podariga* was extracted by CTAB method and analyzed in 1.0% agarose gel electrophoresis. The quality of extracted DNA was shown in Figure 4.6.

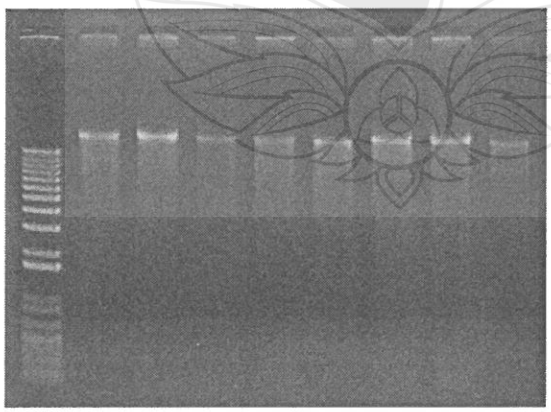

Figure 4.6 Genomic DNA of some accessions in agarose gel electrophoresis.

From the total 64 primer pairs used in this study, only 11 primer pairs could provide polymorphic bands and was selected to use for data analysis (Table 4.4).

Table 4.4 Total 64 Primer combinations used in this study, selected primer pair (√) and non –selected primer pair (x).

Primer	M-CAA	M-CAC	M-CAG	M-CAT	M-CTA	M-CTC	M-CTG	M-CTT
E-AAC	√	x	√	x	x	x	x	x
E-AAG	√	x	√	x	√	√	x	x
E-ACA	x	√	√	x	√	x	x	x
E-ACC	√	x	x	x	x	√	x	x
E-ACG	x	x	x	x	x	x	x	x
E-ACT	x	x	x	x	x	x	x	x
E-AGC	x	x	x	x	x	x	x	x
E-AGG	x	x	x	x	x	x	x	x

From the selected 11 primer pairs, there were 540 bands with size between 50-500 bp (comparing with 25 bp DNA Ladder) with 491 polymorphic bands and the remaining 49 monomorphic bands, resulting in the average of 90.21% polymorphism (Table 4.5, Appendix 2).

Table 4.5 Number of total bands, polymorphic bands, monomorphic bands, and total and average percent polymorphism.

Primer combination	Total bands	Polymorphic band	Monomorphic band	Polymorphism (%)
M-CAA/E-AAC	38	35	3	92.11
M-CAA/E-AAG	44	42	2	95.45
M-CAA/E-ACC	46	45	1	97.83
M-CAC/E-ACA	42	39	3	92.86
M-CAG/E-AAC	51	46	5	90.20
M-CAG/E-AAG	33	25	8	75.76
M-CAG/E-ACA	60	55	5	91.67
M-CTA/E-AAG	55	53	2	96.36
M-CTA/E-ACA	45	40	5	88.89
M-CTC/E-AAG	44	34	10	77.27
M-CTC/E-ACC	82	77	5	93.90
Total	540	491	49	Average = 90.21

Table 4.6 Similarity Index of 22 *Jatropha* accessions using NTSYS (version 2.01e).

Accessions	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1.000																					
2	0.755	1.000																				
3	0.736	0.806	1.000																			
4	0.646	0.668	0.687	1.000																		
5	0.746	0.731	0.798	0.766	1.000																	
6	0.687	0.733	0.832	0.699	0.822	1.000																
7	0.724	0.712	0.764	0.784	0.848	0.780	1.000															
8	0.730	0.737	0.782	0.754	0.866	0.812	0.866	1.000														
9	0.719	0.747	0.783	0.730	0.832	0.820	0.814	0.903	1.000													
10	0.697	0.729	0.807	0.713	0.798	0.813	0.798	0.830	0.838	1.000												
11	0.638	0.697	0.739	0.667	0.721	0.753	0.740	0.762	0.786	0.829	1.000											
12	0.705	0.710	0.765	0.717	0.828	0.795	0.784	0.809	0.847	0.822	0.785	1.000										
13	0.679	0.729	0.796	0.687	0.799	0.842	0.767	0.807	0.835	0.863	0.807	0.874	1.000									
14	0.670	0.736	0.792	0.682	0.774	0.826	0.755	0.798	0.807	0.824	0.795	0.832	0.926	1.000								
15	0.654	0.671	0.681	0.733	0.727	0.684	0.751	0.733	0.739	0.722	0.719	0.765	0.721	0.744	1.000							
16	0.670	0.649	0.664	0.728	0.736	0.704	0.744	0.758	0.747	0.733	0.699	0.783	0.741	0.741	0.843	1.000						
17	0.661	0.657	0.672	0.725	0.728	0.675	0.733	0.756	0.762	0.718	0.684	0.737	0.718	0.709	0.788	0.862	1.000					
18	0.641	0.627	0.634	0.716	0.698	0.672	0.712	0.709	0.695	0.694	0.677	0.715	0.717	0.704	0.786	0.840	0.860	1.000				
19	0.656	0.636	0.644	0.687	0.707	0.667	0.709	0.719	0.712	0.678	0.668	0.711	0.706	0.688	0.757	0.818	0.865	0.868	1.000			
20	0.642	0.605	0.634	0.629	0.690	0.662	0.693	0.698	0.692	0.683	0.643	0.695	0.703	0.671	0.681	0.739	0.772	0.774	0.854	1.000		
21	0.601	0.576	0.610	0.561	0.631	0.633	0.638	0.637	0.623	0.646	0.619	0.660	0.667	0.642	0.608	0.656	0.679	0.698	0.694	0.730	1.000	
22	0.452	0.468	0.477	0.482	0.470	0.449	0.510	0.481	0.491	0.527	0.521	0.505	0.483	0.480	0.527	0.502	0.499	0.522	0.500	0.475	0.497	1.000

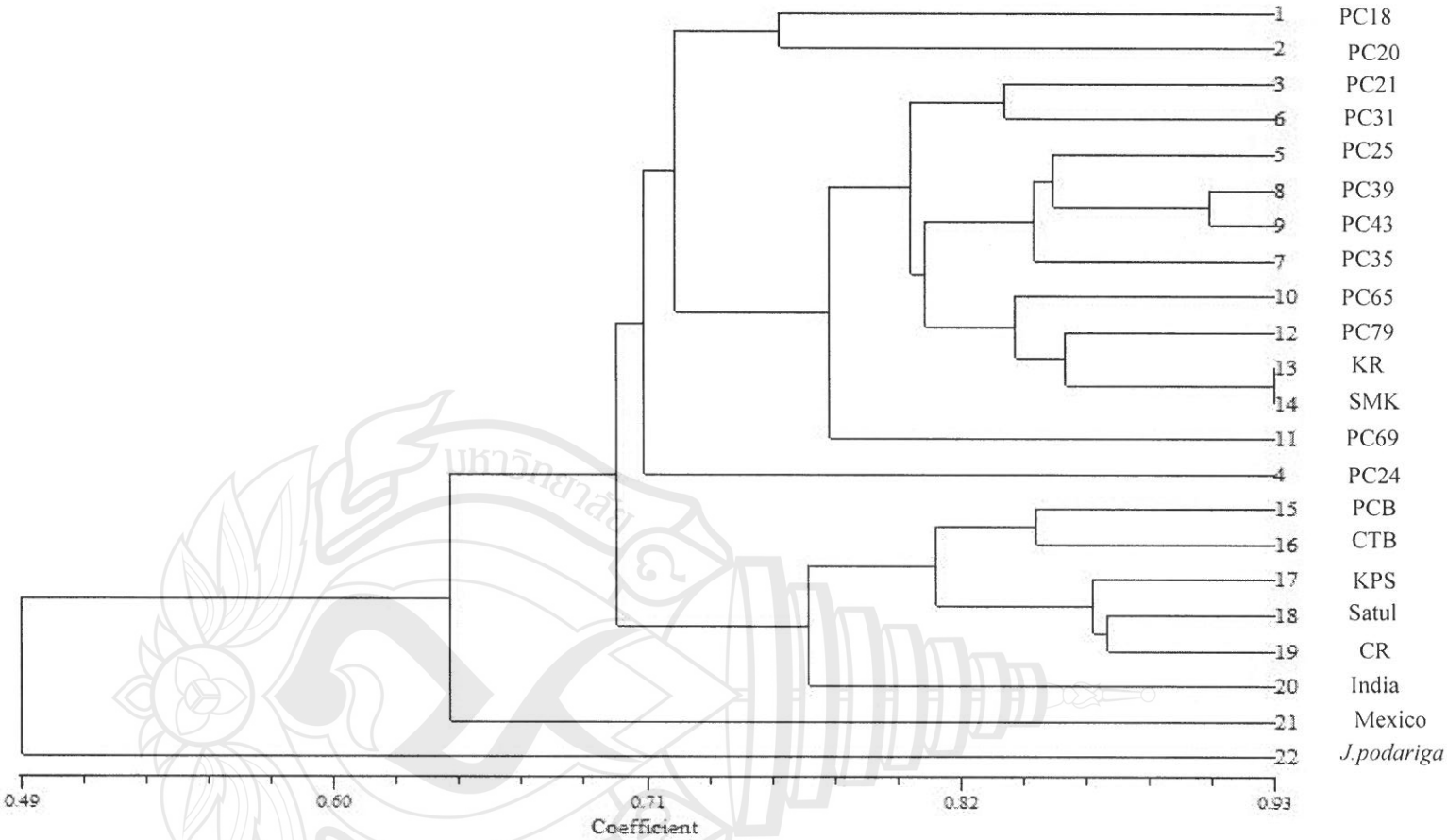

Figure 4.7 Phylogenetic trees of *Jatropha* accessions using UPGMA

The similarity index (Table 4.6) and dendrogram constructed with UPGMA cluster analysis (Figure 4.7) show the genetic similarity and relationships of each accession. *J.podariga* which is the different specie was not clustered into any groups and as different from all the other *J. curcas* accessions with low similarity index ranging from 0.45 to 0.52. Within the *J. curcas* accessions, Mexico accession which is the non-toxic phenotype was clustered into different group from the accessions from Thailand and India which are the toxic plant type. For Thai and Indian accessions, they could be clustered into 2 groups; the group of PC, KR, and SMK accessions which collected from Pakchong and Korat area in Thailand, and the group of PCB, CTB, KPS, Stool, CR, and India which collected elsewhere. However, all *J. curcas* accessions are closely related and not different due to their high similarity index ranging from 0.57 to 0.93.

Result from the dendrogram confirms that the *Jatropha* accessions could be grouped together according to their geographical distribution and their toxicity. The non-toxic Mexico accession that has origin from Mexico has the highest difference from all the toxic Thai and India accessions. This is associated with its non-toxic phenotype which is difference from Thai and India accessions.

The high similarity of Thai accessions suggested that Thailand might not be the center of origin for *J. curcas*. This plant might be introduced to Thailand from other country and then distributed all around the country. This supports the hypothesis that *J. curcas* has origin in Central America and was introduced to Asian (Heller, 1996; Wageningen, 2008). Our result is similar to the previous work done with India accessions reported by Ganesh Ram *et al* (2007), Basha and Sujatha, (2007), and Pamidiamarie *et al*, (2008). They all found differences between the Mexico and the local accessions, but no different among India accessions.

The very high polymorphism found in all *J. curcas* accessions also suggests very low genetic diversity within the global *J. curcas* population. As the consequence, varietal improvement of *J. curcas* for other desirable characteristics other then low toxicity might be impossible to achieve by the conventional breeding program. Biotechnology techniques such as induce mutagenesis to create population genetic diversity, interspecific hybridization or various types of genetically modification techniques would be necessary to introduce new desirable traits in to *J. curcas*

gene pools, and transform this undomesticated plant to be more suitable for large scale biofuel crop production in the future.

4.4 Investigating plant response, in terms of height, to plant-height-related hormones inhibitors

4.4.1. Effect of apical dominant hormones inhibitors on growth of *J. Curcus*

Plant Growth and Development

Table 4.7 shows summary ANOVA of all growth indices of the plants from all treatments all through the experiment. All plants at the start of the experiment (W_0) were not significantly different, suggested that they were uniform and the subsequent growth data could be used instead of the growth increment from the start (W_0). In addition, the interaction between treatment and time were highly significant in most of the indices, except number of leaves, so the means of all treatment combinations were compared instead of the means of the main factors (Table 4.7, 4.8).

Table 4.7 Summary of Analysis of Variance (ANOVA) of all plant growth indices (* = significant at 5% level; ** = significant at 1% level; NS = not significant)

Source of Variation	Index							
	Plant Height (cm)	Number of Leaf	Leaf area (cm^2/plant)	Total dry weight (g/plant)	Leaf dry weight (g/plant)	Shoot dry weight (g/plant)	Root dry weight (g/plant)	Crop growth rate (g/week)
Treatment	**	**	**	**	**	**	NS	**
Time	**	**	**	**	**	**	**	**
Treatment*time	**	NS	**	**	**	**	**	**

Table 4.8 Means of non destructive growth indices in terms of height (cm) and number of leaves per plant of the plants in all treatments during experiment.

Index	Treatment	Week after treated								
		0	1	2	3	4	5	6	7	8
Plant Height (cm)	Control	29.41	36.26	40.87	45.25	48.21	51.39	50.83	53.5	57.67
	IAA	29.74	36.42	40.80	44.63	47.83	52.33	52.44	55.17	59.33
	BA	28.91	30.58 *	33.93 *	37.46 *	40.96*	45.61*	45.50*	48.00*	52.08*
	IAA/BA	28.29	35.81	41.43	45.92	48.88	52.22	53.22	55.83	59.50
	Paclobutazol	28.64	32.94	34.67 *	35.63 *	36.75*	38.06*	37.61*	39.83*	44.17*
Number of leaves	Control	16	19	22	24	26	27	27	28	30
	IAA	17	19	22	25	26	28	28	29	31
	BA	17	15*	18*	22	30	27	26	28	31
	IAA/BA	17	20	23	26	28	30	30	30	32
	Paclobutazol	17	19	21	22	24	27	28	29	33

(* = difference from control of each week which is significant at 5% level)

Table 4.9 Mean of destructive growth indices in terms of leaf area (cm^2), total dry weight (g/plant), leaf dry weight(g/plant), shoot dry weight(g/plant), root dry weight(g/plant), crop growth rate and net assimilation rate of the plants from all treatments (* = difference from control of each week which is significant at 5% level)

Index	Treatment	Week after treated					
		0	1	2	4	6	8
Leaf area (cm2)	Control	909.906	1,080.000	1,320.000	2,030.000	1,810.000	3,060.000
	IAA	870.922	1,070.000	1,720.000	1,890.000	2,090.000	4,270.000*
	BA	940.654	722.471	996.283	892.968*	1,680.000	4,590.000*
	IAA/BA	862.979	1,230.000	1,200.000	1,770.000	2,300.000	4,360.000*
	Paclobutazol	837.081	1,060.000	1,160.000	1,300.000*	1,790.000	4,060.000*
Total dry weight (g/plant)	Control	12.944	19.060	28.836	44.379	49.961	100.107
	IAA	12.872	22.017	25.758	44.409	54.519	134.278*
	BA	12.978	15.193	21.926	27.116*	43.340	133.909*
	IAA/BA	11.677	20.072	26.143	49.046	61.923*	143.948*
	Paclobutazol	14.545	19.320	25.451	47.683	54.637	131.015*
Leaf dry weight (g/plant)	Control	4.524	6.116	8.710	12.652	12.764	23.367
	IAA	4.542	6.514	7.235	12.209	15.169	33.055*
	BA	4.521	4.543	6.393	9.299*	12.457	33.373*
	IAA/BA	4.337	6.792	8.336	13.996	15.843*	35.528*
	Paclobutazol	4.851	6.237	7.401	10.093	13.586	29.998*
Shoot dry weight (g/plant)	Control	4.950	8.113	12.327	20.567	22.577	47.400
	IAA	4.780	9.067	11.313	20.037	24.210	64.470*
	BA	4.990	6.693	9.240	11.723*	19.337	61.847*
	IAA/BA	4.700	8.887	11.573	22.853	27.490	66.300*
	Paclobutazol	5.760	8.453	10.980	23.273	26.558	62.160*
Root dry weight (g/plant)	Control	3.470	4.830	7.800	11.160	14.620	29.340
	IAA	3.550	6.437	7.210	12.163	15.140	36.753*
	BA	3.467	3.957	6.293	6.093	11.547	38.690*
	IAA/BA	2.640	4.393	6.233	12.197	18.590	42.120*
	Paclobutazol	3.933	4.630	7.070	14.317	14.493	38.857*
Crop growth rate (g/week)	Control		6.116	7.946	7.859	6.170	10.895
	IAA		9.145	6.443	7.884	6.941	15.176*
	BA		2.215*	4.474*	3.535*	5.060	15.116*
	IAA/BA		8.395	7.233	9.342	8.374	16.534*
	Paclobutazol		4.776	5.453*	8.284	6.682	14.559*
Net Assimilation Rate (g/week)	Control		0.006	0.029	0.090	0.169	0.390
	IAA		0.010	0.023	0.098	0.183	0.457
	BA		0.003	0.018	0.083	0.143	0.420
	IAA/BA		0.008	0.028	0.119	0.207	0.499
	Paclobutazol		0.005	0.022	0.127	0.193	0.462

Figure 4.8 and 4.9 shows height (cm) of the plant in all treatments. The shortest plant was in the paclobutazol treated group with the final height of 44.17 cm., followed by the plant in the BA treated group with the final height 52.08 cm. Height of the BA treated plant started to reduce significantly from the first week after treated and continued all though the end of the experiment. Height of the paclobutazol treated plants started to significantly reduce at 2 week after treated but the difference from control were higher than those of the BA treated ones (Table 4.8). On the other hand, height of the IAA and IAA/BA treated plant were not significantly different from the control group, and in the final week were higher than those in the control group.

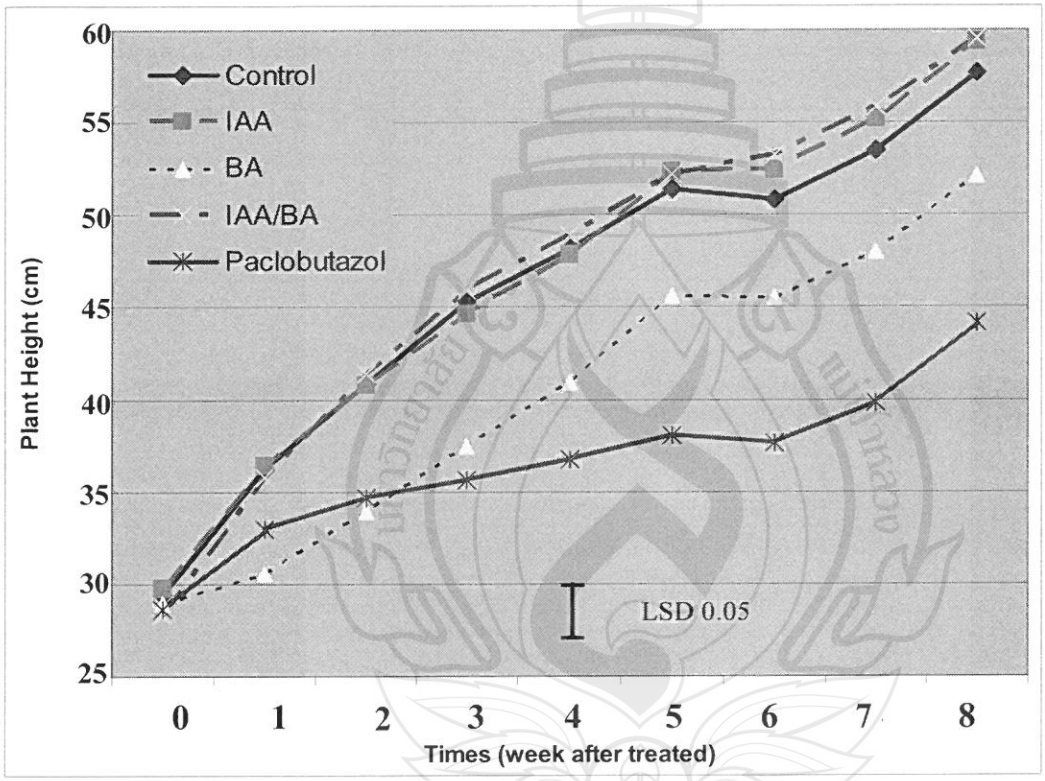

Figure 4.8 Height (cm) of the plants in the untreated control, IAA treated, BA treated, IAA/BA treated, and paclobutazol treated groups.

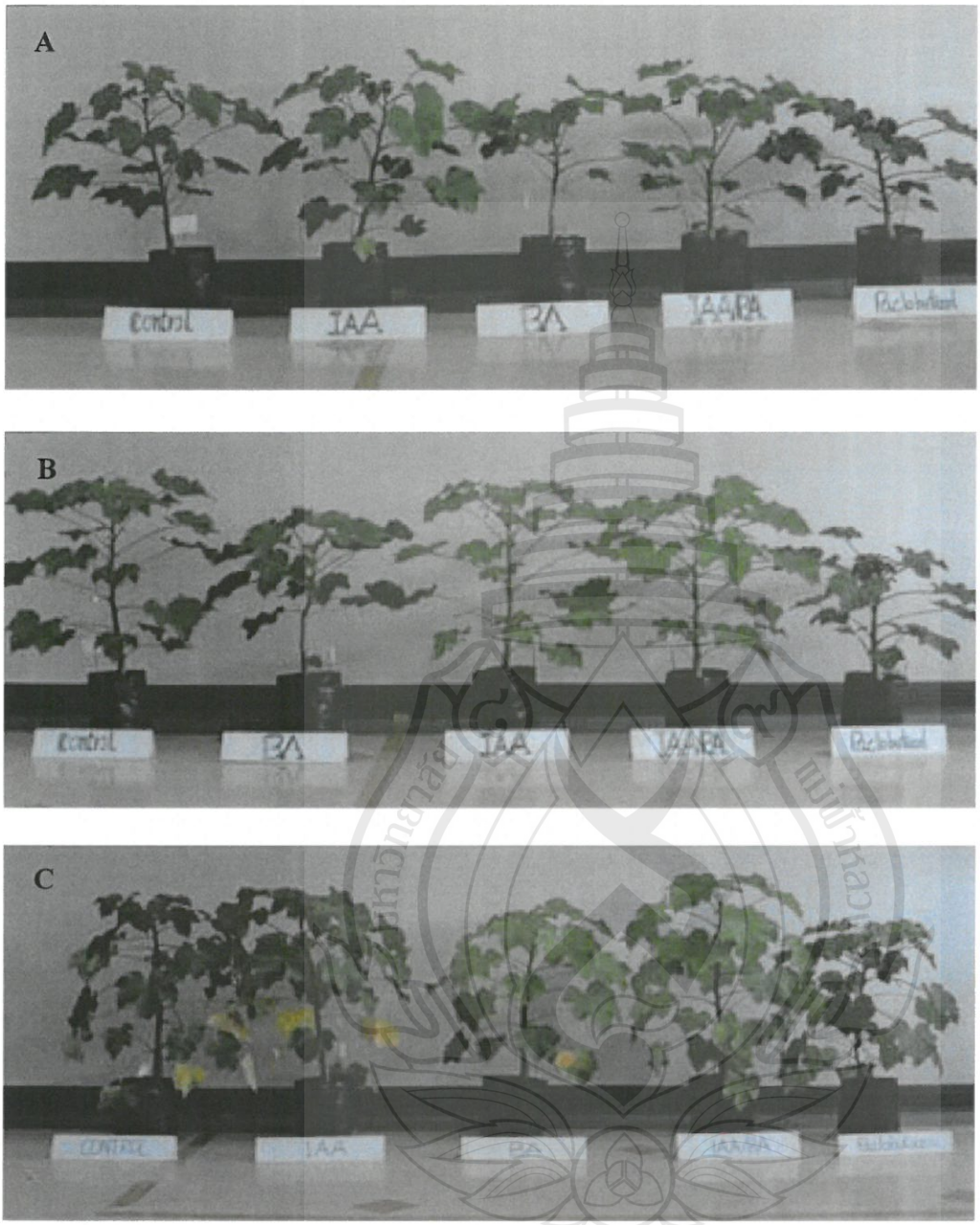

Figure 4.9 Plants at 2 weeks (A), 4 weeks (B) and 6 weeks (C) after treated with apical dominant hormone and inhibitors.

Total dry weight (g/plant) of the plants in all treatment groups were shown in Figure 4.10. Comparing with the control, dry weight of the plants in IAA, BA, IAA/BA, and paclobutazol treated groups were not statistically different from the plant in the controlled group until the final week. Final dry weight of the plants in all treated groups were significantly higher than the control as dry weight the IAA/BA treated plant was 143.948 g., the IAA treated plant was 134.278 g., the BA treated plant was 133.909 g., and the paclobutazol treated plant was 131.015 g. This might be normal for IAA and IAA/BA because both hormones are known to increase plant growth (Hopkins, 1995) but more detail analysis of each growth indices are needed for the BA and paclobutazol treated plants which were shorter than control group.

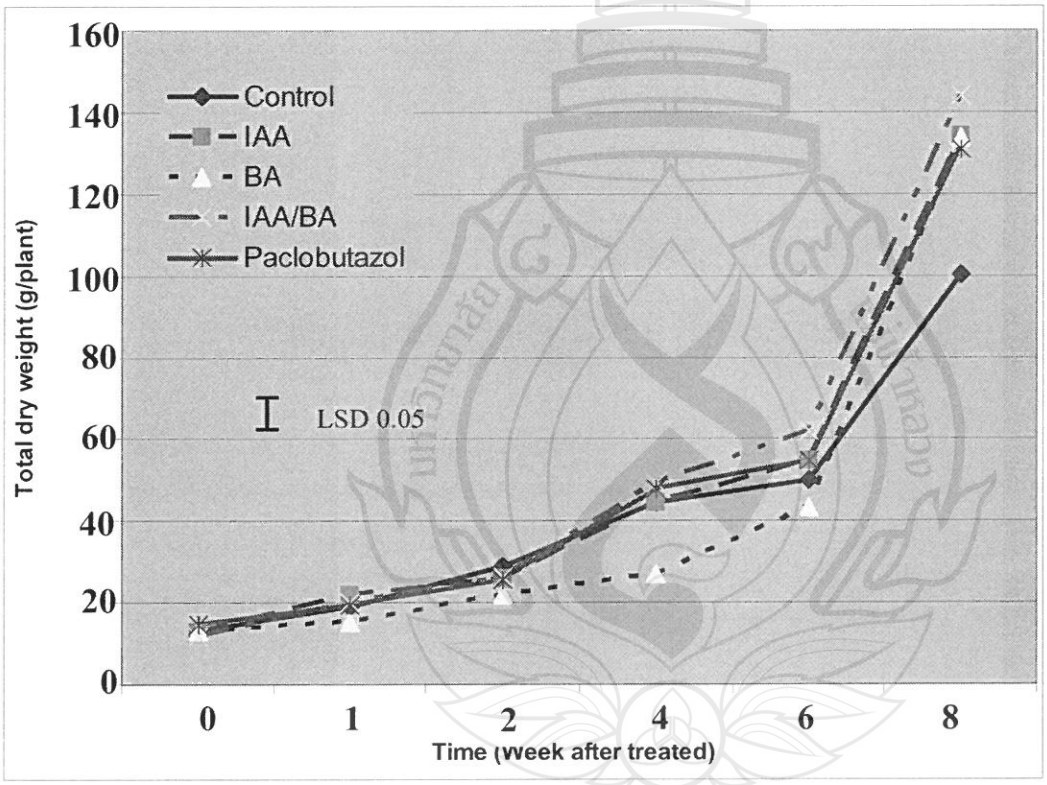

Figure 4.10 Total dry weight (g/plant) of the plants in the untreated control, IAA treated, BA treated, IAA/BA treated, and paclobutazol treated groups all through the experiment.

Figure 4.11 shows number of leaf per plant in each treatment group. Although not being statistically different, paclobutazol treated plant had 33 leaves while the IAA/BA treated plant had 32 leaves, the IAA treated plant had 32 leaves, the BA treated plant had 31 leaves and the untreated control plant had 30 leaves in the final week, respectively. Only the leaves of BA treated plant at week 1 and 2 after treated were significantly lower them the control plant (Table 4.7). This result suggested that although both BA and paclobutazol could inhibit plant height, paclobutazol did not reduce number of leaves and nodes while BA could initially reduce number of leaves and nodes.

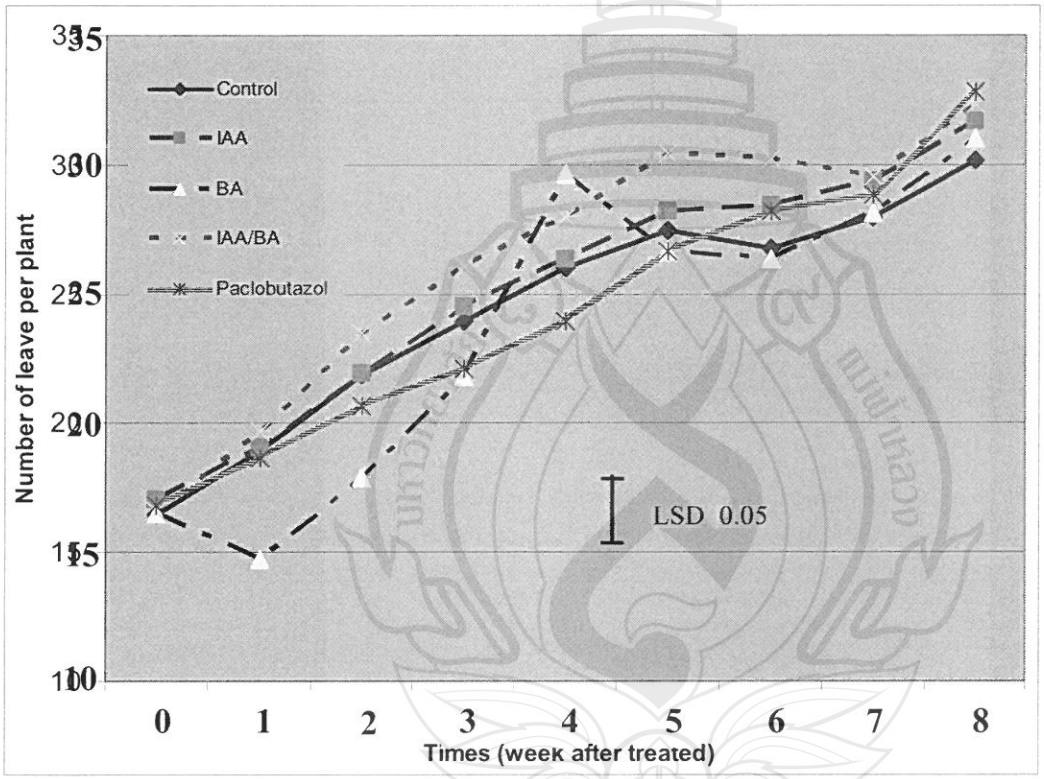

Figure 4.11 Number of leaves of the plant in the untreated control, IAA treated, BA treated, IAA/BA treated, and paclobutazol treated groups all through

Figure 4.12 and Table 4.9 shows total leaf area (cm^2/plant) of the plant in all treatments. They were not statistically different until the final week when leaf area of the control plants was the lowest (3,057 cm^2/plant). The BA treated plant was the highest (4591 cm^2/plant) followed by IAA/BA treated (4362 cm^2/plant), IAA treated (4271 cm^2/plant) and paclobutazol treated (4063 cm^2/plant), respectively. Therefore, paclobutazol and BA did not have effect in reducing total leaf area.

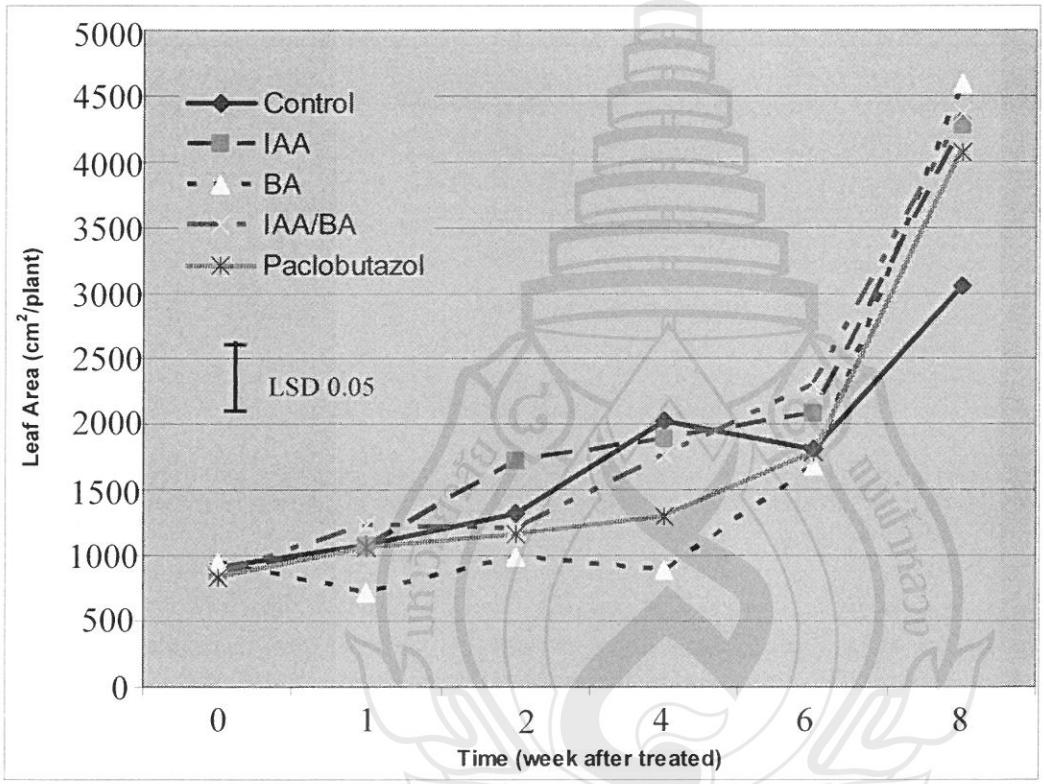

Figure 4.12 Leaf area (cm^2/plant) of the plant in the control, IAA treated, BA treated, IAA/BA treated, and paclobutazol treated groups.

Figure 4.13 and Table 4.10 shows leaf dry weight (g/plant) of the plant in all treatments. Leaf dry weight of the BA treated plant was significant lower than the control plant in week 4 and the IAA/BA treated plant was significantly higher than control in week 6. In the final week, leaf dry weight of the plants in all treated groups were significantly higher than the control (35.528 g for the IAA/BA treated plant, 33.055 g for the IAA treated plant, 33.375 g for the BA treated plant and 29.998 g. for the paclobutazol treated plant, respectively).

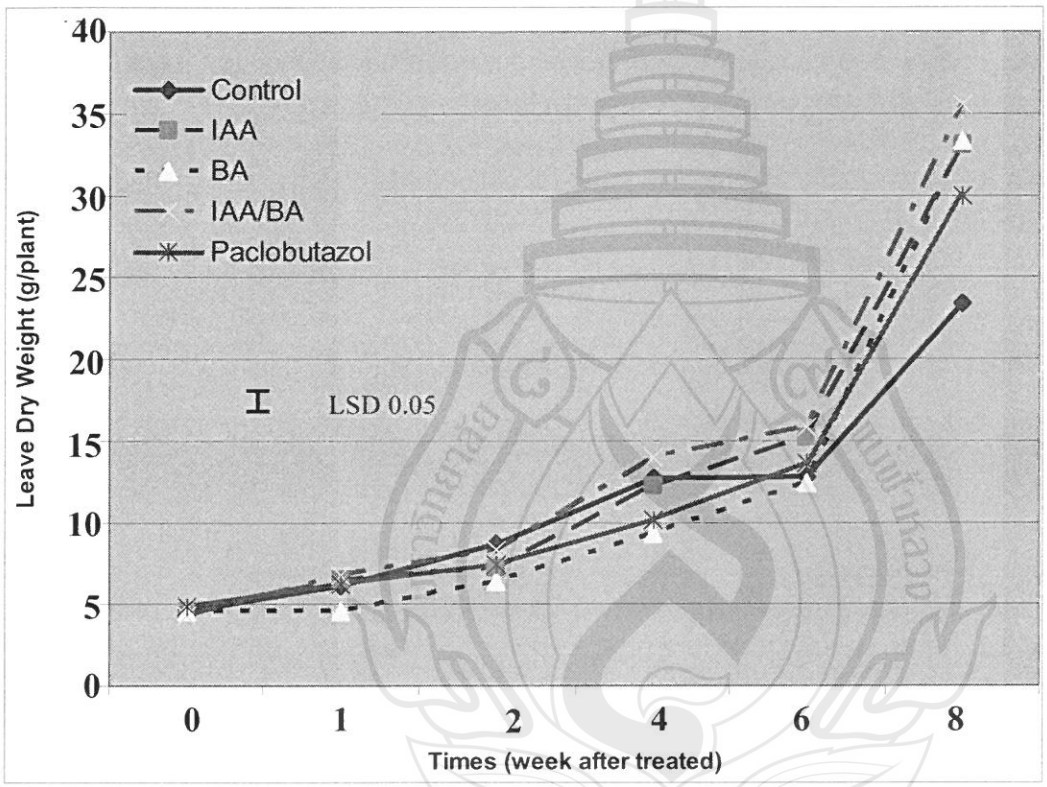

Figure 4.13 Leave dry weight (g/plant) of the plant in the control, IAA treated, BA treated, IAA/BA treated, and paclobutazol treated groups.

Figure 4.14 and Table 4.9 shows stem dry weight (g/plant) of the plants all treatments. Stem dry weight of the plants in all groups were not statistically different from the plant in the control group until the final week, except for the BA treated plant in week 4. Final stem dry weights of plants in all treated groups were significantly higher than the control. They were ranked from the maximum of 66.3 g. in the IAA/BA treated plant, 64.470 g. in the IAA treated plant, 62.160g. in the paclobutazol treated plant and 61.847 g. in the BA treated plant, respectively.

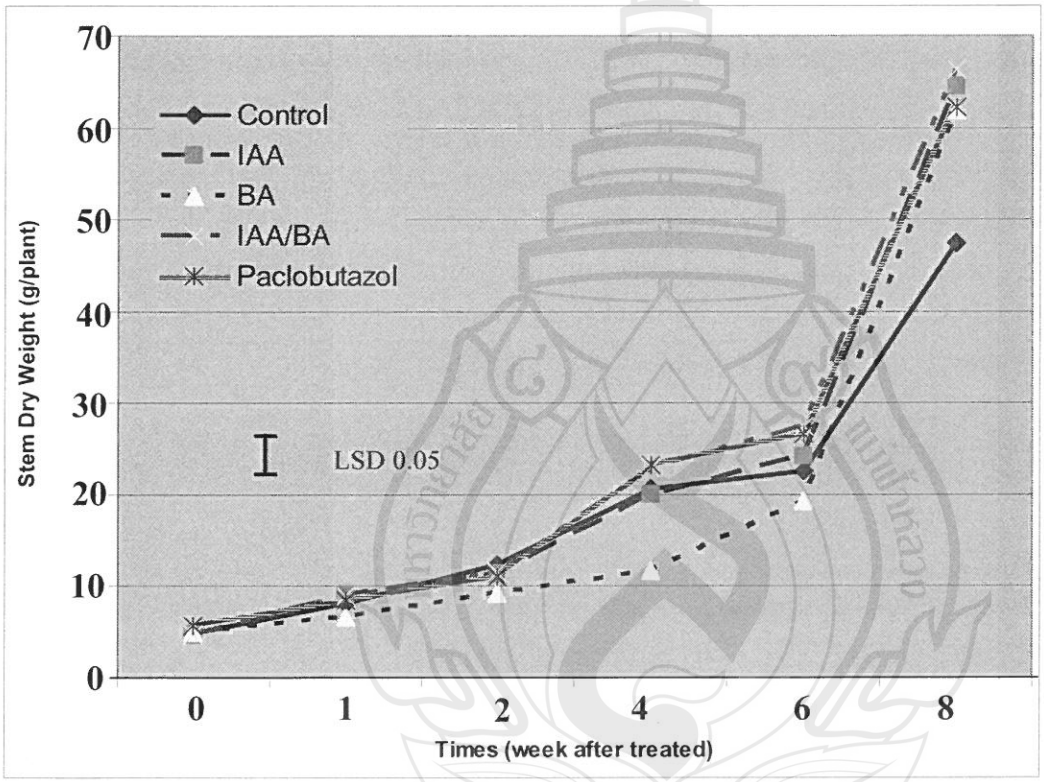

Figure 4.14 Stem dry weight (g/plant) of the plant in the untreated control, IAA treated, BA treated, IAA/BA treated, and paclobutazol treated groups all through the experiment

Figure 4.15 and Table 4.9 shows root dry weight (g/plant) of the plants in all treatment groups. They were significantly different from control group only at the final week, as the IAA/BA treated plant was 42.12 g., the paclobutazol treated plant was 38.85 g., the BA treated plant was 38.69 g. and the IAA treated plant was 36.75 g.

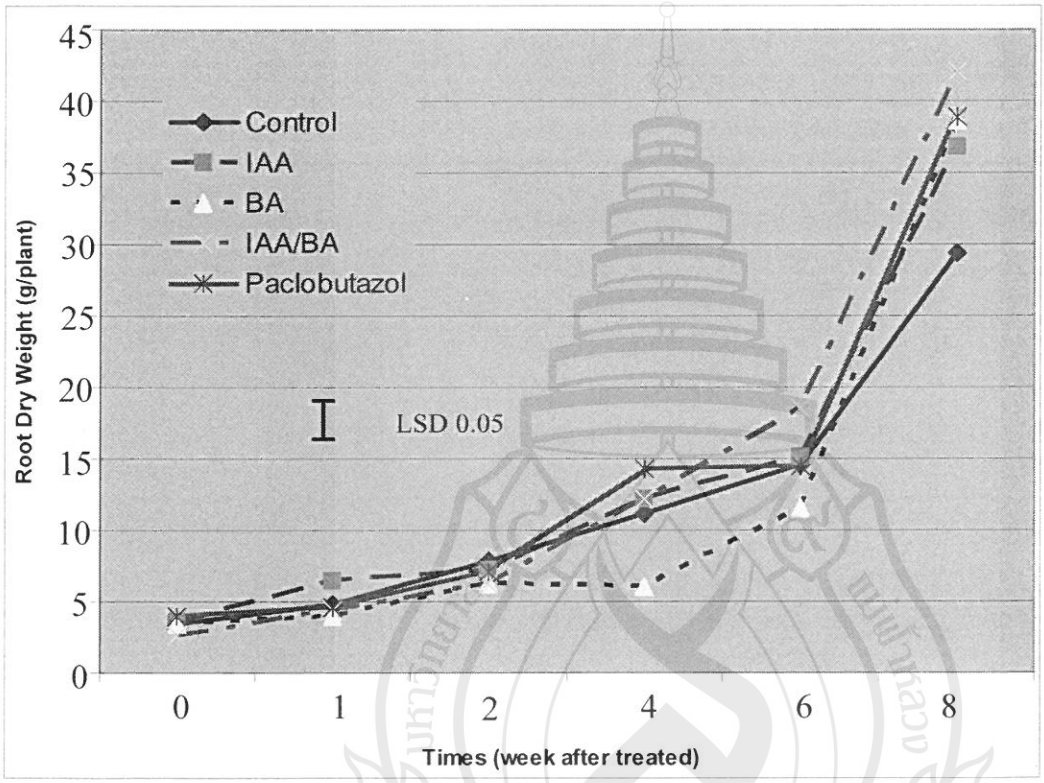

Figure 4.15 Root dry weight (g/plant) of the plant in the untreated control, IAA treated, BA treated, IAA/BA treated, and paclobutazol treated groups all thought the experiment

Therefore, paclobutazol and BA started to inhibit plant height from 2 week after treated until the end of the experiment but total dry weight, number of leaves, leave dry weight, stem dry weight, and root dry weight were increased significantly from control at the end of the experiment. However, BA reduced number of leaves and nodes while paclobutazol did not.

4.5 Growth analysis

Figure 4.16 shows crop growth rate (CGR) of the plant of all the treated groups. They were not statistically different from the plant in the controlled group until the final week when dry weight of the plant in all treated group were significantly higher than those in the control. CGR of the IAA treated plant was 15.176 g/week, the BA treated plant was 15.116 g/week, the IAA/BA treated plant was 16.534 g/week and the paclobutazol treated group was 14.559 g/week. This means every treatment can stimulated plant growth (weight). However, BA treatment in 4 and 6 week after treated have CGR value less than control group, suggesting that BA inhibited plant height and reduced growth rate of the plant at the early stage.

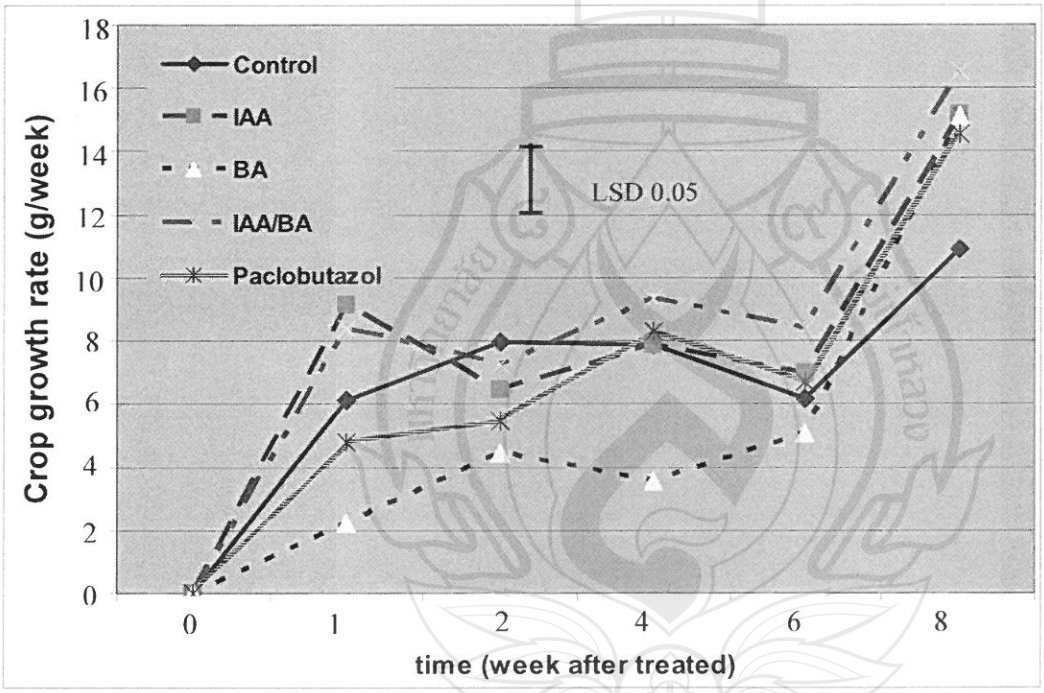

Figure 4.16 Crop growth rate (g/week) of the plant in the untreated control, IAA treated, BA treated, IAA/BA treated, and paclobutazol treated groups all thought the experiment

Figure 4.17 shows net assimilation rate (NAR) of the plant of the entire treatment groups. NAR of every groups were increased according to time, the highest rate was IAA/BA 0.499 g/week, paclobutazol 0.462 g/week, IAA 0.457 g/week, BA 0.420 g/week and control 0.390 g/week. However, NAR of BA and paclobutazol treated plant were not significantly different although number of leaves of the BA treated plant was

less than those of the paclobutazol treated ones. It might be possible that leaves of the BA treated plant were bigger and had more area for receive light for photosynthesis, and so could compensate the effect of lower leaves than the paclobutazol treated plant.

From analysis of all growth indices, it is suggested that paclobutazol had good efficiency to inhibit plant height without reducing plant development (number of leaves and nodes), rate of total dry matter accumulation (CGR) and ability of each unit area of leaf to produce dry matter (NAR). The higher number of leaves and dry weight of the paclobutazol treated plants than the untreated control plants in final week of the experiment suggested that paclobutazol might not only inhibit plant height, but may also accelerated plant dry matter production too.

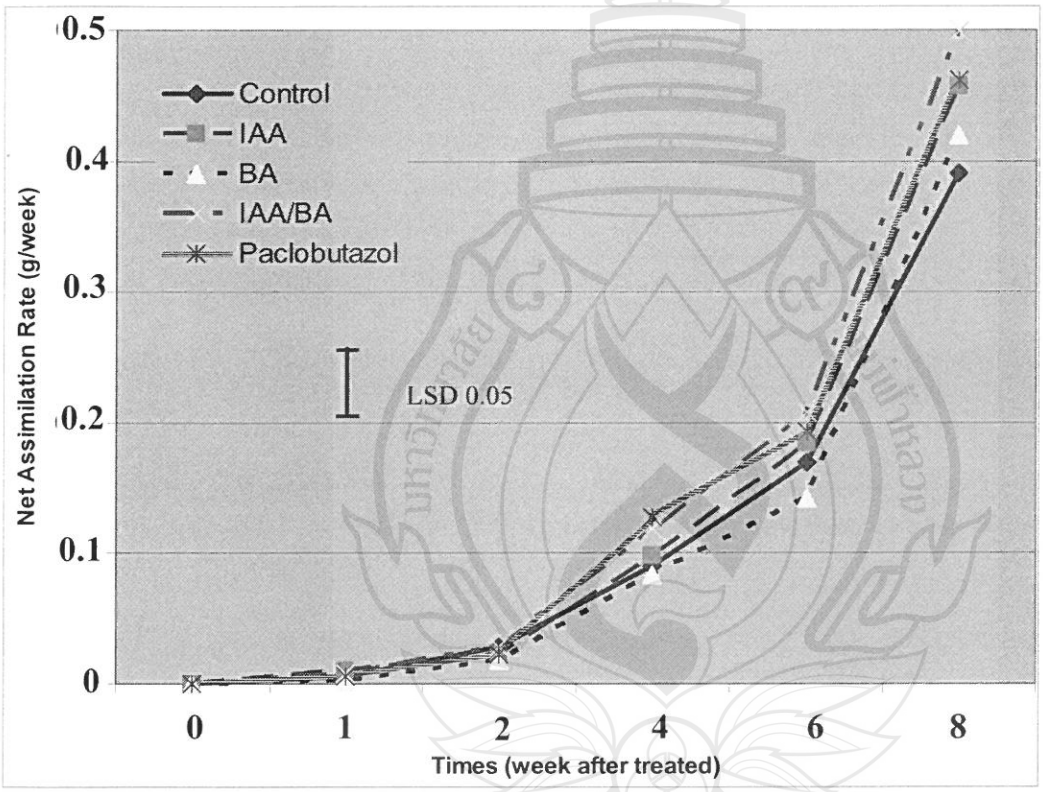

Figure 4.17 Net assimilation rate (g/week) of the plant in the control, IAA treated, BA treated, IAA/BA treated, and paclobutazol treated groups.

4.6 Concentration of IAA and GA in the Plant Tissue

Due to technical problem with the standard, IAA bioassay was not successful, so only GA bioassay can be reported (Figure 4.18). Concentration of GA were 128.8 ppm in the control group, 208.5 ppm in IAA treated group, 636.1 ppm. in BA treated group, 72.4

ppm in IAA/BA treated group and 12.8 ppm in paclobutazol treated group which was the lowest. The results confirmed that paclobutazol might inhibit GA synthetic pathway, resulting in the lowest GA production in these plants.

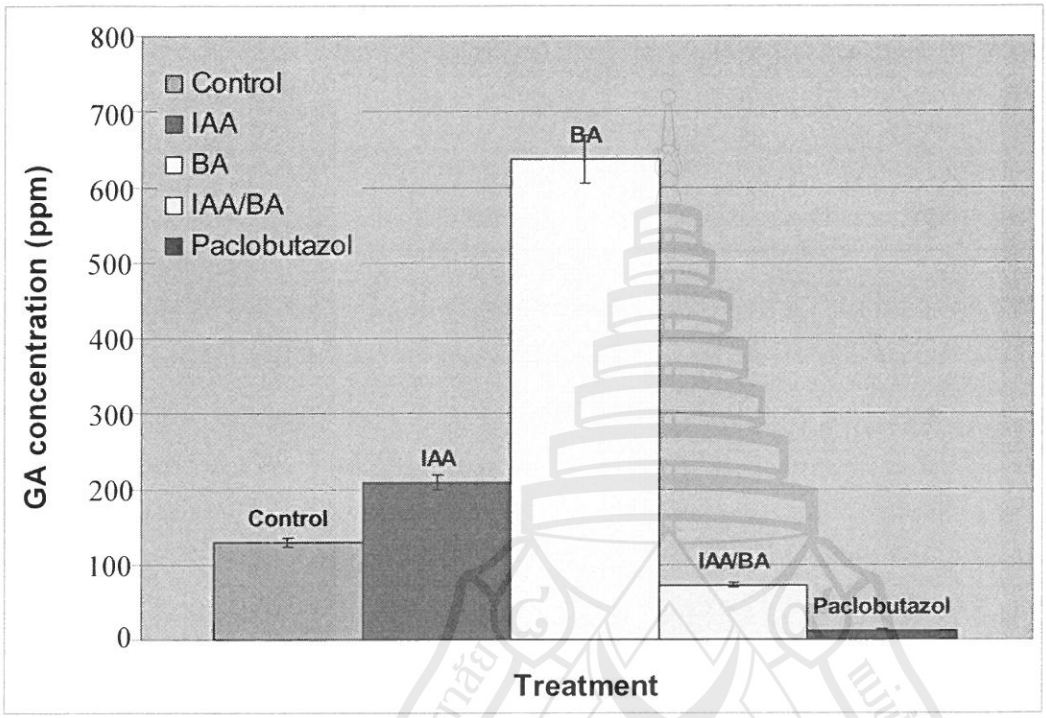

Figure 4.18 Concentration of GA hormone of the plant in the control treated, IAA treated, BA treated, IAA/BA treated, and paclobutazol treated groups from HPLC analysis.

4.7 Discussion

Results from our study suggested that two inhibitors, BA and paclobutazol, could inhibit plant height but did not reduce other growth processes such as leaf area expansion and dry weight accumulation. Therefore, BA and paclobutazol had effect on inhibit plant height but not reduce plant growth.

Although BA and paclobutazol were similar in plant height reduction, the mechanism of these two inhibitors might be different as number of leaves of the BA treated plant was less than the normal plant (compare with control plant), but leaves of the paclobutazol treated plant was not. Normally, numbers of leaves of *Jatropha* plant related to number

of node because each *Jatropha* leaf normally occur at a node and the plant that has less leaves should also has less node too (Heller, 1996).

Our results suggested that BA mechanism for height reduction might be reducing node numbers, while paclobutazol mechanism might be reducing internode length. This finding is similar to Patal and Thaker (2006) study on the effect of IAA and GA_3 on internode elongation of *Merremia emarginata*. They found that GA_3 elongated internode but IAA had effect to increasing number of nodes. Sarket et. al., (2002) also found that IAA increased the plant height, and number of nodes of soybean. Therefore BA's effect on inhibiting IAA that increase plant height through node number could be expressed by reducing number of nodes. On the other hand, paclobutazol has effect on GA which is hormone that increases internode length. Many previous research had been supported this finding. Lange (1998) studied in pumpkin seedlings and found that GA controlled plant height by inhibit internode growth of seeding. Our result is similar to those studies in that paclobutazol could inhibit plant height by reduced internode length but number of nodes is still equal to the normal plant (compare with control plant).

These results might suggest some implication on seed yield too. In *Jatropha*, number of nodes had interaction to number of branches because the bud would occur at node. More nodes should provide more secondary branches which is inflorescent productive, and increase final yield (Hiller, 1996). Although BA could reduce plant height, reducing number of nodes and lateral branches might lead to yield reduction. Paclobutazol, on the other hand, could inhibit *Jatropha* plant height and make it easier to harvest without reducing number of lateral buds which would be developed to inflorescent productive branches; and yield might not be reduced. Quang (1988) treated paclobutazol to tomato and found that tomato treated with paclobutazol provided the highest marketable yield. Fleet and Son (2005) found that using paclobutazol to stop GA activity decreased plant height, but also induced off-season production and increased yield. Therefore paclobutazol might be the suitable plant height inhibitor as well as increasing yield.

Evidence that paclobutazol being an inhibitor of GA synthesis is confirmed by the concentration of GA from the bioassay. The concentration of GA in the tissue of the paclobutazol treated plant was the lowest of all. Janta et. al. (2005) found that paclobutazol stopped GA synthesis pathway at ent-kaurene, ent-kaurenol and ent-kaurenal before they changed into ent-kaurenoic acid which changed to GA in the final step. Ingram and Reid (1987) studied effect of GA with pea in the molecular level using antisense mutants. They found GA deficient genotypes had the lowest internode elongation because *la* and *ls* genes blocked ent-kaurene and *na* blocked ent-7a

hydroxykaureinoic acid in GA synthetic pathway. Their result suggested the possibility for genetically modifying plant with low GA.

Our results which illustrated that paclobutazol might be the best inhibitor to reduce plant height but not reduce plant growth could be important for the large scale production of *Jatropha*. Direct application of paclobutazol to the plant might temporarily decrease plant height and increase yield for a growing season. Biotechnology technique to block genes which control GA synthesis will be possible to provide the permanent result as a new *Jatropha* which is shorter than the original plant.

4.8 Testing effect of GA inhibitors on plant height, growth, photosynthesis, and C sequestration: Paclobutrazol vs. Mapiquep chloride

Table 4.10 shows summary of the ANOVA for all growth indices of *J. curcas* plants which treated with 2 different types of GA inhibitors at 3 different rates. The plant height, stem diameter, number of branches, number of nodes, average internode length and number of leaves were significantly different at different sampling time, rate and interaction between sampling time and application rate, but not significantly different at interaction between time, type of inhibitors and rate. Type of GA inhibitors significantly affect plant height, stem diameter, internode length, stem dry weight, shoot dry weight, total dry weight, carbon sequestration, net assimilation rate, crop growth rate, relative growth rate, leaf area ratio and GA concentration. The different application rate significantly affects most of the indices, except root dry weight, photosynthetic rate, accumulative CO_2 uptake and leaf area duration. Detail analysis of each of all measurements would be discussed as follows.

ble 4.10 Summary of ANOVA analysis of *Jatropha curcas* growth on the first experiment. (ns = not significantly different, * = significantly different at P<0.05, ** = significantly different at P<0.01)

Source of variation	Plant Height	Average Stem Diameter	Number of Branches	Number of Node	Average Internodes Length	Number of Leaves
	cm/plant	cm/plant			cm/plant	
ıibitors	**	**	*	*	**	*
ncentrations	**	**	**	**	**	**
ıibitors x Concentrations	**	**	ns	ns	ns	ns

Source of variation	Leaf Dry Weight	Stem Dry Weight	Root Dry Weight	Shoot Dry Weight	Total Dry Weight	Total Leaf Area
	g/plant	g/plant	g/plant	g/plant	g/plant	cm^2/plant
ıibitors	ns	**	ns	**	**	ns
ncentrations	**	**	**	**	**	*
ıibitors x Concentrations	ns	**	**	**	**	ns

Source of variation	Photosynthetic Rate	Net CO_2 Assimilation Rate	Accumulative CO_2 uptake	Carbon Sequestration	GA Concentration	GA Content
	µmol/m^2/s	µmol/s	mol	g C	µg /g dry matter	µg
ıibitors	ns	ns	ns	**	**	**
ncentrations	ns	**	ns	**	**	**
ıibitors x Concentrations	*	**	**	**	**	**

Source of variation	Net Assimilation Rate	Crop Growth Rate	Relative Growth Rate	Leaf Area Ratio	Leaf Area Duration
	g/week	g/week	g/week	cm^2/week	cm^2/week
ıibitors	**	**	**	**	ns
ncentrations	ns	**	ns	*	ns
ıibitors x Concentrations	*	**	*	**	ns

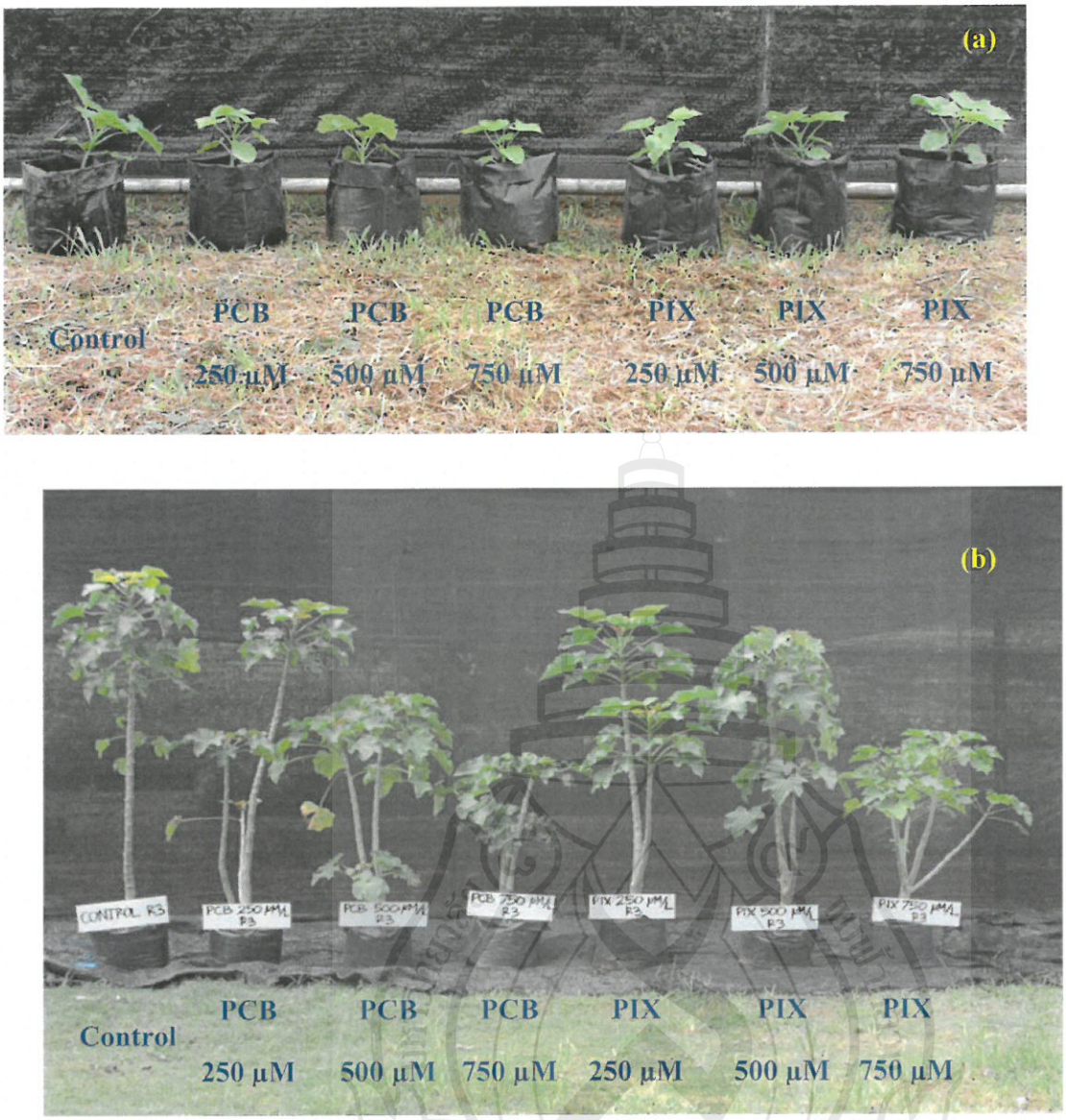

Figure 4.19 *Jatropha curcas* treated with different types and rates of GA inhibitors at (a) the first week and (b) the week 20 after application of experiment 1.

4.8.1 Plant growth

Figure 4.19 (a) and (b) illustrated the plants in all treatments at the start and 20 weeks after application, repectively. The control untreated plants had the highest plant height among all the treated plants at all sampling times (Figure 4.20). The plants treated with 250 µM of GA inhibitors were higher than plants treated with 500 µM. *J. curcas* plants which treated with 750 µM of GA inhibitors had the lowest plant height. Among the treated plants, the plants treated with mepiquat chloride were higher than the plants treated with paclobutrazol. The treated plants started to reduce its height from the first week after treated and continued all through the end of experiment. Highly

significant differences between type of GA inhibitors, application rate and their interactions suggests that GA inhibitors application might be able to reduce *J. curcas* plant height.

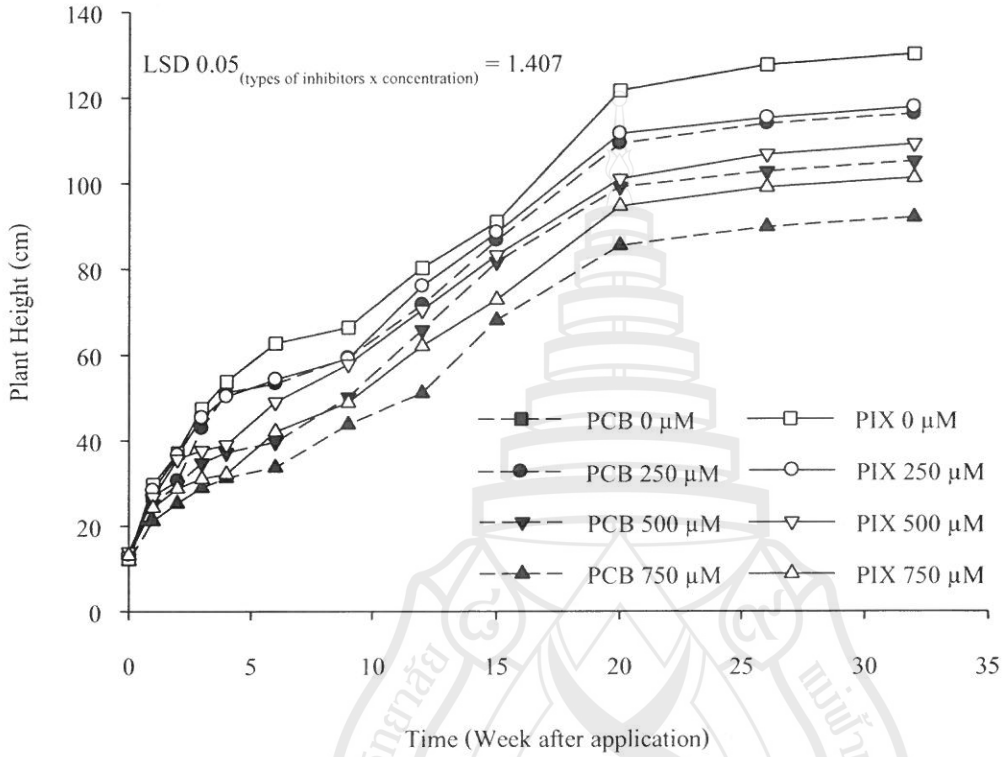

Figure 4.20 Height of *J. curcas* treated with different types and rates of GA inhibitors.

Highly significant difference of the number of nodes only occurred for plants treated with 750 µM in the first 12 weeks (Figure 4.21), but the plants could increase their nodes to be similar to the other treatments after weeks 15. The nodes were not significantly different between the control untreated plants and plants which treated with GA inhibitors at rate 250 and 500 µM. After weeks 15 there was no significant difference among treated plants, but their number of nodes increased slightly above the control untreated plants. This result indicates that GA inhibitors could reduce the plant height without reducing the number of nodes.

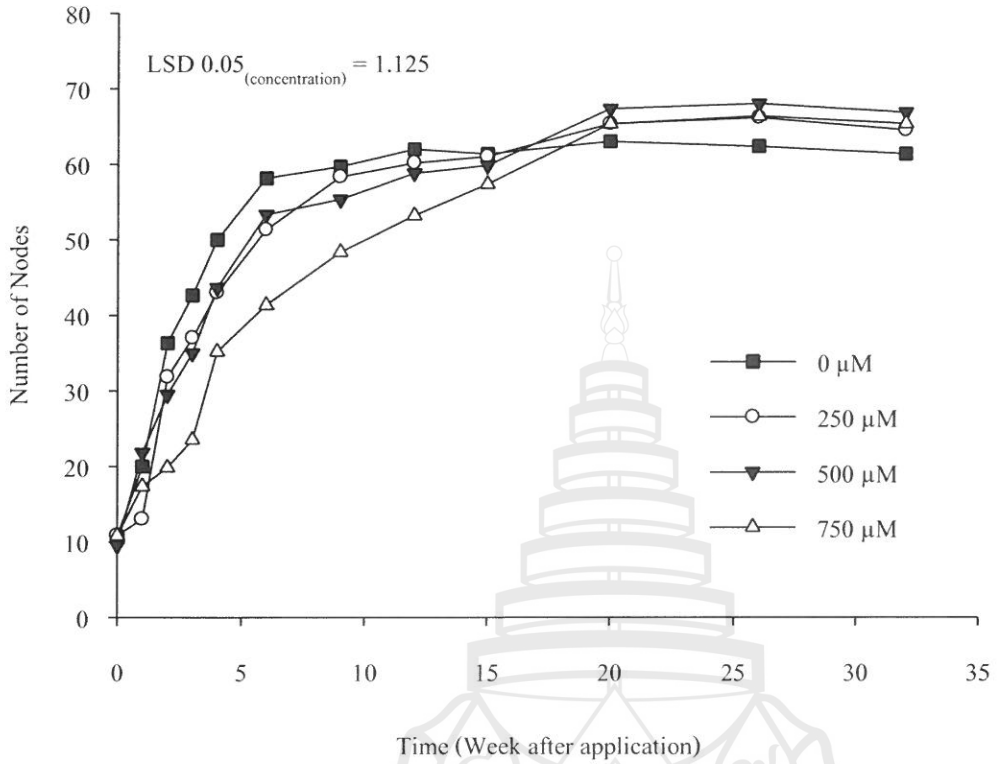

Figure 4.21 Number of nodes of *J. curcas* treated with different types and rates of GA inhibitors.

The treated plants had significantly lower average internode length than the control untreated plants from the weeks 3 after treatment until the end of sampling time (Figure 4.22). The plants treated with 500 µM of GA inhibitors had the lower average internode length than the plants treated with 250 µM of GA inhibitors. The treated plants with the highest rate (750 µM) had the lowest average internode length. This result suggests that GA inhibitors application might be able to reduce main stem height but the plants still produced the same number of node as untreated plants by reducing the internode length.

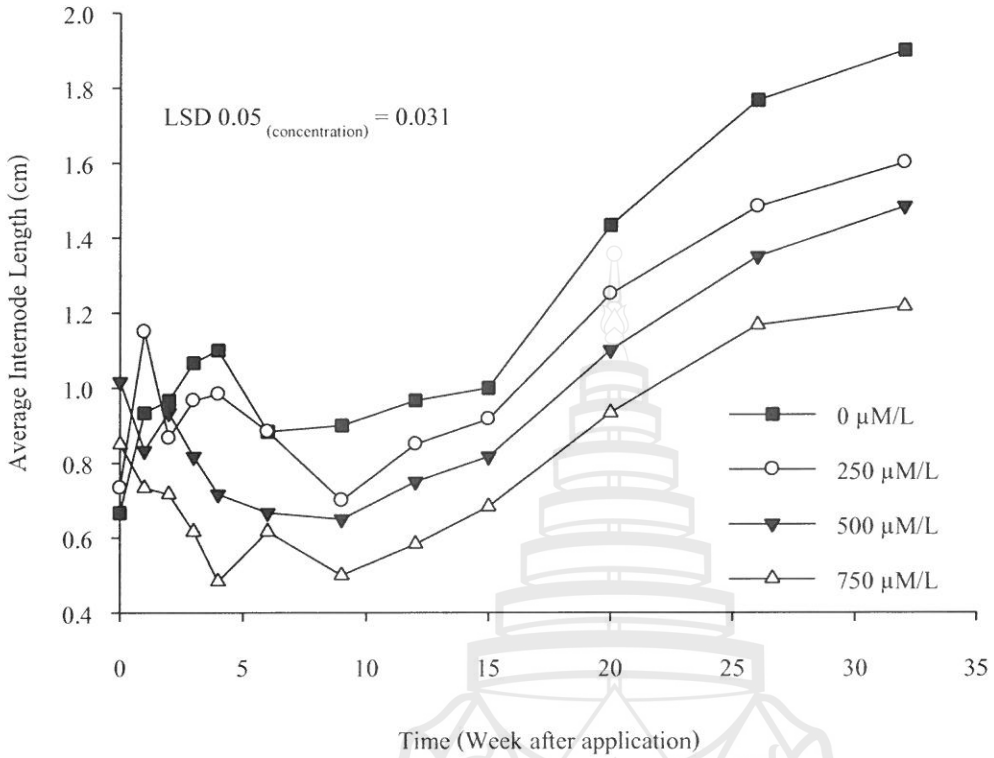

Figure 4.22 Average internode length of *J. curcas* treated with different types and rates of GA inhibitors.

The treated plants started to produce branches at 3 weeks after treated, while the control untreated plants had its first branch on 26 weeks after the treatment, but not as much as the treated ones (Figure 4.23). The treated plants with the highest rate (750 µM) had the higher number of branches than the plants treated with the lower rates. The plants treated with 500 µM of both inhibitors had higher number of branches than plants treated with 250 µM. Highly significant difference between the control untreated plants and treated plants suggests that GA inhibitors might inhibit GA hormone which reduced main stem height and stimulate branches production as well as the number of nodes.

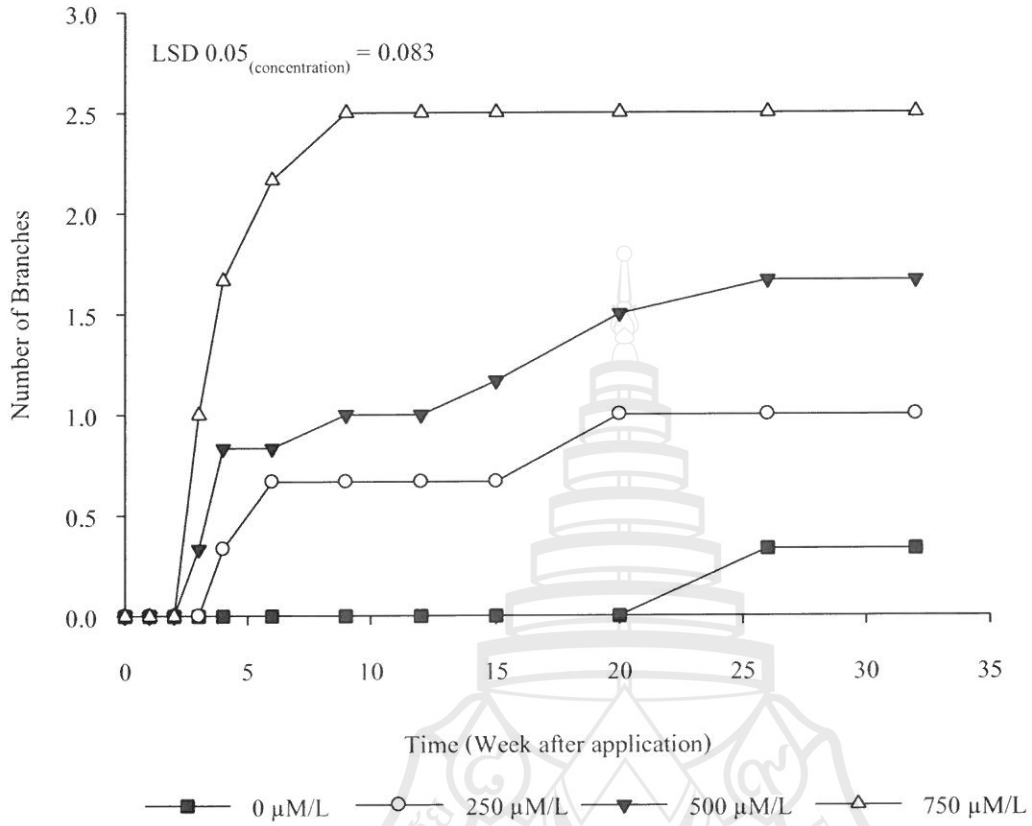

Figure 4.23 Number of branches of *J. curcas* treated with different type and rates of GA inhibitors.

Total dry weight of the treated plants were highly significant different from the untreated plants at first 15 weeks (Figure 4.24). The control untreated plants had the highest total dry weight among all the treated plants. After weeks 15, the treated plants had total dry matter higher than the treated control plants. *J. curcas* plants which treated with the highest inhibitors rate (750 µM) produced the lowest total dry weight. There was no significant difference between plants treated with 250 and 500 µM of GA inhibitors. Plants treated with paclobutrazol had higher total dry weight than plants treated with mepiquat chloride especially from week 3 after treatment until week 15. This result suggests that GA inhibitors application might affect the *Jatropha* total dry matter production at the early stage, but not at the later stage of growth.

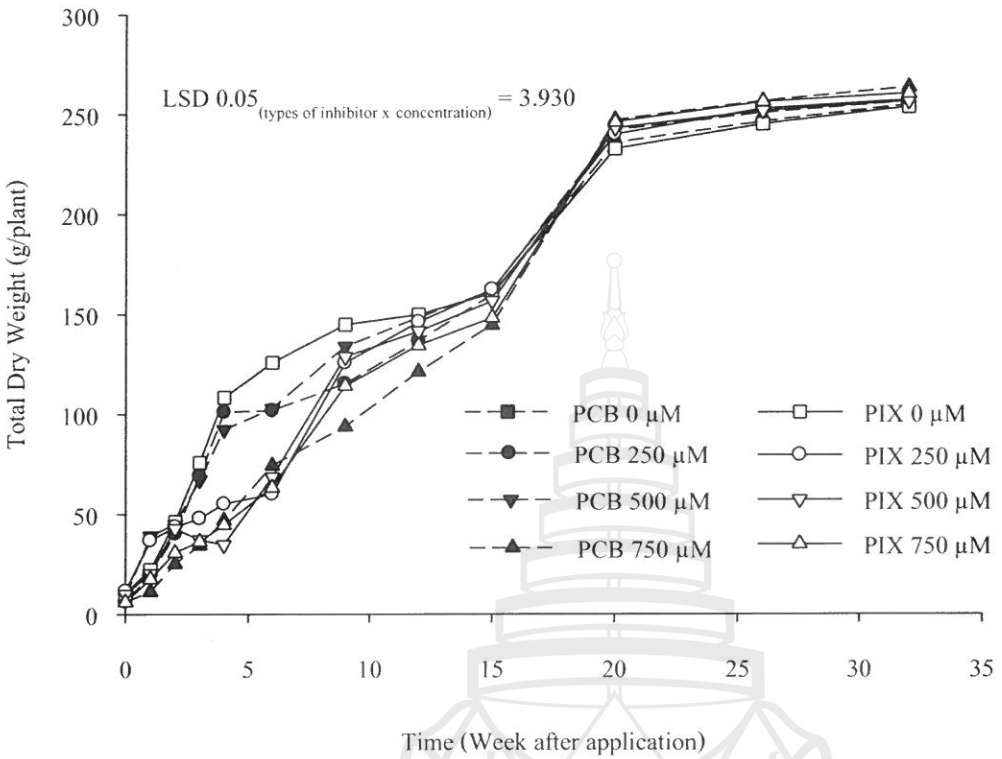

Figure 4.24 Total dry weight (g/plant) of *J. curcas* treated with different types and rates of GA inhibitors.

Stem diameter of the plants treated with paclobutrazol at the rate of 250 and 500 µM were not significantly different at the first 9 weeks, but after weeks 15 there was significantly different among all plants (Figure 4.25). The average stem diameters of *J. curcas* were significantly different between the control untreated plants and the treated ones. The control untreated plants had the lowest stem diameters and plants which treated with the highest rate (750 µM) had the highest stem diameters. The plants treated with 250 µM of GA inhibitors had the lower stem diameters than plants treated with 500 µM of GA inhibitors. Furthermore, the plants treated with paclobutrazol had the higher stem diameters than plants treated with mepiquat chloride. This result suggests that the increase of GA inhibitors application rate might decrease the main stem height but increase the stem diameters. Stem of the treated plants were shorter but fatter, thus leverage the affect on total dry matter.

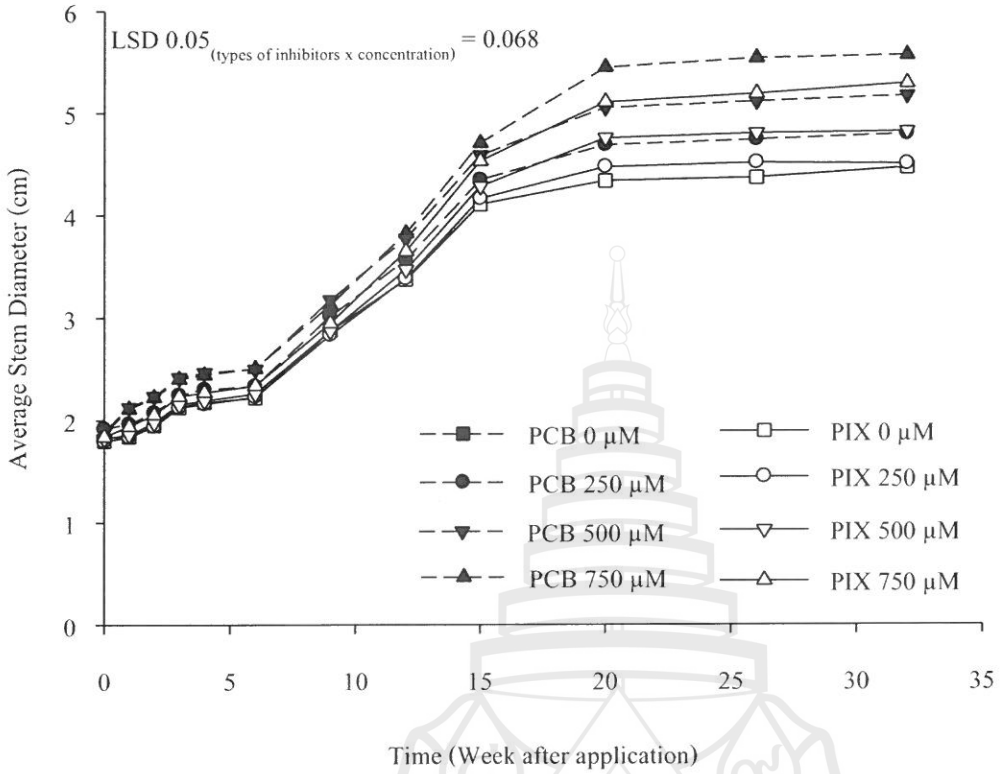

Figure 4.25 Stem diameter of *J. curcas* treated with different types and rates of GA inhibitors, experiment 1.

The shoot dry weight was significantly different at different types of inhibitors, rate of application as well as their interactions only at the early growth stage, but not at the later stage (Figure 4.26). The difference started from week 3 after treatment application which plants treated with paclobutrazol had higher shoot dry weight than the plants treated with mepiquat chloride until week 12. There was no significant difference of shoot dry weight between plants treated with 250 and 500 µM of GA inhibitors.

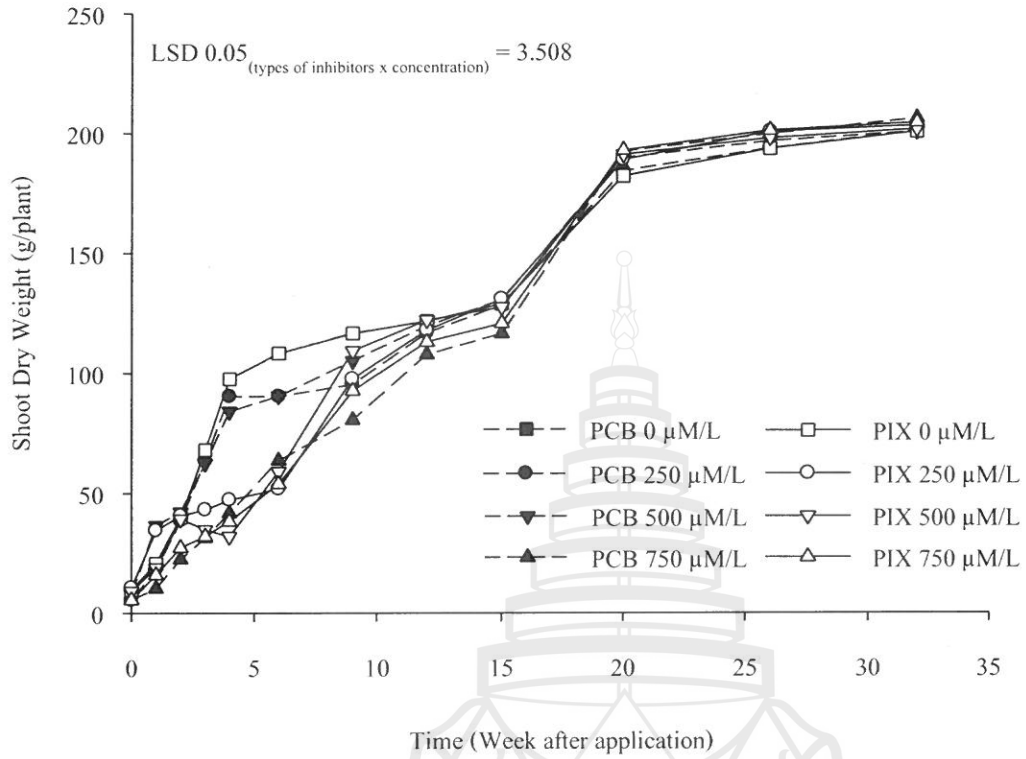

Figure 4.26 Shoot dry weight (g/plant) of *J. curcas* treated with different types and rates of GA inhibitors.

The difference of stem dry weight between the control untreated plants and treated plants started on week 3, which is the last week of GA inhibitors application, and lasted until week 12 when the difference among all treatment became less (Figure 4.27). At the first 15 weeks, the control untreated plants had the highest stem dry weight and the plants treated with 750 µM of GA inhibitors had the lowest stem dry weight. There was no significant difference between plants treated with 250 and 500 µM of GA inhibitors. The plants treated with paclobutrazol had higher stem dry weight than the plants treated with mepiquat chloride. The similar result from stem dry weight, shoot dry weight and total dry matter production suggests the significant influence of stem dry weight on shoot and total dry weight as well.

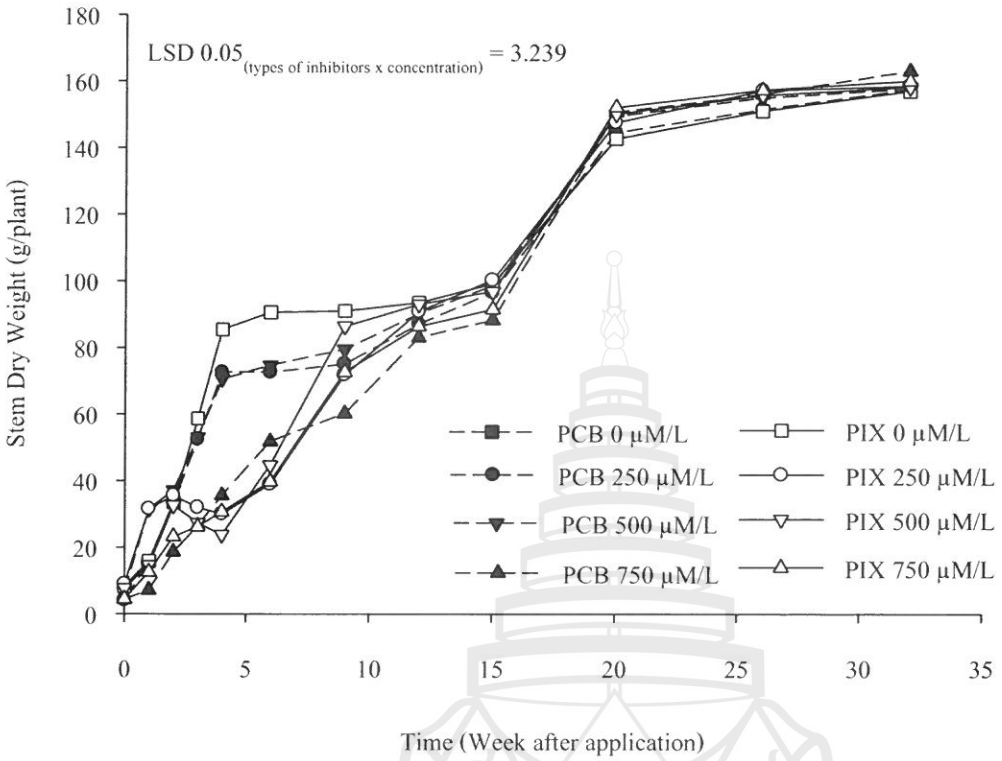

Figure 4.27 Stem dry weight (g/plant) of *J. curcas* treated with different types and rates of GA inhibitors.

Leaf dry weight of the control untreated plants was significantly different from all the treated plants Table 4.11(a). Types of inhibitors did not significantly affect the leaf dry weight, but the application rate did significantly. The plants treated with the highest rate (750 µM) produced the lowest leaf dry weight from the other plants, but there was no significant difference between the control untreated plants and plants treated with 250 and 500 µM of GA inhibitors at week 12, 26 and 32. As in nodes number, number of leaves of the control plants was significantly higher than all the treated plants until after week 15 when their leave number started to be slightly lower than the other treated plants Table 4.11 (b), but there is no significant difference between the control plants and plants treated with 250 and 500 µM of GA inhibitors. Among the treated plants, GA inhibitors only reduced leaves number of the plants which treated with the highest rate (750 µM) until after week 15 when their leave number started to keep up with the other plants. The similar result between number of nodes and number of leaves might be due to the fact that the *J. curcas* leaves arrangement is an alternative

type which each leaf is produced on each of its node, and therefore number of leaves has to be according to number of nodes of that plant.

ble 4.11 Mean of (a) leaf dry weight (g) and (b) number of leaves at each sampling week from experiment 1

Treatment	Week after application											
	0	1	2	3	4	6	9	12	15	20	26	32
a. Leaf Dry Weight (g)												
ιM	46	.83	·7	9.33	2.20	7.68	5.68	;.15	29.77	9.38	:.35	43.58
) μM	46	.82*	)8*	11.23*	17.48*	15.35*	23.04*	;.43	31.27*	40.47*	:.70	43.94
) μM	36	.62	;3*	8.33*	10.82*	15.07*	4.35	).29	31.30*	40.52*	.86	43.14
) μM	12*	.21*	)3*	5.37*	6.80*	12.95*	20.22*	25.71*	28.74*	41.21*	43.51*	43.62
D 0.05	21	.51	;7	0.65	0.65	0.65	1.42	.15	0.90	0.95	.14	1.19
b. Number of Leaves												
ιM	).7	0.0	;.3	42.7	50.0	57.0	59.7	62.0	;1.3	63.0	62.3	61.3
) μM	).8	3.0*	.8*	37.0*	43.0*	51.3*	58.3	60.2	;1.0	65.3*	66.2*	64.5*
) μM	9.5*	1.8*	).5*	35.0*	43.7*	53.3	55.3*	58.8*	)9.8	67.3*	68.0*	66.8*
) μM	).8	7.3*	).8*	23.5*	35.2*	41.3*	48.3*	53.2*	57.3*	65.3*	66.3*	65.3*
D 0.05	.2	1.6	·.5	1.4	3.6	3.9	3.8	2.3	2.4	0.8	1.2	1.5

Leaf dry weight and number of leaves * = different from the control treatment of each week which is significant at LSD $_{(concentration)}$ 0.05; the interaction between types of inhibitor and concentrations was not significant.

There was a significant difference on total leaf area between treated and untreated plants from 2 weeks after treatment until weeks 15 (Figure 4.28). Start from weeks 20 the total leaf area had no significant difference between the treated and untreated plants. Type of inhibitors did not significantly affect the total leaf area as well as interactions between type of inhibitors and the application rate. Total leaf area of treated plants was slightly less than those of the untreated control plants. The control untreated plants had wider leaf area than the treated plants especially plants treated with the highest rate of inhibitors (750 µM) which had the lowest leaf area. There was no significant difference between plants treated with 250 and 500 µM of GA inhibitors. This result suggest GA inhibitors application at rate 250 and 500 µM did not affect the leaf dry weight, number of leaves and total leaf area of *Jatropha* plants.

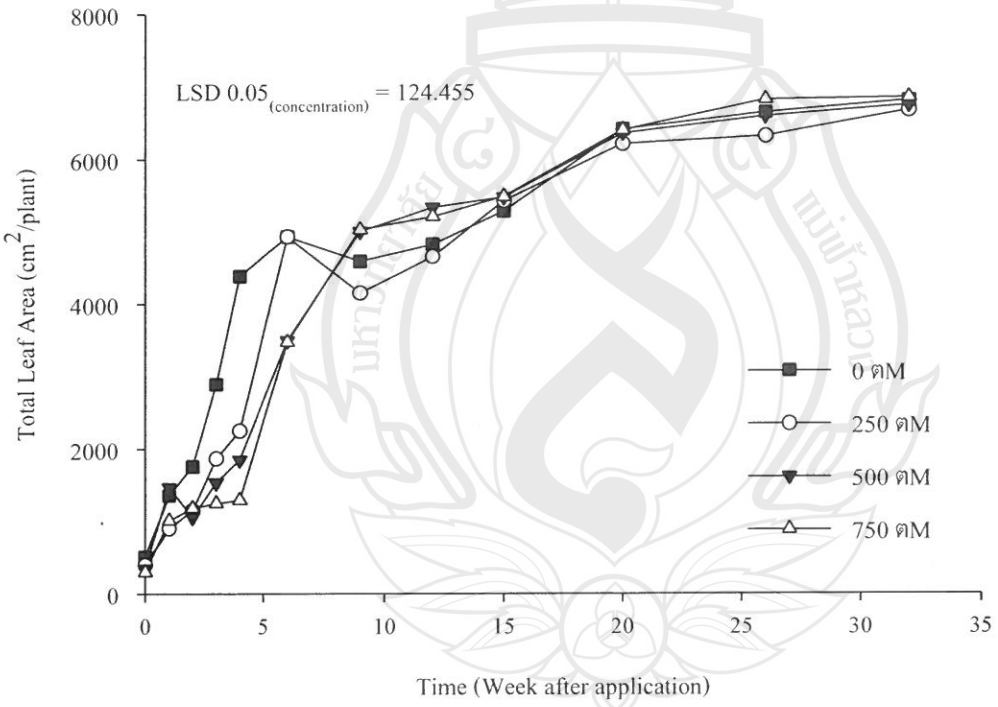

Figure 4.28 Total leaf area (cm^2/plant) of *J. curcas* treated with different types and rates of GA inhibitors.

The control untreated plant had the highest root dry weight than all the treated plants at the first 20 weeks, before the treated plants had higher root dry weight than the control untreated plants (Figure 4.29). Type of inhibitors did not significantly affect the root dry weight, but the application rate did significantly. The difference on root dry

weight among the application rate especially happened from week 2 until week 12, where the untreated control plants and plants treated with 500 µM of GA inhibitors were significantly higher than other plants. The plants treated with the highest rate of GA inhibitors (750 µM) had the lowest root dry weight from weeks 9 until weeks 15, but at week 32 after application the plants treated with 500 µM of GA inhibitors had no significant difference from plants treated with 750 µM and 250 µM of GA inhibitors.

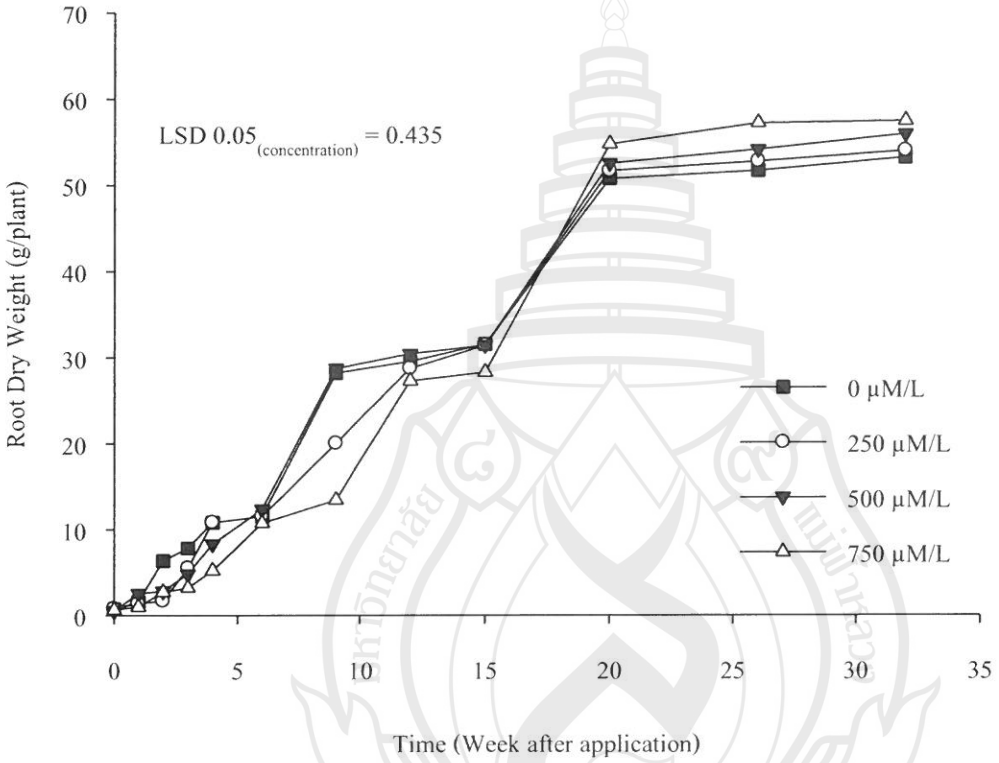

Figure 4.29 Root dry weight (g/plant) of *J. curcas* treated with different types and rates of GA inhibitors.

4.8.2 Plant growth analysis

Plants treated with paclobutrazol had the higher net assimilation rate than plants treated with mepiquat chloride (PIX). The difference was started from first week after treatment where the plants treated with paclobutrazol had significantly the higher net assimilation rate than plants treated with mepiquat chloride. The net assimilation rate per plants of *J. curcas* plants did not significantly different at different rate, but type of inhibitors did significantly. The control untreated plants had lower ability of each unit area of leaf to produce dry matter than plants treated with paclobutrazol (Figure 4.30).

This result indicates that *Jatropha* plants treated with paclobutrazol had better ability to produce dry matter from each unit area of leaf.

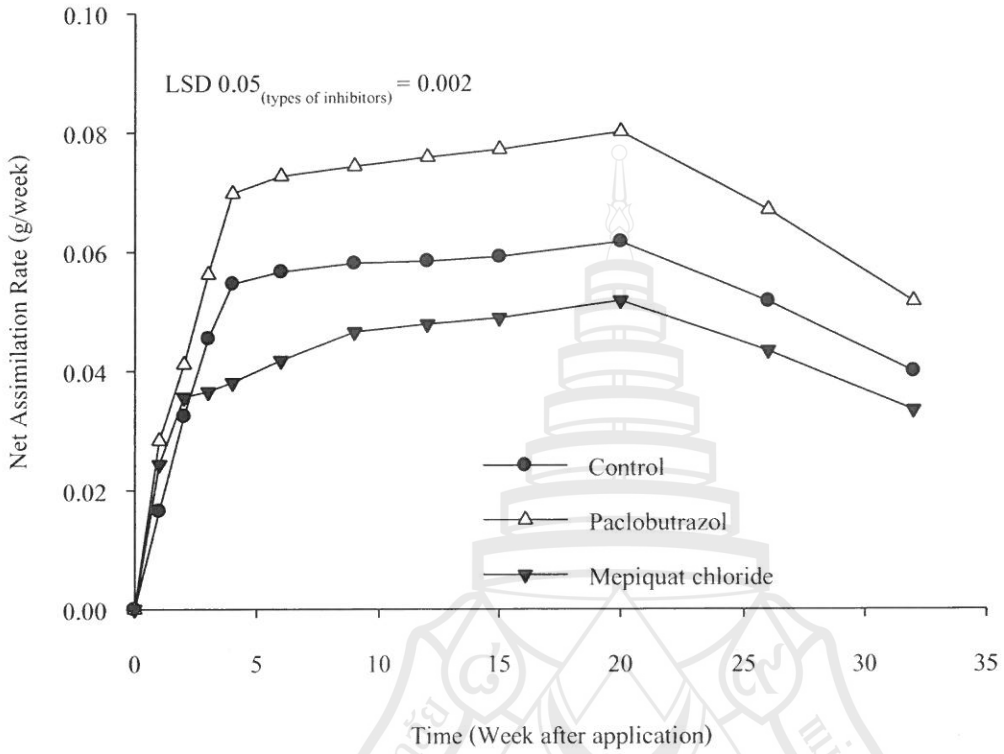

Figure 4.30 Net assimilation rate (g/week) of *J. curcas* treated with different types and rates of GA inhibitors.

The control untreated plants had higher crop growth rate and plants treated with the highest rate of GA inhibitors had the lowest crop growth rate all through the experiment. There was no significant difference between plants treated with 250 and 500 µM of GA inhibitors. Plants treated with paclobutrazol had higher crop growth rate than plants treated with mepiquat chloride (PIX). At 3 weeks after treatment, crop growth rate of the plants treated with paclobutrazol were significantly higher than plants treated with PIX (Figure 4.31). Highly significant difference between different type and rate of inhibitors suggest that GA inhibitors application might affect the rate of total dry matter accumulation of *J. curcas* plant.

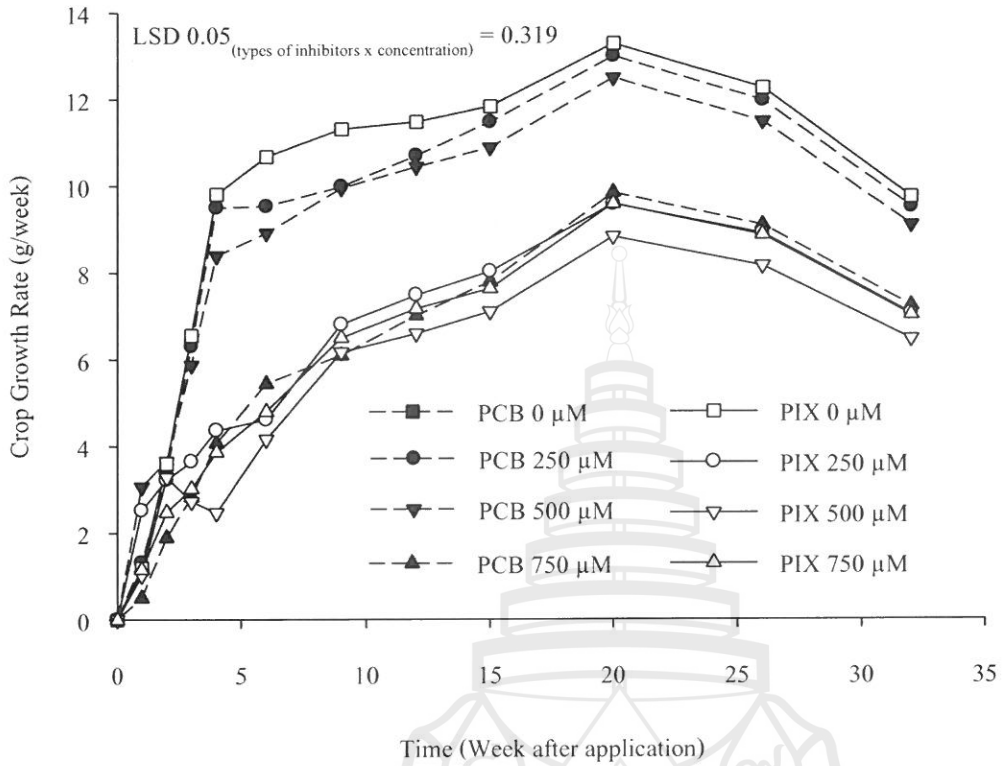

Figure 4.31 Crop growth rate (g/week) of *J. curcas* treated with different type and rates of GA inhibitors.

Relative growth rate of the untreated control plants was not significant different from the treated plants (Table 4.12). However, although the relative growth rate of *Jatropha* plants did not significantly different at different rate, the type of inhibitors did significantly. Plants treated with paclobutrazol had higher relative growth rate than plants treated with mepiquat chloride started from 3 weeks after treatment. This result suggests the *Jatropha* plants which treated with paclobutrazol accumulating dry matter higher than plants treated with PIX.

There were significant difference between treated plants and control untreated plants at first 20 weeks on the leaf area ratio (Table 4.13 a). The control untreated plants leafiness was no significantly different from plants treated with 250 and 500 µM of GA inhibitors. After weeks 20 the leaf area ratio between the control untreated plants and the treated plants had no significant difference. The different type of inhibitors significantly affects the leaf area ratio as well as the interaction between type of inhibitors and rate. The difference especially occurred at week 3 until week 15. The plants treated with

paclobutrazol 250 µM had the highest leaf area ratio at week 6 until week 9, in the other hand plants treated with paclobutrazol 500 µM hand the lowest leaf area ratio from week 3 until week 6 *J. curcas* plants which treated with the highest inhibitors rate (750 µM) produced the highest of leaf area ratio.

The leaf area duration of the control untreated plants and the plants treated with 250 µM of GA inhibitors were significantly lower than the plants treated with 500 µM at the first 3 weeks, but not significantly different from the plant treated with 750 µM during that time (Table 4.13 b). At the week 9 the plants treated with 500 µM of GA inhibitors had higher leaf area duration among the treated plants. However, the overall average of the leaf area duration between the control untreated plants and the treated plants was not significantly different. This result indicates GA inhibitors application did not affect the increase of leaf area over time.

ble 4.12 Mean of relative growth rate (g/week) at each sampling week from experiment 1

Treatment	Week after application											
	0	**1**	**2**	**3**	**4**	**6**	**9**	**12**	**15**	**20**	**26**	**32**
lative Growth Rate (g/week)												
ntrol	0	:9	)7	;6	)2	)9	)4	)5	)7	5	;3	:0
:lobutrazol	0	:9*	3	:7	)2	)2	)9	5	9	:8	'4	:9
:piquat chloride	0	:9	;6*	)0*	)0*	9*	·1*	·5*	·9*	:8*	5*	:0*
D 0.05	0	9	5	5	5	5	5	5	5	5	5	5

;R * = different from the control treatment of each week which is significant at $LSD_{(\text{types of inhibitor})}$ 0.05; the concentrations was not significant

Table 4.13 Mean of (a) leaf area ratio (cm^2/week) and (b) leaf area duration(cm^2/week) at each sampling week from experiment 1

Treatment	Week after application											
	0	1	2	3	4	6	9	12	15	20	26	32
a. Leaf Area Ratio (cm^2/week)												
'B 0 µM	0	.31^{abc}	.21^{ab}	.73^{a}	.11^{b}	.83^{b}	.49^{b}	.61^{b}	.47	.99	.55	.71
'B 250 µM	0	.91^{bc}	.86^{cd}	.58^{b}	.52^{cd}	.12^{b}	.38^{b}	.75^{b}	.51	.82	.67	.89
'B 500 µM	0	.75^{bc}	.77^{d}	.49^{b}	.77^{d}	.34^{b}	.06^{b}	.22^{b}	.21	.68	.95	.10
'B 750 µM	0	.38^{a}	.04^{a}	.35^{a}	.82^{c}	.94^{b}	.87^{ab}	.10^{a}	.18	.06	.87	.00
X 0 µM	0	.31^{abc}	.21^{ab}	.73^{a}	.11^{b}	.83^{b}	.49^{b}	.61^{b}	.47	.99	.55	.71
X 250 µM	0	.85^{c}	.91^{bcd}	.68^{a}	.66^{ab}	.40^{a}	.91^{a}	.93^{a}	.18	.34	.05	.48
X 500 µM	0	.81^{abc}	.47^{abc}	.63^{a}	.79^{ab}	.99^{a}	.57^{ab}	.35^{a}	.57	.01	.19	.43
X 750 µM	0	.23^{ab}	.56^{ab}	.53^{a}	.21^{a}	.10^{a}	.19^{ab}	.18^{ab}	.54	.75	.84	.52
Leaf Area Duration (cm^2/week)												
ıM	0	3	556	325	540	)32	859	128	163	247	182	372
) µM	0	7*	1132*	1986*	3288*	25	)23	460	583	974	734	897
) µM	0	9	1366*	1446*	1658*	5436*	497	405*	)96*	343*	885	)51
) µM	0	5*	1133*	1362*	1689*	5257*	996*	253	562	538	)65	361
D 0.05	0	3	164	194	96	52	853	965	)38	)90	934	940

Leaf Area ratio = Means comparison within a column by DMRT at 5% level.

Leaf Area Duration * = different from the control treatment of each week which is significant at $LSD_{(concentration)}$ 0.05; the interaction between types of inhibitor and concentrations was not significant.

4.8.3 GA concentration

Soon after applying GA inhibitors, the GA concentration in the plant dropped significantly (Figure 4.32). The control untreated plants had GA concentration significantly higher than treated plants. The plants treated with higher rate of GA inhibitors had the lowest GA concentration. The plants treated with 250 µM of GA inhibitors had higher GA concentration than plants treated with 500 µM. *J. curcas* plants which treated with 750 µM of GA inhibitors had the lowest GA concentration. Among the treated plants, the plants treated with mepiquat chloride had the higher GA concentration than plants treated with paclobutrazol. GA concentration of treated plants started decreasing from first week after treatments, might be due to the GA inhibitors application.

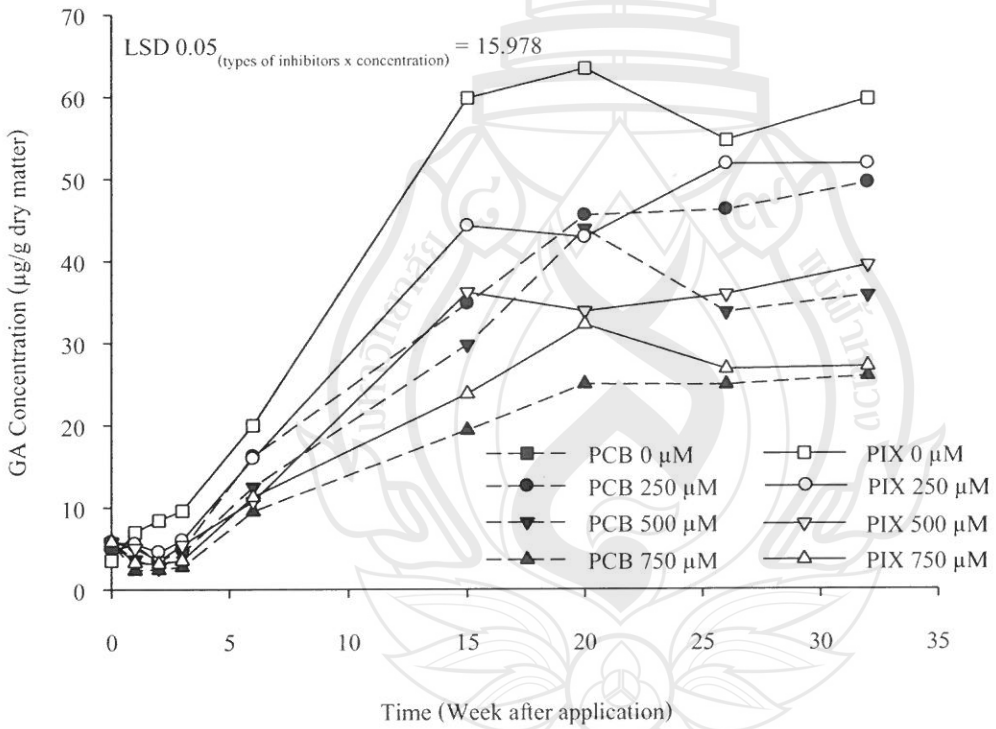

Figure 4.32 GA concentration (µg/g dry matter) of *J. curcas* treated with different types and rates of GA inhibitors.

The control untreated plants had GA content per plant significantly higher than the treated plants (Figure 4.33). The plants treated with higher rate of GA inhibitors had the lowest GA content per plant. The plants treated with 250 µM of GA inhibitors had higher GA content per plant than plants treated with 500 µM. *J. curcas* plants which treated with 750 µM of GA inhibitors had the lowest GA content per plant. Among the

treated plants, the plants treated with mepiquat chloride had higher GA content per plant than plants treated with paclobutrazol. GA content of the control untreated plants decreasing at the week 26.

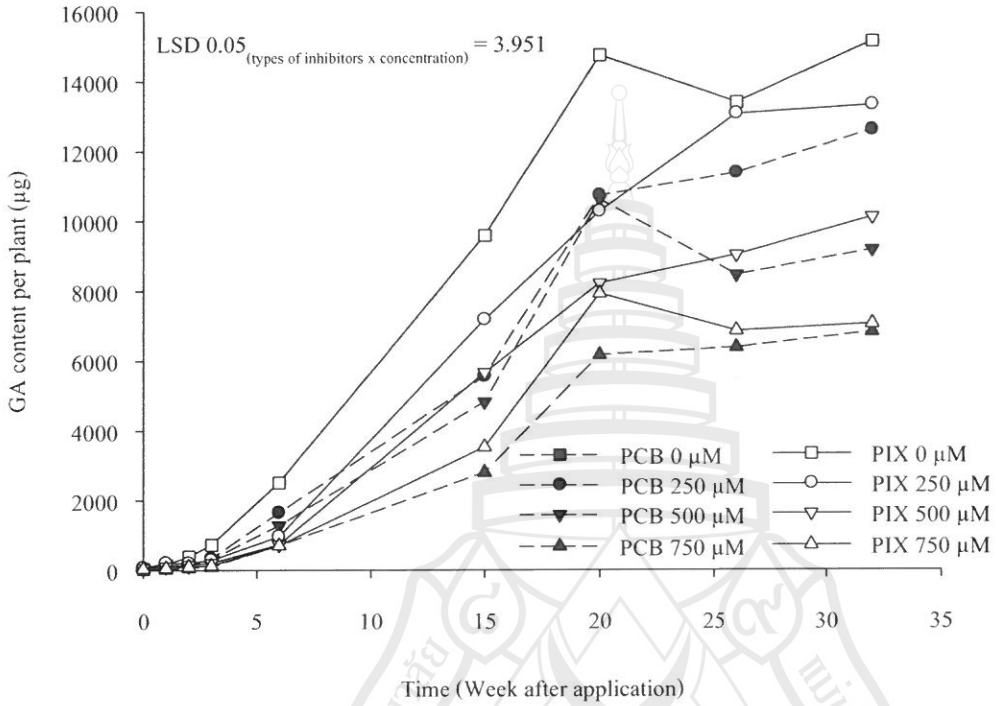

Figure 4.33 GA content per plant (µg) of *J. curcas* treated with different types and rates of GA inhibitors.

4.8.4 CO_2 assimilation and carbon sequestration

The type of inhibitors did not significantly affect net CO_2 assimilation rate, but the application rate did significantly (Figure 4.34). At the first 6 weeks, the control untreated plant had the highest net CO_2 assimilation rate than treated plants, but then decreasing to be lower than all the treated plants on weeks 15 and weeks 20. At weeks 32 there was no significant difference between the control untreated plants and the treated plants.

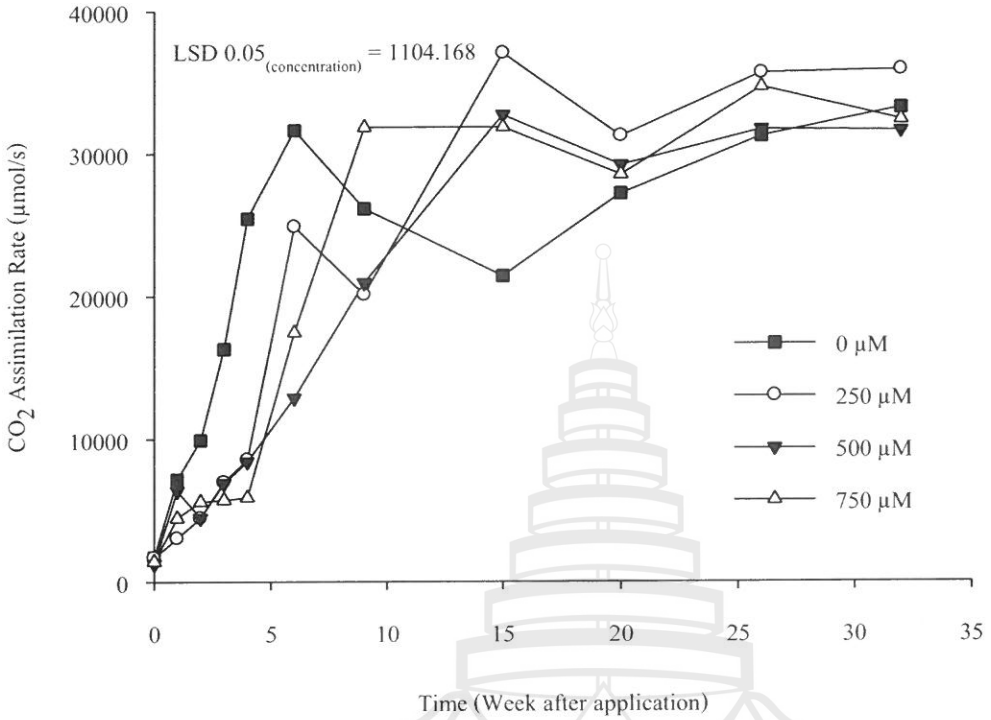

Figure 4.34 CO_2 assimilation rate (µmol/s) of *J. curcas* treated with different types and rates of GA inhibitors.

At the first 3 weeks after treatment there was no significant difference between the control untreated plants and treated plants at accumulative CO_2 uptake per plants (Figure 4.35). The control untreated plants had the higher accumulative CO_2 uptake at weeks 4 after treatment. At week 15 the treated plants had the higher accumulative CO_2 uptake than the control untreated plants, but at the weeks 32 there was no significant difference between the control untreated plants and the treated ones.

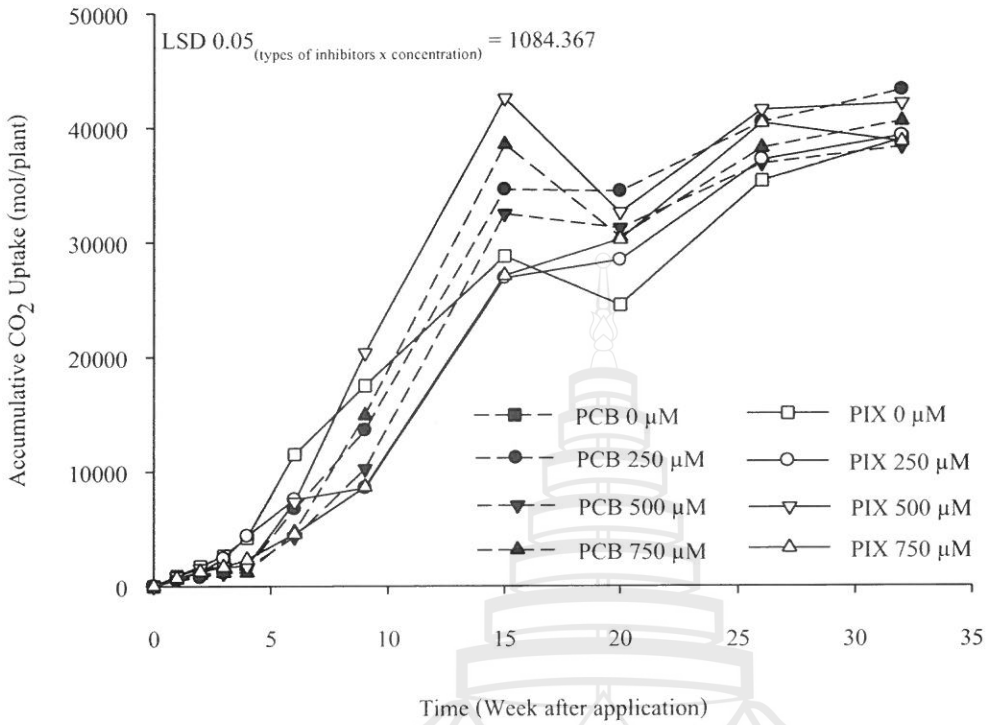

Figure 4.35 Accumulative CO_2 uptake (mol/plant) of *J. curcas* treated with different types and rates of GA inhibitors.

The significant difference in carbon sequestration especially occurred at the first 15 weeks, where the control plants had the highest carbon sequestration than treated plants (Figure 4.36). Type of inhibitors and rate significantly affected carbon sequestration. The plant treated with the highest rate of GA inhibitors had the lowest carbon sequestration. *J. curcas* plants which treated with the highest inhibitors rate (750 µM) produced the lowest plant carbon sequestration. There was no significant difference between plants treated with 250 and 500 µM of GA inhibitors. After weeks 15 the treated plants had carbon sequestration higher than the control untreated plants. Plants treated with paclobutrazol had higher plant carbon sequestration than plants treated with PIX. This result might due to the fact that the control plants had higher leaf area fix CO_2 from atmosphere and convert it into dry matter production.

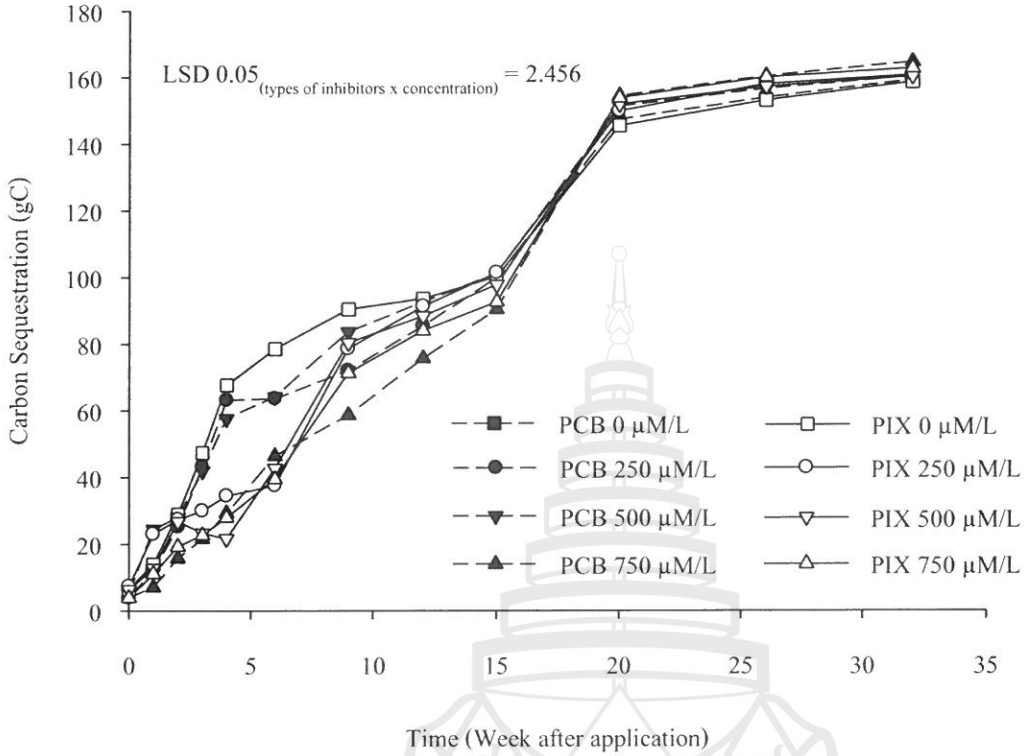

Figure 4.36 Carbon sequestration (g C) of *J. curcas* treated with different types and rates of GA inhibitors.

Result from first experiment suggested GA inhibitors application at different types and different rates affect the *Jatropha* plant growth. The GA inhibitors application could reduce the *Jatropha* plant height without reduce the number of node. Number of nodes of the control untreated plants was not significantly different from the plants treated with 250 and 500 µM of GA inhibitors. The plant height reduction was caused by the internode length reduction. The GA inhibitors application also stimulates the number of branch production.

Furthermore, the GA inhibitors application also affects the total dry weight at the first 15 weeks, but there was no significant difference between plants treated with 250 and 500 µM of GA inhibitors. Although the GA inhibitors application could reduce the plant height, the stem diameter of those treated plants was higher than the control untreated plants. The *Jatropha* plant which treated with GA inhibitors had shorter and fatter stem than the control untreated plants. The GA inhibitors at application rate 250 and 500 µM also did not significantly different on leaf dry weight, number of leaves, and leaf area.

J. curcas plants which treated with paclobutrazol had higher net assimilation rate, crop growth rate and relative growth rate than plants treated with mepiquat chloride. This result suggests that plants treated with paclobutrazol could produce dry matter higher than plants treated with Mepiquat chloride.

The GA inhibitors also affect the GA concentration and GA content of the *Jatropha* plants. Soon after the GA inhibitors application, the plants GA concentration dropped significantly. The higher application rate of GA inhibitors decrease the plant GA concentration and GA content much more.

The control untreated plants had the highest CO_2 assimilation rate than the treated plants. This result might be due to the fact that the control untreated plants had the highest leaf area per plant would have more leaf surface to fix CO_2 and thus assimilated much more CO_2 than the treated plants. The control untreated plants also had the highest carbon sequestration at the first 15 weeks which might be the consequence of absorbing carbon from the atmosphere; with the highest leaf area the control plants at first 15 weeks could absorb carbon much more from the atmosphere.

Except plant height, the other growth indices of the plants that treated with 250 and 500 µM of GA inhibitors were similar. This result indicates the plants treated with 500 µM of GA inhibitors could reduce the *Jatropha* plant height much better than plants treated with 250 µM of GA inhibitors, but had no significantly difference on number of node, total dry weight, leaf dry weight, number of leaves, and leaf area. On the other hand, GA inhibitors application with rate 750 µM had the lowest plant height and in most all of the experiment indices.

We then concluded that the GA inhibitors at 500 µM were more suitable to be used on the second experiment.

4.9 Effects of different types and application modes of giberellin inhibitors on Jatropha growth, photosynthesis and tissue GA concentrations

Table 4.5 shows summary of the ANOVA for all growth indices of *J. curcas* plants which treated with 2 different types of GA inhibitors and 3 different application modes. Type of inhibitors did not significantly affect almost all of indices, except for the plant height and internodes length. The GA inhibitors application mode significantly affected almost all of indices, except number of nodes, number of leaves, root dry weight, photosynthetic rate, and leaf area ratio. Detail analysis of each of all measurements would be discussed as follows.

ble 4.14 Summary of ANOVA analysis of *Jatropha curcas* growth on the second experiment. (ns = not significantly different, * = significantly different at P<0.05, ** = significantly different at P<0.01)

Source of variation	Plant Height	Average Stem Diameter	Number of Node	Average Internodes Length	Number of Leaves
	cm/plant	cm/plant		cm/plant	
ıibitors	**	**	ns	**	ns
ncentrations	**	**	ns	**	ns
ıibitors x Concentrations	*	ns	ns	ns	ns

Source of variation	Leaf Dry Weight	Stem Dry Weight	Root Dry Weight	Shoot Dry Weight	Total Dry Weight	Total Leaf Area
	g/plant	g/plant	g/plant	g/plant	g/plant	cm^2/plant
ıibitors	ns	ns	ns	ns	ns	ns
ncentrations	**	**	ns	**	**	**
ıibitors x Concentrations	ns	ns	ns	ns	ns	ns

Source of variation	Photosynthetic Rate	Net CO_2 Assimilation Rate	Accumulative CO_2 uptake	Carbon Sequestration	GA Concentration	GA Content
	µmol/m^2/s	µmol/s	mol	g C	µg /g dry matter	µg
ıibitors	ns	ns	ns	ns	ns	ns
ncentrations	ns	**	**	**	**	**
ıibitors x Concentrations	ns	ns	ns	ns	ns	ns

Source of variation	Net Assimilation Rate	Crop Growth Rate	Relative Growth Rate	Leaf Area Ratio	Leaf Area Duration
	g/week	g/week	g/week	cm^2/week	cm^2/week
ıibitors	ns	ns	ns	ns	ns
ncentrations	**	**	**	ns	**
ıibitors x Concentrations	ns	ns	ns	ns	ns

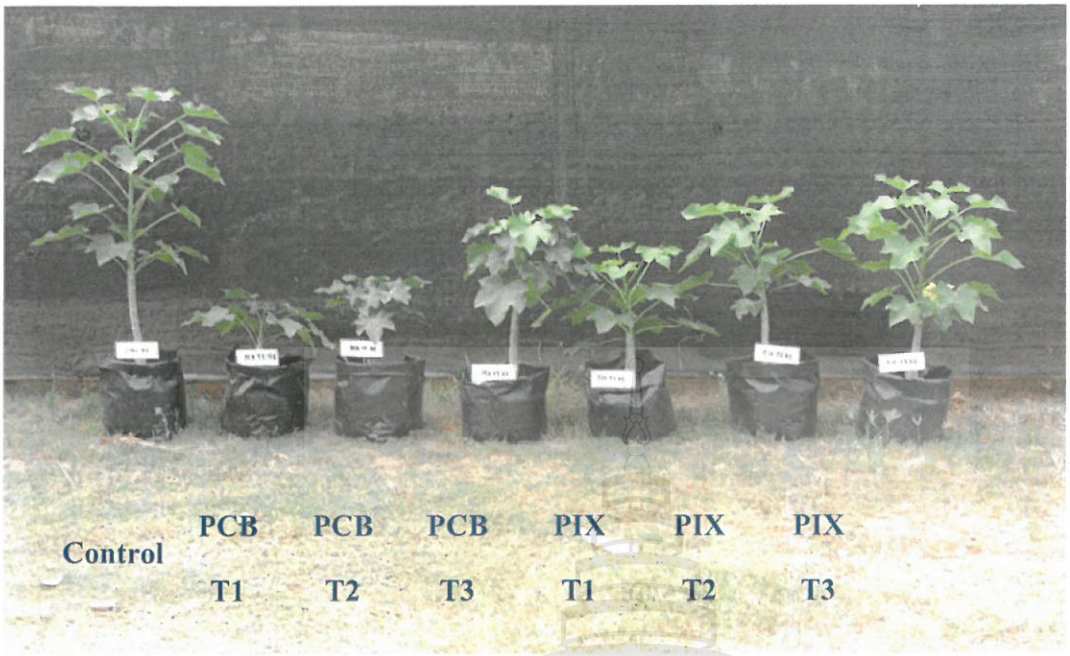

Figure 4.37 *Jatropha curcas* treated with different types and application modes of GA inhibitors at the 6 weeks after application of experiment 2.

4.9.1 Plant growth

Figure 4.37 illustrated the plants in all treatments at 6 weeks after application. The control untreated plants had the highest plant height among all the treated plants through all the experiment period (Figure 4.38). *J. curcas* plants which treated with all GA inhibitors on first week (T1) had the lowest plant height. Plants treated with application mode T2 were shorter than plants treated with application mode T3. The type of inhibitors only slightly affected the plant height. Plants treated with mepiquat chloride were higher than plants treated with paclobutrazol. This result suggests that the application of high amount GA inhibitors on the early stage of growth could reduce *J. curcas* plant height more efficient than three separate applications.

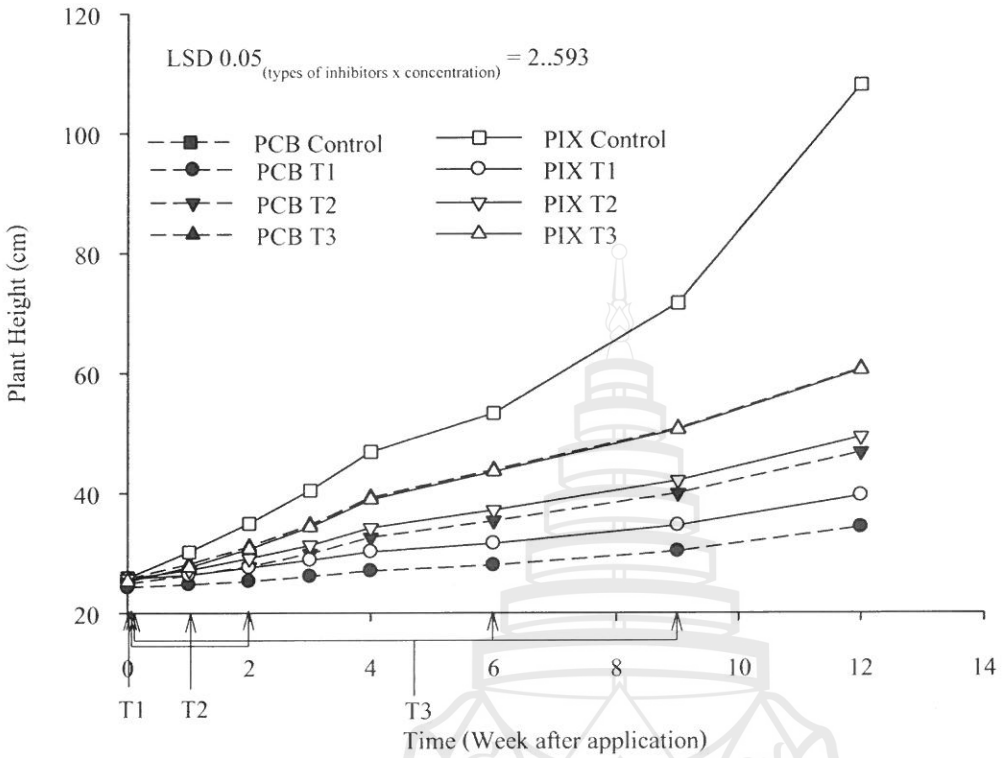

Figure 4.38 Plant height of *J. curcas* treated with different types and application modes of GA inhibitors, experiment 2.

At the first 4 weeks the control untreated plants had no significant difference on the number of nodes with the treated plants (Figure 4.39), but from the week 6 after application the control plants increased the number of nodes and had the highest number of nodes than the treated plants. Although slightly different, the overall average of the number of nodes of the control plants was not significantly different from the treated plants, as well as different types and modes of inhibitors application. The result indicates that the GA inhibitors application with different application modes did not affect the number of node.

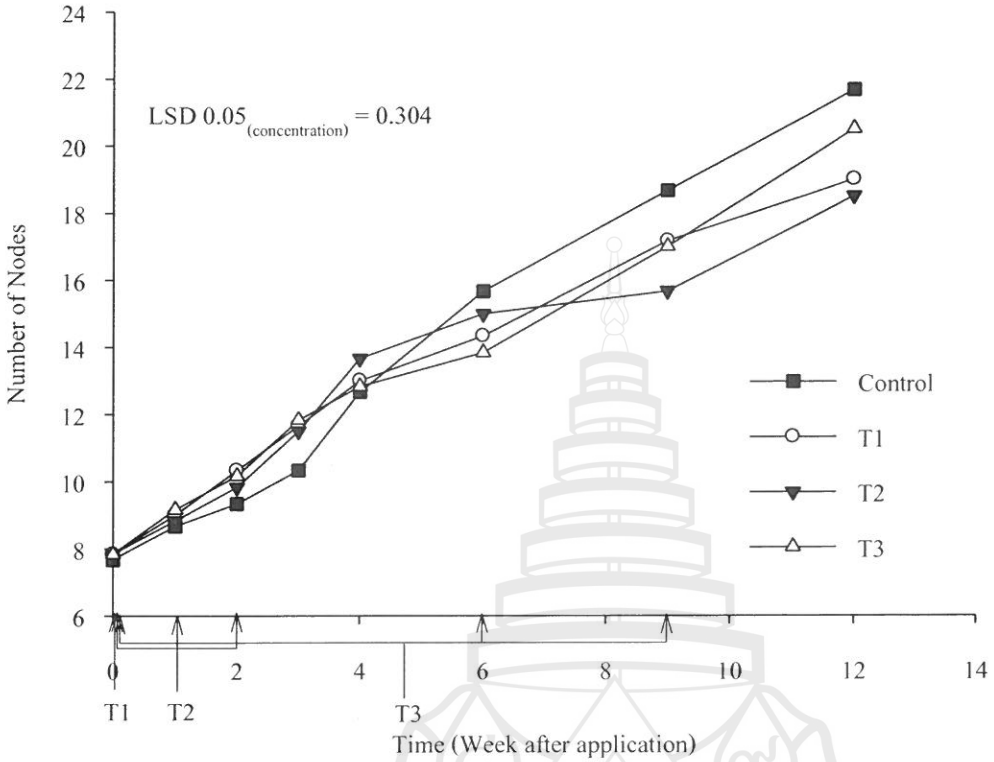

Figure 4.39 Number of nodes of *J. curcas* treated with different types and application modes of GA inhibitors, experiment 2.

The average internode length was significantly different between the control plants and the treated plants through all the experiment period (Figure 4.40). The control plants had higher average internode length than the treated ones. The plant which treated with application mode T1 had the lowest average internode length. The plants treated with application mode T3 had higher average internode length than plants treated with application mode T2. Type of inhibitors and application mode significantly affected the average internode length, but there was no interaction between type of inhibitors and application mode. This result indicates that GA inhibitors application with different application modes could decrease the *Jatropha* plant height but did not decrease the number of nodes. The decreasing of treated plants height was due to average internode length reduction.

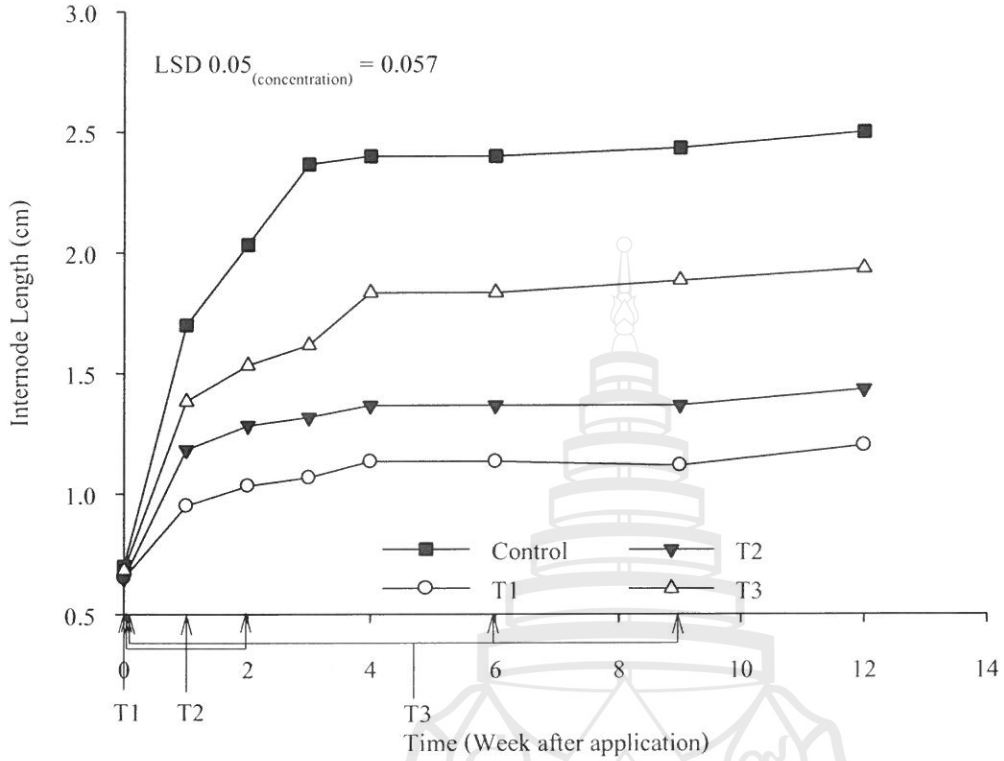

Figure 4.40 Internode length of *J. curcas* treated with different types and application modes of GA inhibitors, experiment 2.

At the first 3 weeks there was no significant difference on the total dry weight between treated and untreated plants (Figure 4.41). Types of inhibitors did not significantly affect the total dry weight, but the application modes did significantly through all the experiment period. The plant which treated with application mode T1 had the lowest total dry weight. The control plants had the highest total dry weight. The plants treated with application mode T3 had higher total dry weight than plants treated with application mode T2. This result suggests the different application modes of GA inhibitors could manipulate *J. curcas* dry matter production.

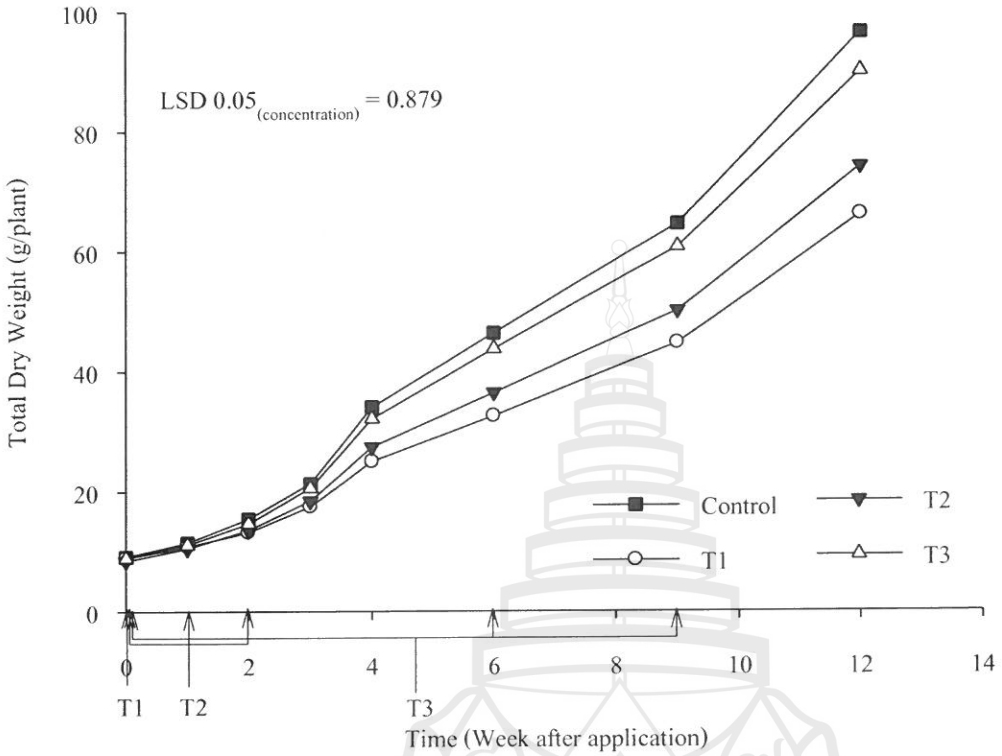

Figure 4.41 Total dry weight (g/plant) of *J. curcas* treated with different types and application modes of GA inhibitors, experiment 2.

The stem diameter of *J. curcas* was significantly different between the control plants and the treated ones (Figure 4.42). The control plants had the lowest stem diameter than the treated plants. At the first 5 week there was significant difference on stem diameter between treated plants. Start from week 9 after treatment the treated plants with application mode T3 had the highest stem diameter among the other plants. The different type of inhibitors did not significantly affect the stem diameter, but the application time did significantly. According to the overall average, the plant which treated with application mode T1 had the highest stem diameter. This result indicates the GA inhibitors with different application mode could decrease the plant height but increase the stem diameter. The treated plants had shorter height but fatter stem.

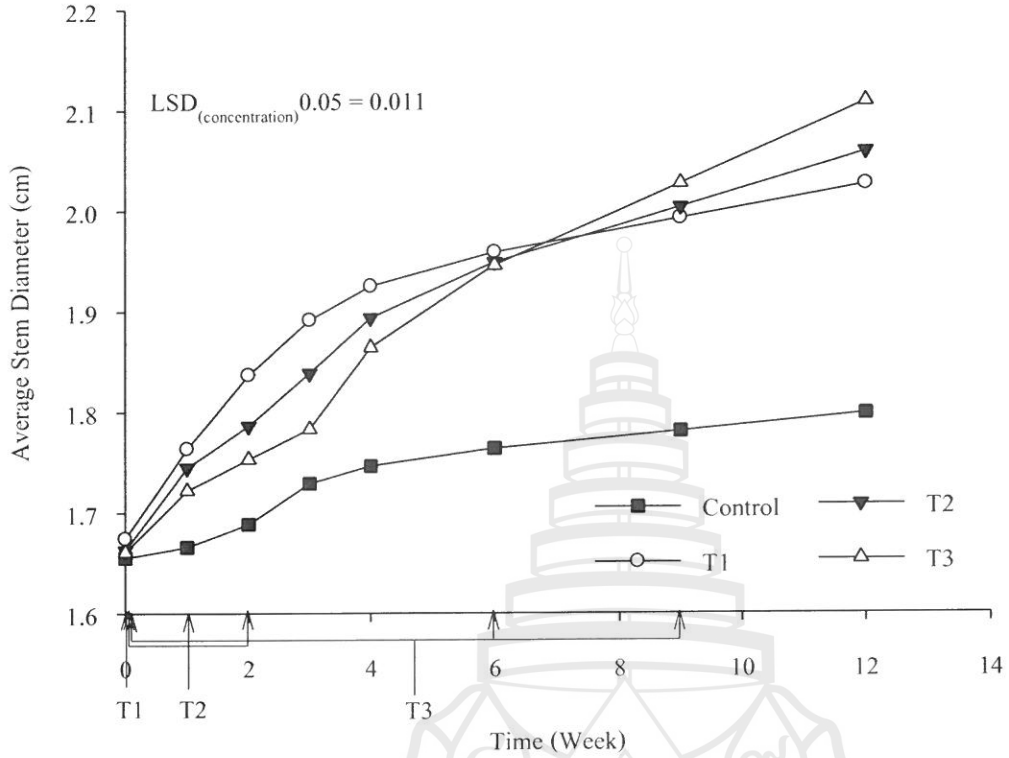

Figure 4.42 Stem diameter of *J. curcas* treated with different types and application modes of GA inhibitors, experiment 2.

There was no significant difference in shoot dry weight at the 2 first weeks among experimental plants, but the plants became significantly different from week 3 until the end of experiment (Figure 4.43). The control plants had the highest shoot dry weight. The plant which treated with application mode T1 had the lowest shoot dry weight. The plants treated with application mode T3 had higher shoot dry weight than plants treated with application mode T2. The plants treated with application time T3 had the highest shoot dry weight from the other treated plants. Types of inhibitors did not significantly affect the shoot dry weight, but the application modes did significantly.

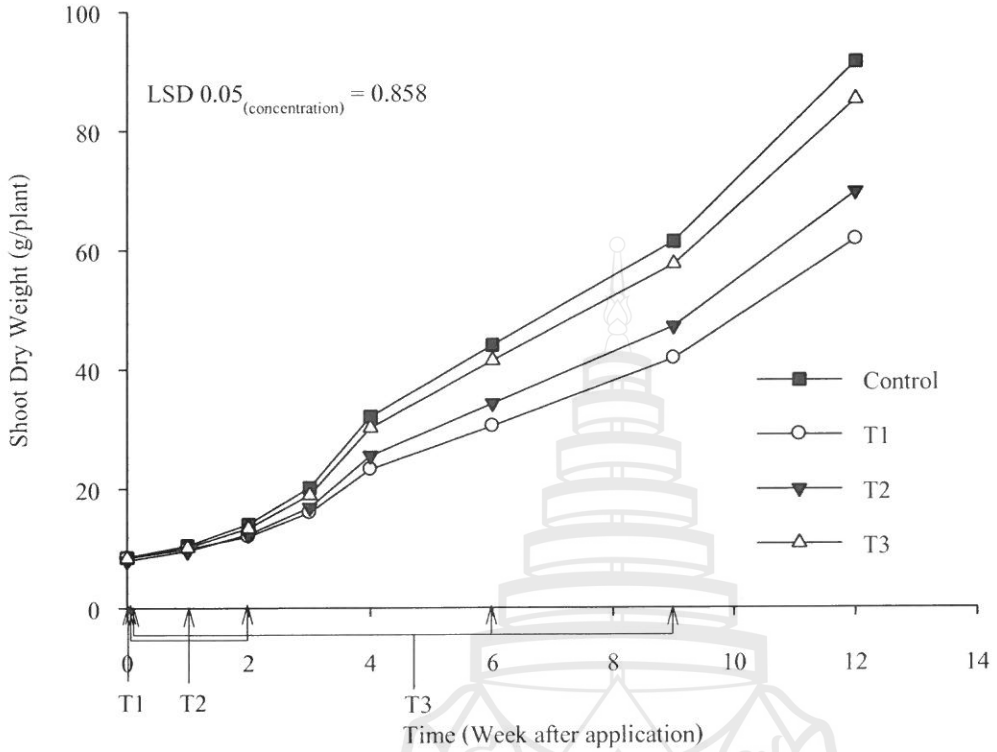

Figure 4.43 Shoot dry weight (g/plant) of *J. curcas* treated with different types and application modes of GA inhibitors, experiment 2.

The control plants had the highest stem dry weight through all the experiment time (Figure 4.44). Type of inhibitors did not significantly affect the stem dry weight, but the application modes did significantly. The plant which treated with application mode T1 had the lowest stem dry weight. The plants treated with application mode T3 had higher stem dry matter than plants treated with application mode T2. The similarity result among stem dry weight, shoot dry weight and total dry matter production suggest the significant influence of stem dry weight on shoot and total dry weight as well.

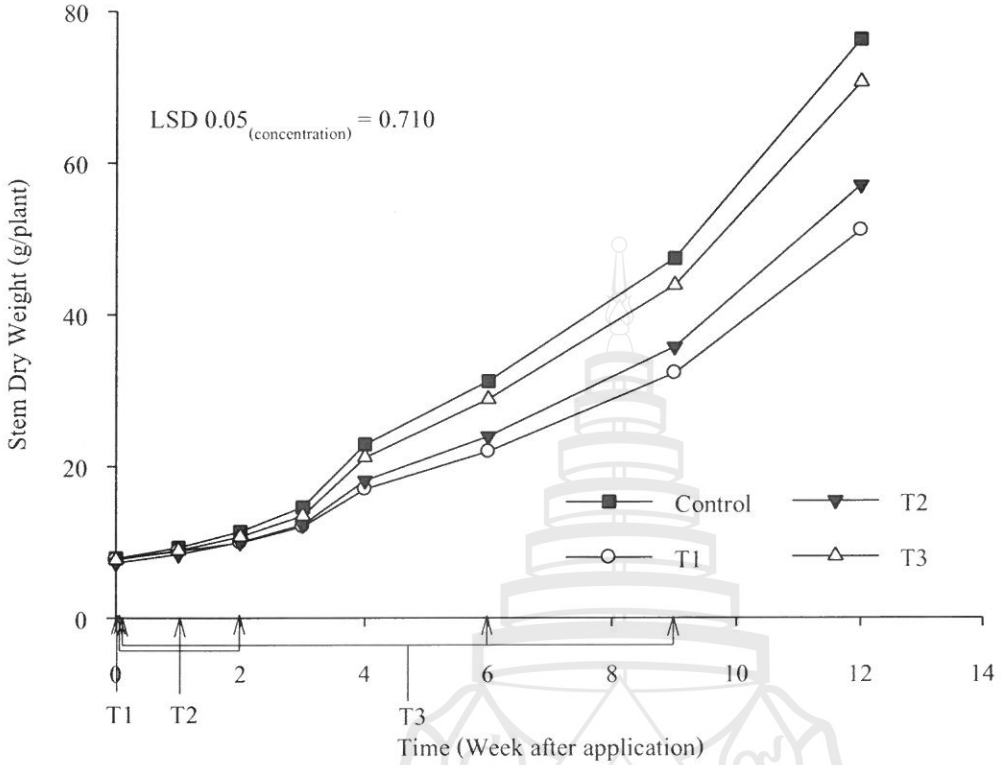

Figure 4.44 Stem dry weight (g/plant) of *J. curcas* treated with different types and application modes of GA inhibitors, experiment 2.

There was no significant difference in leaf dry weight between the treated and untreated plants at the first week, but significant difference occurred from the week 2 until the end of sampling time (Table 4.15a). Type of inhibitors did not significantly affect the leaf dry weight, but the application time did significantly. The plant which treated with application mode T1 had the lowest leaf dry weight. The control plants and plants treated with application mode T3 had the highest leaf dry weight. There were no significant difference between control plants and plants treated with application mode T3.

The numbers of leaves of the control plants were not significantly different than the treated plants (Table 4.15b). At the first 4 weeks the control untreated plants had the lowest number of leaves, but the control plants increase the number of leaves and had the highest number of leaves than the treated plants from the week 6 after application. Different type of inhibitors and different application time did not significantly affect the number of leaves, as well as their interaction.

Table 4.15 Mean of (a) leaf dry weight (g) and (b) number of leaves at each sampling week from experiment 2

Treatment	Week after application							
	0	1	2	3	4	6	9	12
a. Leaf Dry Weight (g)								
Control	0	179.3	354.7	712.2	1299.2	3671.8	6195.5	6382.4
T1	0	159.6	281.6*	505.5*	900.3*	2431.4*	4178.0*	4390.7*
T2	0	168.7	309.5	555.8*	954.4*	2661.0*	4527.1*	4709.5*
T3	0	177.6	314.8	622.3	1162.6*	3321.3*	5585.3*	5649.1*
LSD 0.05	0	23.6	55.8	92.9	101.9	253.5	400.7	371.6
b. Number of Leaves								
Control	5.68	7.08	9.63	13.31	21.28	28.97	40.36	60.28
T1	5.57	6.58*	8.23*	10.90*	15.62*	20.35*	27.92*	41.32*
T2	5.28*	6.64*	8.46*	11.50*	17.12*	22.75*	31.30*	46.31*
T3	5.55	7.16	9.15*	12.84*	20.08*	27.33*	37.94*	56.22*
LSD 0.05	0.29	0.16	0.23	0.32	0.39	0.50	0.54	0.82

* = different from the control treatment of each week which is significant at $LSD_{(application\ modes)}$ 0.05

The significant difference in total leaf area between the treated and the control untreated plants was started from week 2 until the rest of sampling period (Figure 4.45). The control plants had the highest leaf area. Type of inhibitors did not significantly affect total leaf area, but the application modes did significantly. The plant treated with application time T1 and the plants treated with application time T2 had the lowest total leaf area. The plants treated with application mode T3 had the highest total leaf area from the other treated plants.

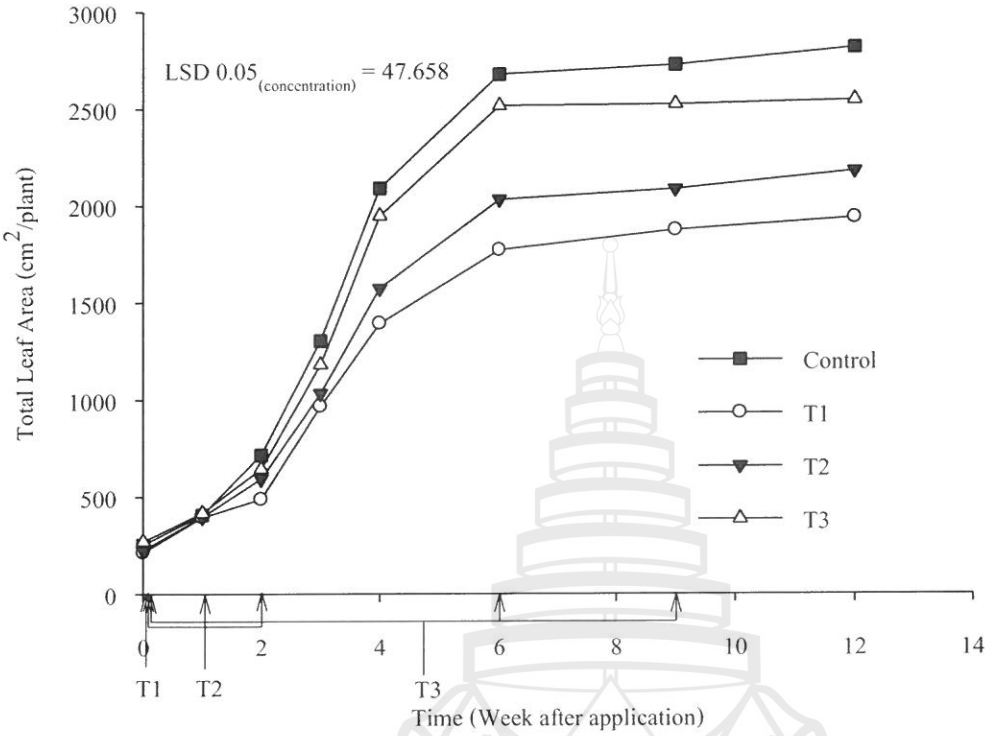

Figure 4.45 Total leaf area (cm^2/plant) of *J. curcas* treated with different types and application modes of GA inhibitors, experiment 2.

At the last 8 weeks of the experiment, there is significant different in root dry weight between the control untreated plants and the plants treated with application mode T1 and T2 (Figure 4.46). The control plants and plants treated with application mode T3 had the highest root dry weight than plants treated with application mode T1 and T2. Different type of inhibitors and different application modes did not significantly affect root dry weight, as well as their interaction.

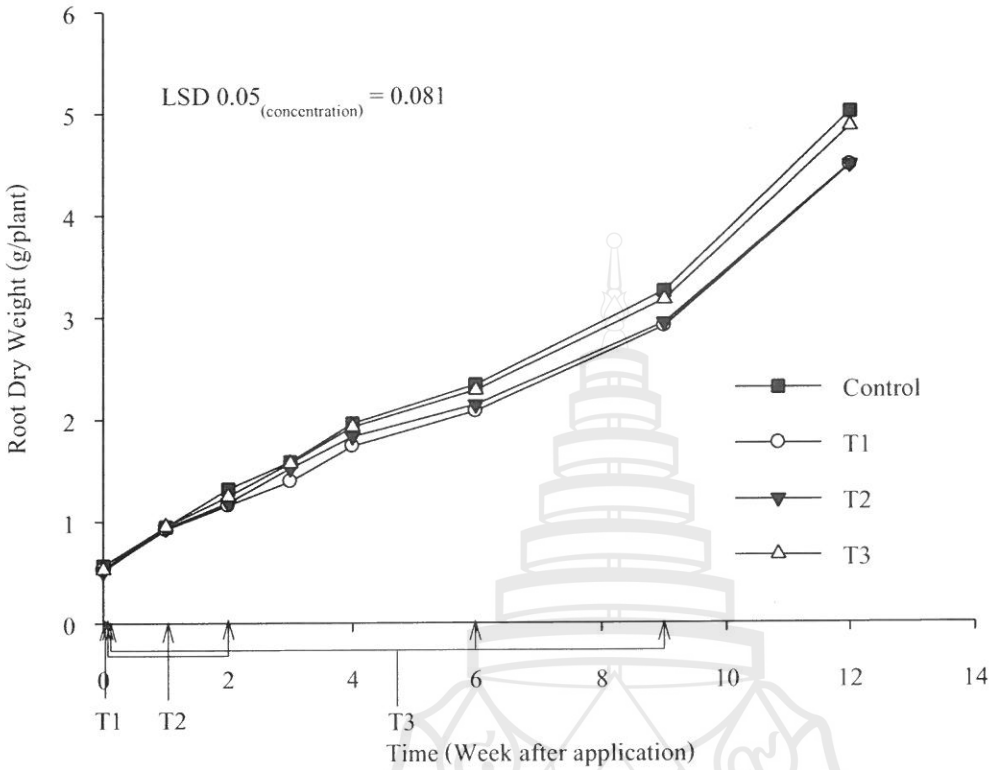

Figure 4.46 Root dry weight (g/plant) of *J. curcas* treated with different types and application modes of GA inhibitors, experiment 2.

4.9.2 Plant growth analysis

The control and plants treated with application mode T3 had no significant difference on net assimilation rate (Figure 4.47). The plants treated with application mode T1 and T2 slightly less than those of the control plants. The plants treated with application mode T3 had the highest net assimilation rate among the other treated plants at the week 2 after treatment until the end of sampling time. The result suggested that the control untreated plants had no significant different ability of each unit area of leaf to produce dry matter than the plants treated with application mode T3.

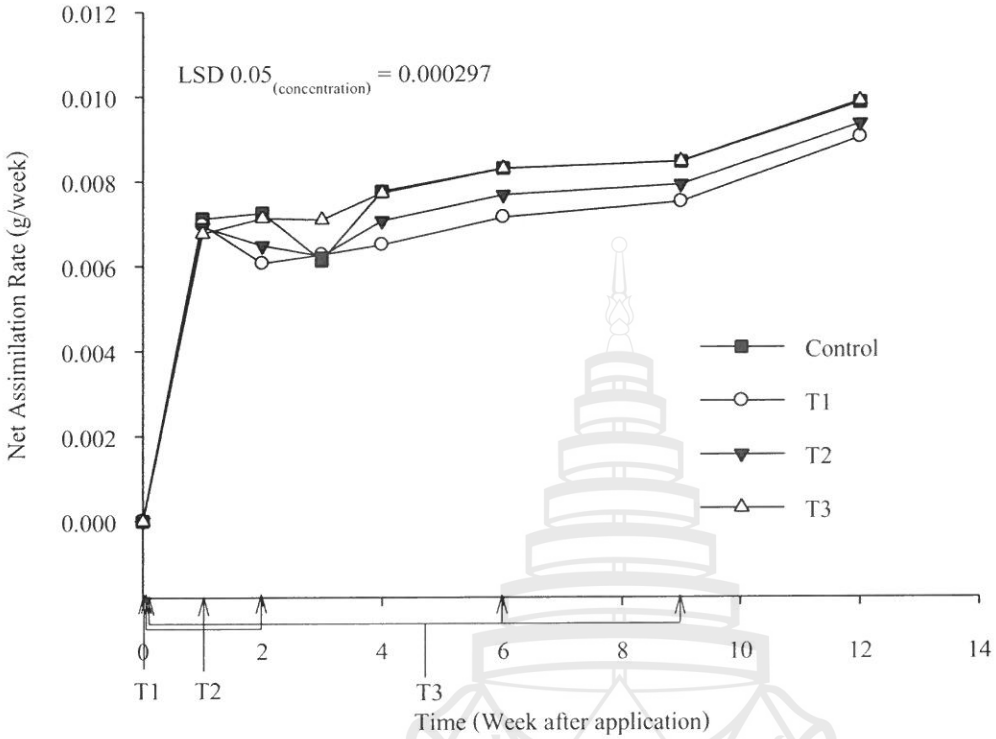

Figure 4.47 Net assimilation rate (g/week) of *J. curcas* treated with different types and application modes of GA inhibitors, experiment 2.

There was no significant difference in crop growth rate between the control untreated plants and plants treated with application mode T3 at the first week and week 3 after treatment, but from week 4 after treatment there was significant difference between them until the rest of the experimental period (Figure 4.48). The control untreated plants had higher crop growth rate than treated plants. The treated plants had lower rate of total dry matter accumulation. Types of inhibitors did not significantly affect crop growth rate, but the application modes did significantly. The plants treated with application mode T1 had the lowest crop growth rate. The plants treated with application mode T3 had higher crop growth rate than plants treated with application mode T2. This result suggests that different application time of GA inhibitors significantly affected the rate of total dry matter accumulation of *J. curcas* plant.

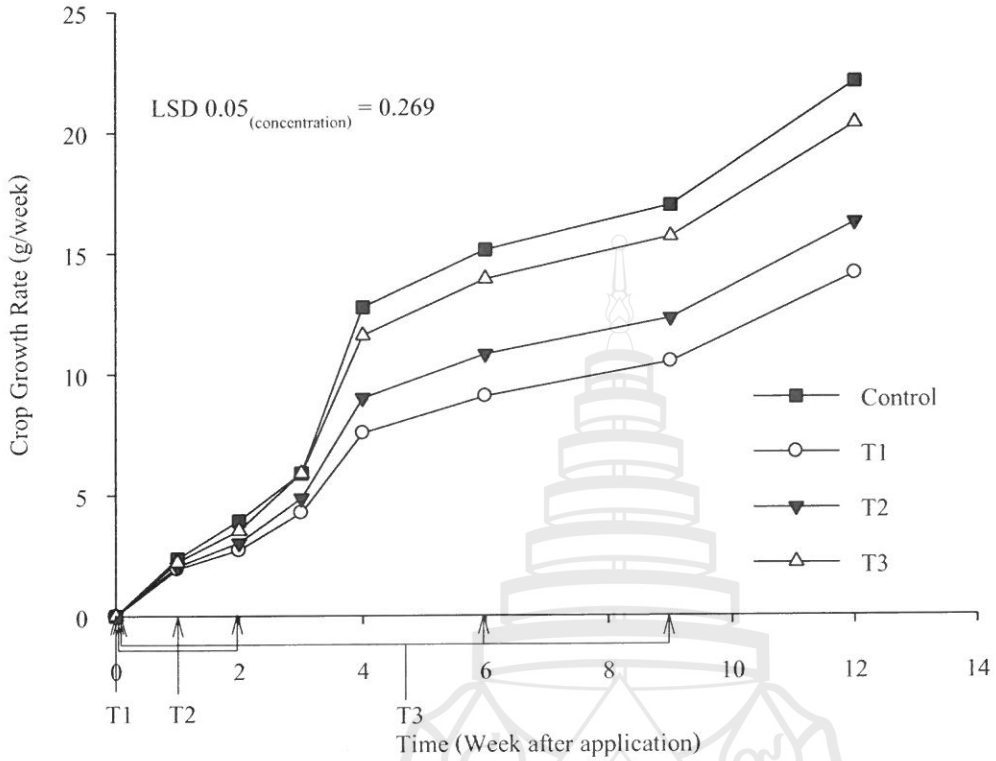

Figure 4.48 Crop growth rate (g/week) of *J. curcas* treated with different types and application modes of GA inhibitors, experiment 2.

The control plants and plants treated with application mode T3 had the highest relative growth rate from the week 2 after treatment until the end of sampling time (Table 4.16). At the week 3 after treatment the relative growth rate of the control untreated plant had decrease below the plant treated with application mode T3, but after that the control plants increase the relative growth rate above all treated plants. Types of inhibitors did not significantly affect the relative growth rate, but the application modes did significantly. The plant which treated with application mode T1 had the lowest relative growth rate.

Table 4.16 Mean of relative growth rate (g/week) at each sampling week from the second

Treatment	Week after application							
	0	1	2	3	4	6	9	12
ıtive Growth Rate (g/week)								
Control	0	0.21	0.30	0.32	0.47	0.50	0.49	0.50
T1	0	0.21	0.22*	0.28*	0.36*	0.39*	0.40*	0.42*
T2	0	0.23	0.25*	0.31*	0.40*	0.43*	0.43*	0.45*
T3	0	0.23	0.28*	0.34*	0.45*	0.48*	0.47*	0.48*
LSD 0.05	0	0.05	0.01	0.01	0.01	0.01	0.01	0.01

* = different from the control treatment of each week which is significant at $LSD_{(application\ modes)}$ 0.05

The leaf area ratio increasing rapidly at the early weeks, but started to decrease at week 6 (Table 4.17a). On the first 2 weeks, leaf area ratio of the control plants was not significant different of leafiness with the treated plants, but started to be significantly different from weeks 2 after application until the end of the experiment. The plants treated with application mode T3 was the lowest among the other plants all through the experiment. The leaf area ratio of *Jatropha* plants did not significantly different at different types of inhibitors and different application modes. This result indicates the GA inhibitors application affect the leaf area ratio for the second experiment.

There was no significant difference on leaf area duration between the control untreated plants and treated plants from the first week until the end of the experiment period (Table 4.17b). This result suggests that GA application on different type and modes did not affect on the leaf area duration.

Table 4.17 Mean of (a) leaf area ratio (cm^2/week) and (b) leaf area duration (cm^2/week) at each sampling week from the second experiment

Treatment	Week after application							
	0	1	2	3	4	6	9	12
a. Leaf Area Ratio (cm^2/week)								
Control	0	0.21	0.30	0.32	0.47	0.50	0.49	0.50
T1	0	0.21	0.22*	0.28*	0.36*	0.39*	0.40*	0.42*
T2	0	0.23	0.25*	0.31*	0.40*	0.43*	0.43*	0.45*
T3	0	0.23	0.28*	0.34*	0.45*	0.48*	0.47*	0.48*
LSD 0.05	0	0.05	0.01	0.01	0.01	0.01	0.01	0.01
b. Leaf Area Duration (cm^2/week)								
Control	0	25.88	50.93	75.09	91.29	115.75	98.71	84.59
T1	0	27.15	41.83	78.45	90.15	116.78	104.24	89.40
T2	0	19.59	45.01	68.81	85.60	113.63	97.91	84.80
T3	0	19.75	40.21	65.57	87.64	113.69	95.11	79.89
LSD 0.05	0	10.34	14.99	11.57	9.26	10.23	8.45	6.13

* = different from the control treatment of each week which is significant at $LSD_{(application\ modes)}$ 0.05

4.9.3 GA concentration

The control plants had GA concentration significantly higher than treated plants (Figure 4.49). Type of inhibitors did not significantly affect the GA concentration, but the application time did significantly. At the week 1 after treatment the GA concentration of all of the plants treated with application mode T1 decreased significantly, which might be due to the high rate of GA inhibitors application at the week 0. The plants treated with application mode T1 had the lowest GA concentration and plants treated with application mode T2 and T3 not significantly different.

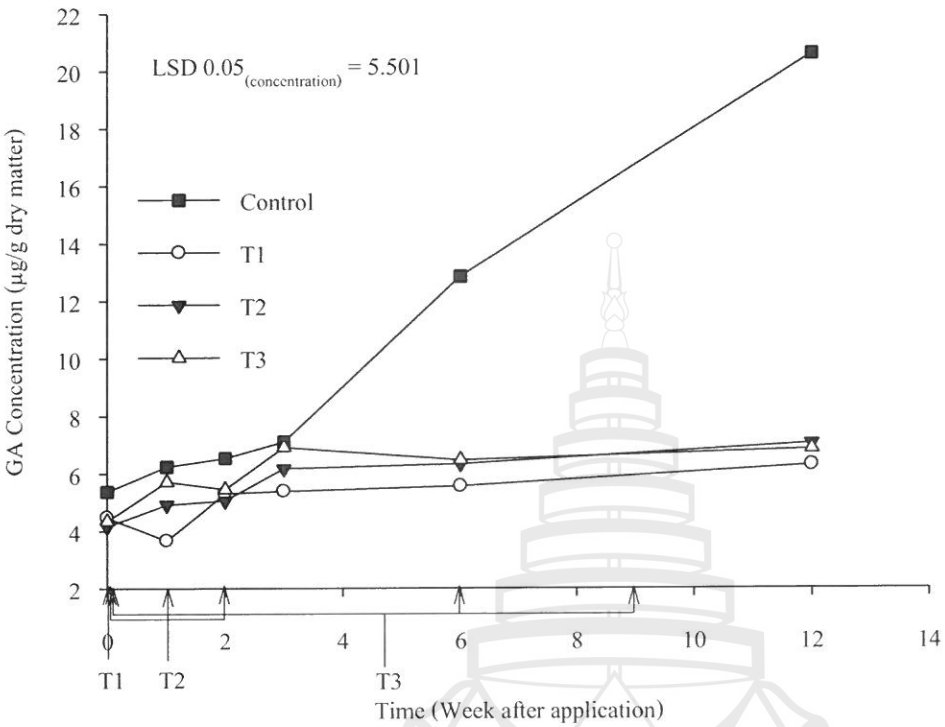

Figure 4.49 GA concentration (µg/g dry matter) of *J. curcas* treated with different types and application modes of GA inhibitors, experiment 2.

The significant difference in GA content per plant between the control untreated plants and the treated plants was started from first after treatment until the rest of experiment time (Figure 4.50). The control plants had GA content per plant significantly higher than treated plants. Types of inhibitors did not significantly affect the total dry weight, but the application modes did significantly. The plants treated with GA inhibitors with application time T1 had the lowest GA content. Plants treated with application mode T2 and T3 not significantly different. This result illustrates the decreasing of GA content on treated *J. curcas* plants due to GA inhibitors application.

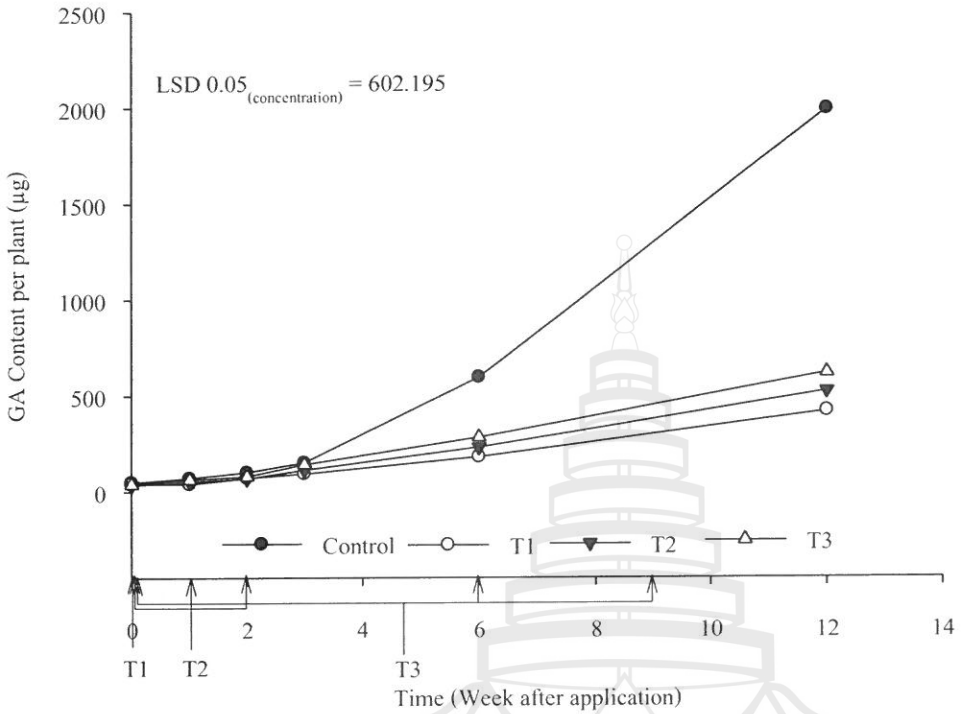

Figure 4.50 GA content per plant (µg) of *J. curcas* treated with different types and application modes of GA inhibitors, experiment 2.

4.9.4 CO_2 assimilation and carbon sequestration

There was significantly difference on net CO_2 assimilation rate between the control treated plants and treated plants from the weeks 4 until the end of experiment time (Figure 4.51). At the first 3 weeks there was no significant difference between the control untreated plants and the treated plants. Types of inhibitors did not significantly affect CO_2 assimilation rate, but the application modes did significantly. The control plants and plants treated with application time T3 had the highest CO_2 assimilation rate. The plants treated with application time T1 and T2 had the lowest CO_2 assimilation rate. This result similar with the first experiment, the control untreated plants had wider leaf area to fix CO_2 than the treated plants.

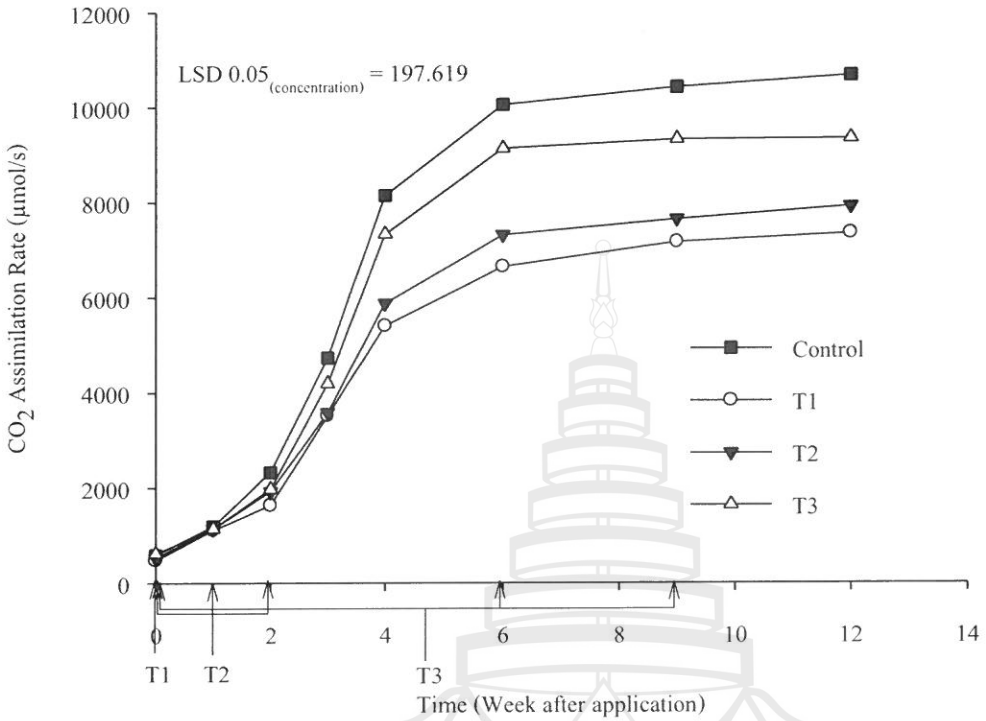

Figure 4.51 Net CO_2 assimilation rate (µmol/s) of *J. curcas* treated with different types and application modes of GA inhibitors, experiment 2.

At the first 3 weeks there was no significant difference in accumulative CO_2 uptake between the untreated control and the treated plants (Figure 4.52), but the significant difference on accumulative CO_2 uptake between the control untreated plants and the treated plants started from the week 3 after treatment. Type of inhibitors did not significantly affect accumulative CO_2 uptake per plant, but the application modes did significantly. The control plants and plants treated with application time T3 had the highest accumulative CO_2 uptake per plant than the plants treated with application time T1 and T2.

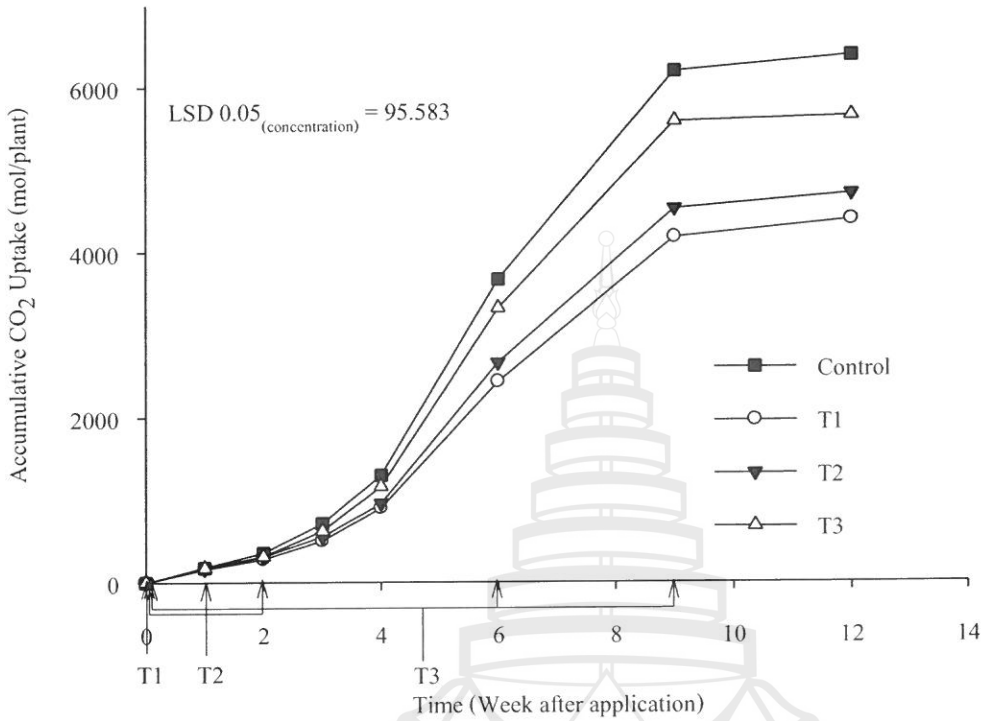

Figure 4.52 Accumulative CO_2 uptake (mol/plant) of *J. curcas* treated with different types and application modes of GA inhibitors, experiment 2.

There was no significant difference in carbon sequestration between the untreated and the treated plants at the first 2 weeks (Figure 4.53), after which the different started until the end of the experiment period. The control plants had the highest carbon sequestration than treated plants. Type of inhibitors did not significantly affect carbon sequestration, but the application modes did significantly. The plant which treated with application mode T1 had the lowest carbon sequestration. The plants treated with application mode T3 had higher carbon sequestration than plants treated with application mode T2. This result might be due to the higher leaf area of the control plants had the widest leaf area to absorb carbon from the atmosphere.

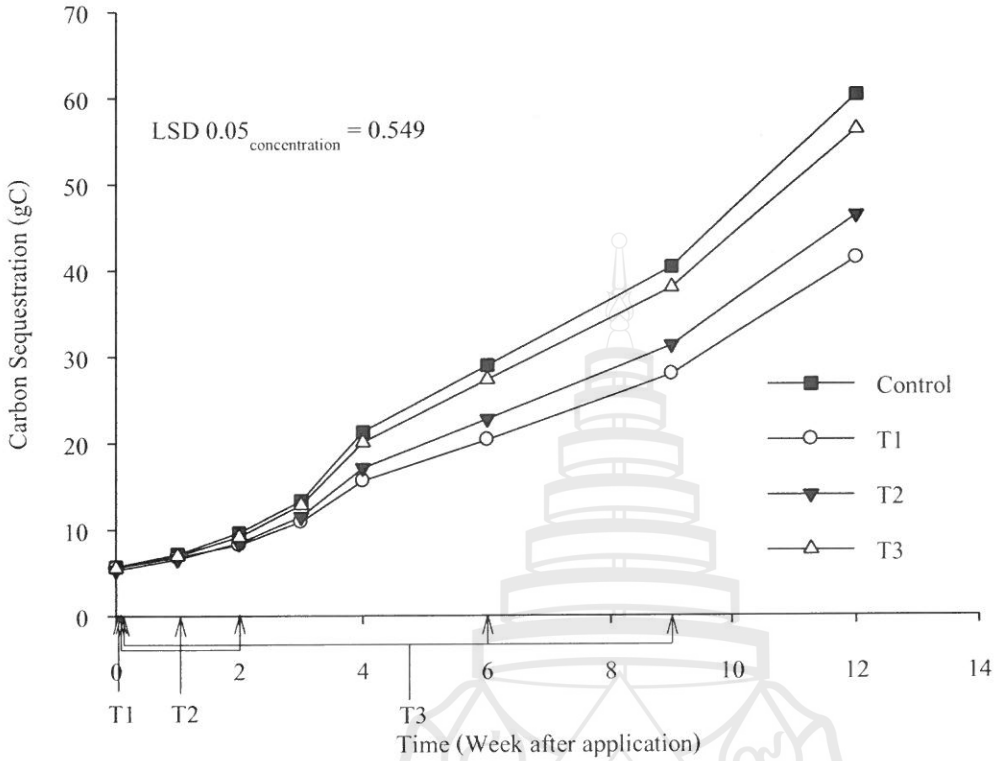

Figure 4.53 Carbon sequestration (g C) of *J. curcas* treated with different types and application modes of GA inhibitors, experiment 2.

In conclusion, these results were similar to the first experiment. Most of the growth indices were not significantly different in the same type of GA inhibitors application 500 µM, except the plant height. Different modes of GA inhibitors application could decrease the *Jatropha* plant height, especially for the plant treated application mode T1 which had the lowest plant height but had the same number of node with the control plants. The height reduction was because the treated plants had shorter internodes length than the untreated plants.

Different mode of application affects the *Jatropha* dry weight only at the early stage when the control untreated plants had the highest total dry weight at the first 15 weeks, then became similar to the other treatments afterwards. The plants treated with the application mode T1 had the widest stem diameter. The application of high rate of inhibitors at the first week could produce the shortest and the fattest stem of *Jatropha* plants. Although the application mode could affect the growth pattern of *Jatropha*

plants, it only occurred on above-ground plant part. The root dry weight did not affect with the different application mode of GA inhibitors application.

Although there was significant difference between the crop growth rate and relative growth rate of the untreated and treated plants, the plants treated with application mode T3 had no significant difference from the untreated plants. This result indicates that plants treated with application mode T3 produced dry matter from single unit leaf area more efficient than the other treated plants.

The GA concentration of the treated plants was also lower than the untreated plants, but interestingly, there was no significant difference between plant treated with application mode T2 and T3. This suggests that applying GA inhibitors either at three consecutive weeks (T2) or at week 0, 6 and 9 (T3) provided the same effect on GA concentration and GA content in the plant tissue. The plant treated with application mode T1 had the lowest GA concentration and content.

The control untreated plants had the highest CO_2 assimilation rate, accumulative CO_2 uptake and carbon sequestration. This result might be because the untreated plants had wider leaf area than treated plants. Wider leaf area allows the untreated control plants absorb CO_2 much more than the treated plants.

Even though the height of untreated plants was significantly different from the plants treated with application mode T3, there was no significant difference on the leaf dry weight and net assimilation rate. The plants treated with the application mode T3 had the highest total dry weight, CO_2 assimilation rate, accumulative CO_2 uptake and carbon sequestration than the other treated plants. On the other hand, the plants treated with application mode T1 had the lowest total dry weight. This result suggests that the high rate of GA inhibitors application at the early week (T1) could only reduce plant height without maintaining the dry matter production, whereas the 3 separate applications at 3 consecutive weeks (T2) could reduce the *Jatropha* plant height but still maintaining the dry matter production.

4.10 Relationship between CO_2 assimilation, dry matter and carbon sequestration

4.10.1 Relationship between dry matter and carbon sequestration

The total dry matter production was significantly correlated with carbon sequestration ($r=1^{**}$) (Figure 4.54). This might be due to the fact that, according to Albrecth and Kandji (2003), the carbon sequestration was calculated directly from dry

matter, which 50% of total dry matter for above-ground carbon sequestration and 25% of above-ground carbon sequestration for the below-ground carbon sequestration.

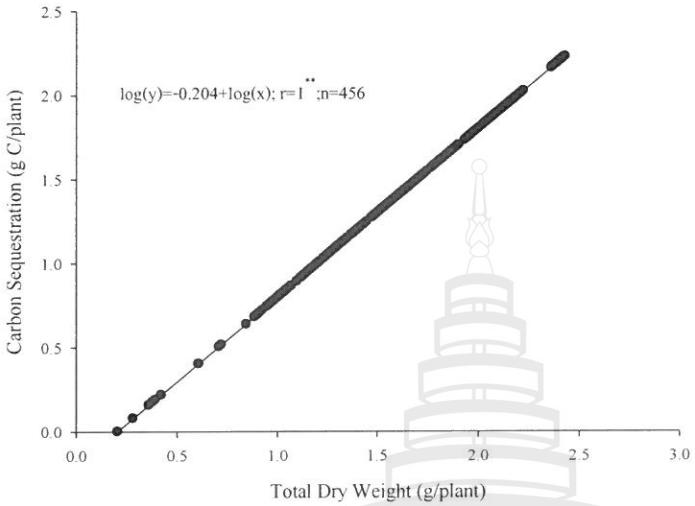

Figure 4.54 Relationship between total dry matter production (g/plant) and carbon sequestration (g C/plant) of *Jatropha* plant.

4.10.2 Relationship between CO_2 assimilation, dry matter and carbon sequestration from the first experiment

At the first experiment CO_2 assimilation rate per plant was highly correlated with total dry matter production ($r=0.93^{*}$) (Figure 4.55) and carbon sequestration ($r=0.93^{*}$) (Figure 4.56). *J. curcas* biomass production and net CO_2 assimilation was also highly correlated ($r=0.95^{**}$) (Figure 4.57). The positive correlation suggested that the most of CO_2 assimilated by *Jatropha* plant would be sequestrated as dry matter. This assumption is supported by the high significant correlation between carbon sequestering and total CO_2 uptake ($r=0.95^{**}$) (Figure 4.58).

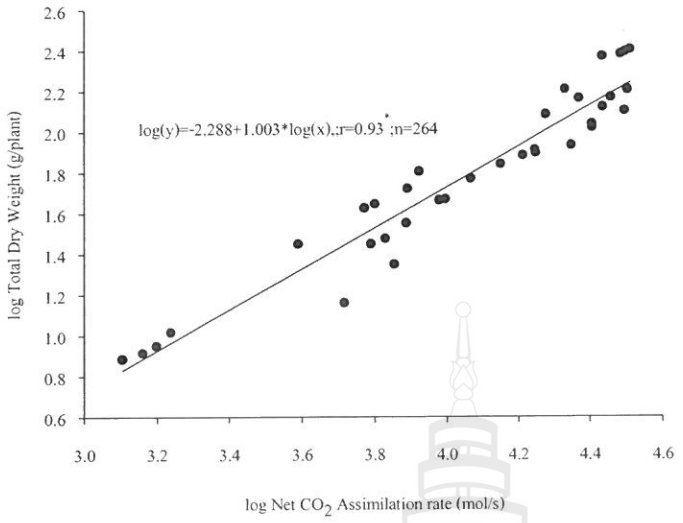

Figure 4.55 Relationship between net CO_2 assimilation rate (mol/s) and total dry matter production (g/plant) of *Jatropha* plant from the first experiment.

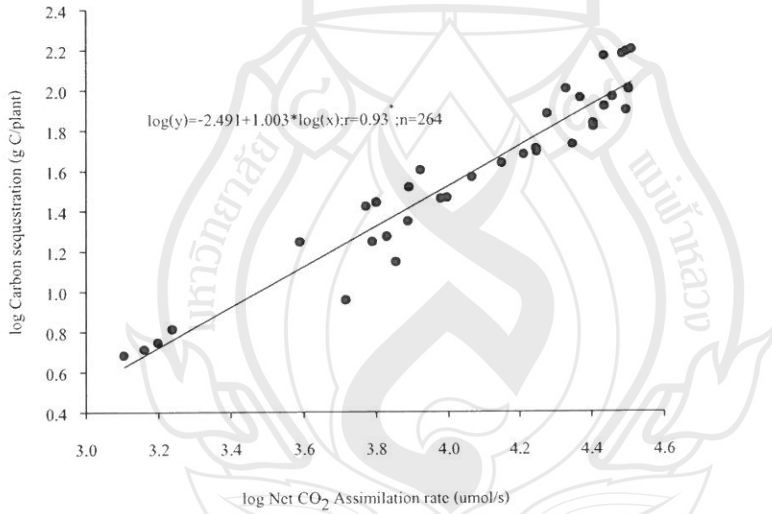

Figure 4.56 Relationship between net CO_2 assimilation rate (mol/s) and carbon sequestration (g C/plant) of *Jatropha* plant from the first experiment.

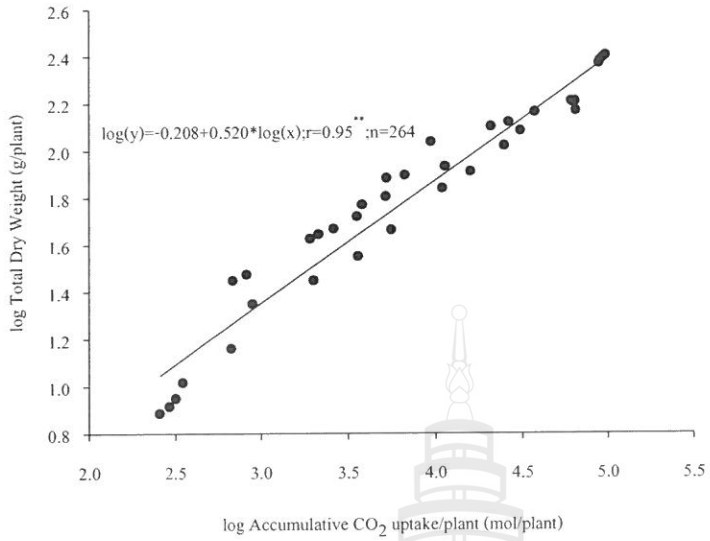

Figure 4.57 Relationship between accumulative CO_2 uptake (mol/plant) and total dry matter production (g/plant) of *Jatropha* plant from the first experiment.

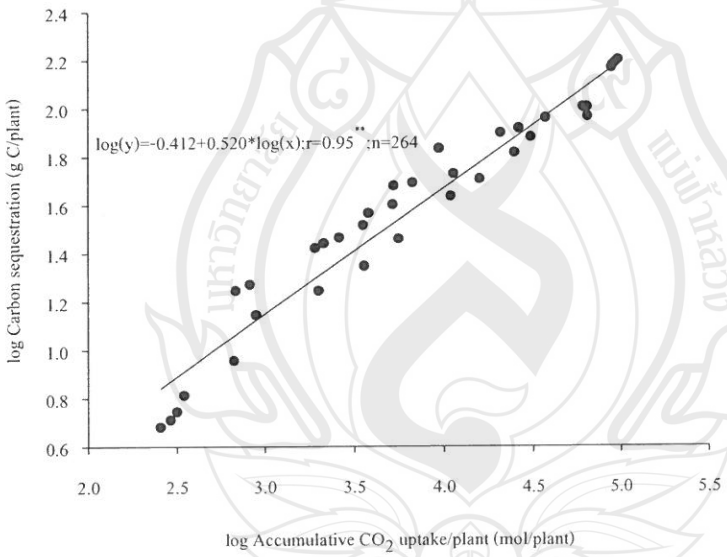

Figure 4.58 Relationship between accumulative CO_2 uptake (mol/plant) and carbon sequestration (g C/plant) of *Jatropha* plant from the first experiment.

4.10.3 Relationship between CO_2 assimilation, dry matter and carbon sequestration from the second experiment

Similar to the first experiment, CO_2 assimilation rate per plant from the second experiment was highly correlated with total dry matter production ($r=0.95^{**}$) (Figure 4.59) and carbon sequestration ($r=0.94^{*}$) (Figure 4.60). *J. curcas* biomass production and net CO_2 assimilation was also highly correlated ($r=0.96^{**}$) (Figure 4.61).

The positive correlation suggested that most of CO_2 assimilated by *Jatropha* plant would be sequestrated as dry matter. This assumption is supported by the high significant correlation between carbon sequestering and total CO_2 uptake ($r=0.94^{*}$) (Figure 4.62).

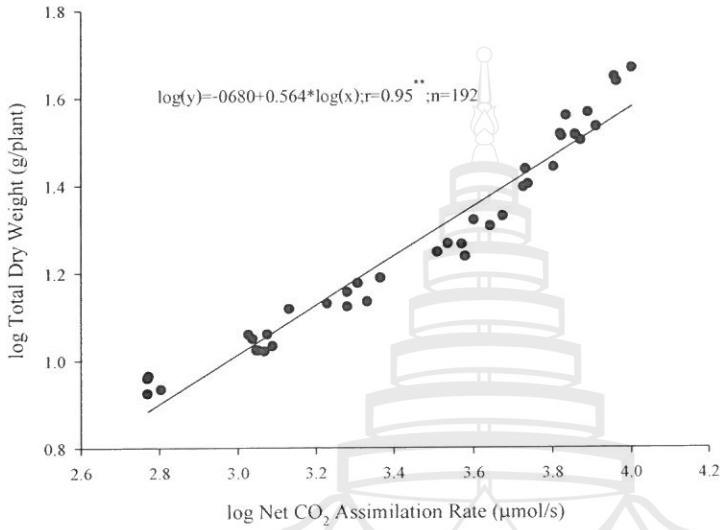

Figure 4.59 Relationship between net CO_2 assimilation rate (mol/s) and total dry matter production (g/plant) of *Jatropha* plant from the second experiment.

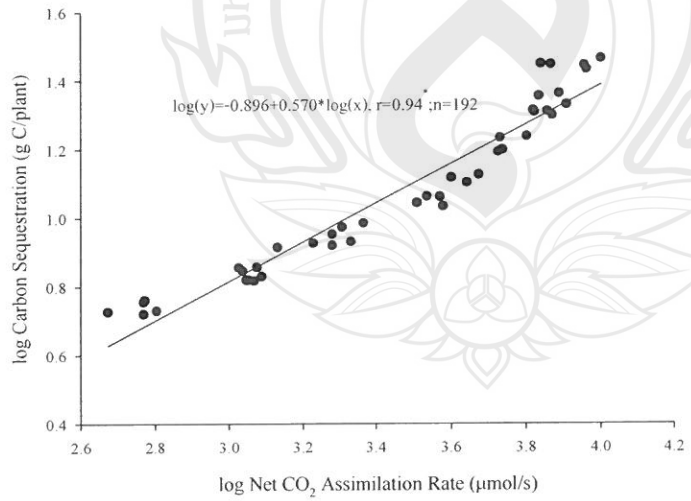

Figure 4.60 Relationship between net CO_2 assimilation rate (mol/s) and carbon sequestration (g C/plant) of *Jatropha* plant from the second experiment.

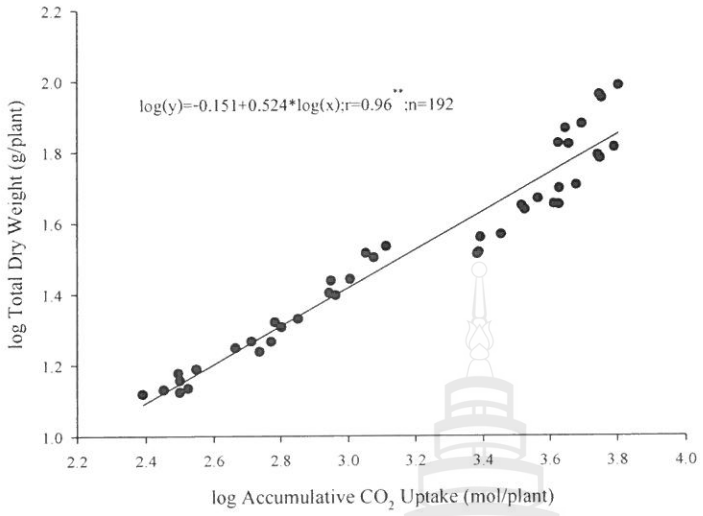

Figure 4.61 Relationship between accumulative CO_2 uptake (mol/plant) and total dry matter production (g/plant) of *Jatropha* plant from the second experiment.

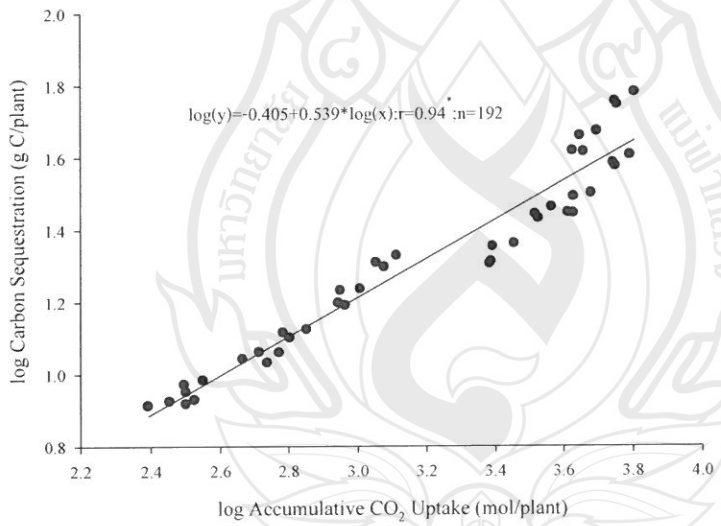

Figure 4.62 Relationship between accumulative CO_2 uptake (mol/plant) and carbon sequestration (g C/plant) of *Jatropha* plant from the second experiment

4.10.4 Relationship between CO_2 assimilation, dry matter and carbon sequestration from the both experiments.

The pooled data from both experiments shows the highly correlation between CO_2 assimilation rate, dry matter production and carbon sequestration. CO_2 assimilation rate per plant was highly correlated with total dry matter production ($r=0.91^*$) (Figure 4.63) and carbon sequestration ($r=0.91^*$) (Figure 4.64). *J. curcas* biomass production and

net CO_2 assimilation was also highly correlated (r=0.94*) (Figure 4.65). The highly significance of the positive correlation suggested that the most of CO_2 assimilated by *Jatropha* plant would be sequestrated as dry matter. This assumption is supported by the high significant correlation between carbon sequestering and total CO_2 uptake (r=0.94*) (Figure 4.66).

Most importantly, the highly correlation between carbon sequestration and CO_2 uptake suggest the possibility of using CO2 uptake and photosynthetic data to estimate carbon sequestration of the *Jatropha* plant. This issue will be further discussed in 4.4.3.

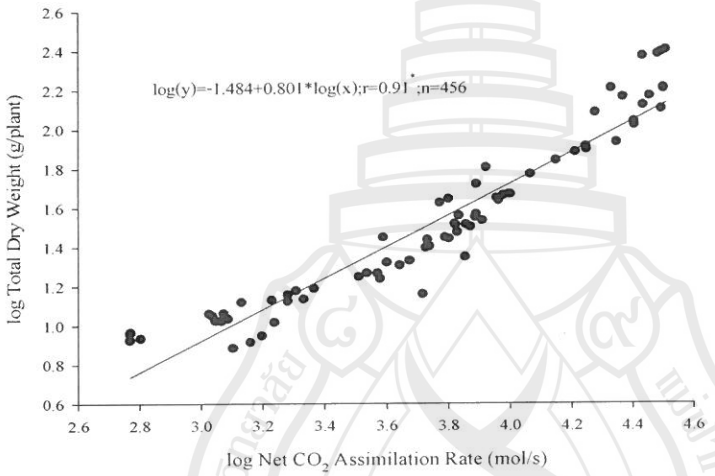

Figure 4.63 Relationship between net CO_2 assimilation rate (mol/s) and total dry matter production (g) of *Jatropha* plant from both experiments.

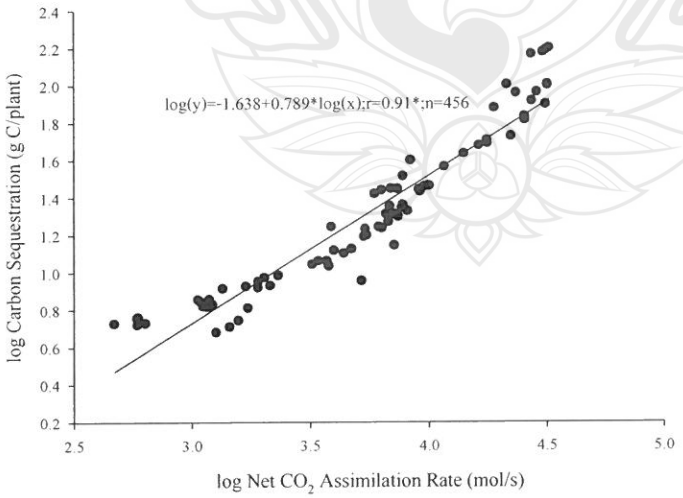

Figure 4.64 Relationship between net CO_2 assimilation rate (mol/s) and carbon sequestration (g C/plant) of *Jatropha* plant from both experiments.

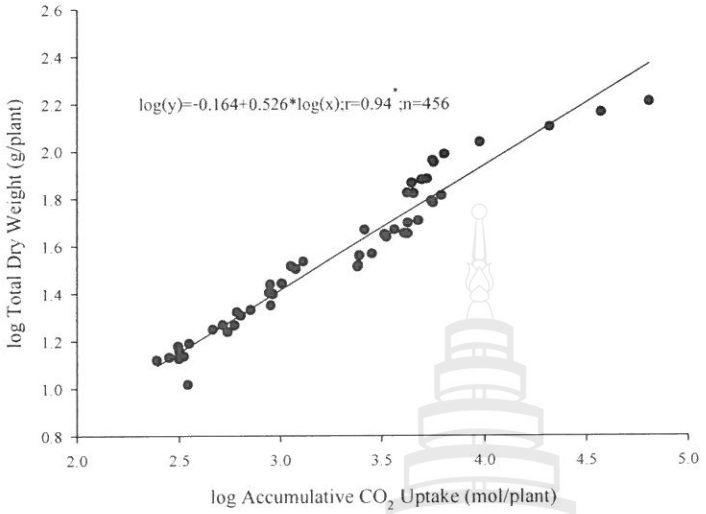

Figure 4.65 Relationship between accumulative CO_2 uptake (mol/plant) and total dry matter production (g/plant) of *Jatropha* plant from both experiments.

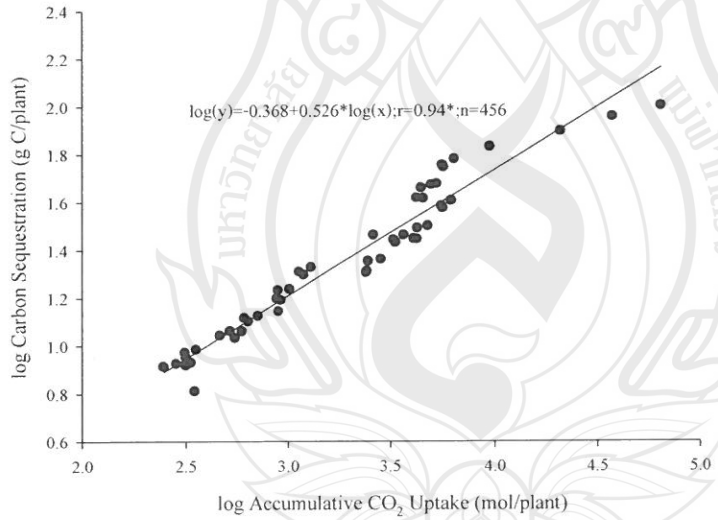

Figure 4.66 Relationship between accumulative CO_2 uptake (mol/plant) and carbon sequestration (g C/plant) of *Jatropha* plant from both experiments.

4.11 Discussion

4.11.1 Effect of GA inhibitors on plant height, growth and GA content in the plant

We found from the first experiment that the GA inhibitors at the rates of 250 and 500 µM provided shorter *Jatropha* plant height, but similar dry matter production, total leaf area, number of nodes and number of leaves compared to the control untreated

plants. On the other hand, GA inhibitors at the rate of 750 μM provided shortest plant height but also lower dry matter production. In the second experiment, the application of GA inhibitors at the 3 separate times with at least 1 week intervals might be the most appropriate to reduce *Jatropha* plant height because it could reduce the plant height while maintaining the same the dry matter production.

Results from both experiments suggest that the application of GA inhibitors such as paclobutrazol and mepiquat chloride at the appropriate concentrations could reduce the *Jatropha* plant height without causing the reduction of the total biomass. Stems of the plant treated with GA inhibitors were shorter but fatter than the control plants, with the same number of nodes and leaves. No difference in of the number of nodes between the treated and untreated plants suggests that the GA inhibitors application might reduce the *Jatropha* plant height by reducing internodes length, but not number of nodes. The treated plants also had higher number of branches compared to the untreated plants, suggest that GA inhibitors application could reduce the stem elongation and stimulate the lateral branch production. Reddy *et al.* (2004) also found in cotton that mepiquat chloride application could reduce the plant height while maintaining the total biomass production at same rate with the control plant.

This ability of GA inhibitors to reduce the *Jatropha* plant growth without reduce its dry matter confirm previous experiments by Saengow (Unpublished data). He tested the effect of a GA and an IAA inhibitors and found that IAA inhibitor reduced plant height by reducing number of nodes while the GA inhibitor reduce plant height by reducing internodes length. We used 2 different types of GA inhibitors at 3 different rates to determine the appropriate type and rate of GA inhibitors for *Jatropha* plant. Furthermore, plants treated with paclobutrazol had higher net assimilation rate, crop growth rate and relative growth rate than plants treated with mepiquat chloride. These results suggest that paclobutrazol might be more suitable to reduce the *Jatropha* plant height.

Our result on GA bioassay further suggests that the stem height reduction might be due to the fact that GA inhibitors could inhibit the GA synthesis in the *Jatropha* plant. The GA concentration in the plant treated with the GA inhibitors was decreased soon after applying inhibitors. The reduction of GA concentration in *Jatropha* plant was followed by the reduction of the plant height, suggests that the *Jatropha* plant height reduction was the effect of the reducing GA concentration. GA has the major role as a growth regulator in plant which had effect on cell elongation of the stem (Ono *et al.*, 2000); therefore it is likely that inhibiting of GA biosynthesis reduce the plant height by

reducing internode elongation. Rademacher (2000) found that mepiquat chloride could block the cyclases copalyl-diphosphate synthase and *ent*-kaurene synthase involved in the early steps of GA metabolism on plants using fungi *Gibberella fujikuroi* culture, while paclobutrazol stopped GA synthesis pathway at ent-kaurene, ent-kaurenol and ent-kaurenal before they changed to GA in the final step. Our results are consistent to those of Nichols (2003) who treated cotton plants with mepiquat chloride and got shorter plant by increase the number of sympodial nodes and reproductive branches, and also decrease internodes length. This is the first time these effects have been illustrated in *Jatropha*.

4.11.2 CO_2 assimilation

There was no significant difference of CO_2 assimilation rate between the treated and untreated plants. This might be because the photosynthetic rate (A) of the untreated plants was not different from the treated plants. Since there was no significant difference on total leaf area, the CO_2 assimilation rate between the treated and untreated plants was also not significantly different. Leaf area determines the leaf surface to fix CO_2 from atmosphere as Thomas *et al.*, (2006) showed that higher leaf area could increase the overall CO_2 assimilation and the fraction of total biomass on *Eucalyptus grandis* plant. Wieland and Wample (1985) also found that the apple plant treated with 0.8 g a.i./cm paclobutrazol application could maintain plant CO_2 assimilation rate.

With the similar ability of each plant to absorb CO_2 from the atmosphere with the control untreated plants, the treated plants might have similar ability to reduce atmospheric CO_2 which is the one of the major greenhouse gas. Our result suggests that the plants treated with GA inhibitors could reduce the CO_2 at the same amount of normal plant. Furthermore, with the smaller plants induced by GA inhibitors application, we might be able to increase the plant density of *Jatropha* per hectare and thus, increase the absorption of more CO_2 from atmosphere than the normal *Jatropha* plants.

4.11.3 Possibility to estimate carbon sequestration using CO_2 assimilation

Our result shows the significant relationships between CO_2 assimilation and total dry matter of *Jatropha* plant ($r=0.91^{*}$). The total dry matter is usually used to estimate the carbon sequestration (Villalobos *et al.*, 2006). The significant relationship between CO_2 assimilation rate and total dry matter production leads to the relationship between CO_2 assimilation and carbon sequestration. The highly significant relationship between carbon sequestration and CO_2 assimilation as well as accumulative CO_2 uptake

($r=0.94^{*}$), suggested that the carbon sequestration could be estimated using CO_2 assimilation instead of using plant total dry matter.

Photosynthetic carbon sequestration determines net CO_2 assimilation primary products which is the result of photosynthesis during the growing period minus carbon losses via respiration (Villalobos *et al.,* 2006). The significant relationship between plant carbon sequestration and total CO_2 uptake suggests that the increase of accumulative plant CO_2 uptake would represent the amount of carbon which converted into plant biomass. This result supporting Arroja *et al.,* (2006) that the photosynthetic products or tree dry matter should be considered as a major component of carbon sequestration in the forest.

The highly significant correlation both net CO_2 assimilation rate and accumulative CO_2 uptake with carbon sequestration suggests that both indices might be more suitable as a non-destructively estimation of carbon sequestration of *J. curcas* plant than dry matter production which is more destructive.

4.11.4 Practical application

Our findings that GA inhibitors at application rate 250 and 500 µM could reduce the plant height of *Jatropha curcas* without reduce the total dry matter, growth rate, CO_2 assimilation and carbon sequestration could be useful in controlling *Jatropha* plant height. Furthermore, paclobutrazol might be more suitable for reducing *Jatropha* plants height than mepiquat chloride since plants treated with paclobutrazol has higher ability to produce dry matter accumulation. Applying GA inhibitors to reduce *Jatropha* plant height might be easier than pruning, especially at large scale plantation because it could reduce the high cost and labor for pruning. Moreover, pruning *Jatropha* plant could cause dry matter reduction which might effect yield, as well as increase exposure to toxicity due to many carcinogenic substances in jatropha plant, namly, curcin, jatrophine and phorbolesters which is cancer trigger (Heller, 1996; Thongbai *et al.*, 2006; Francis, 2007)

Since there was no seed produced during the experiment, we could not evaluate the effect of the GA inhibitors on the seed yield production. Study on the effect of GA inhibitors on the seed yield could lead to more appropriate practices to reduce jatropha plant height without reduce the seed yield. Biotechnology technique to block genes which control GA synthesis might also be possible to provide the permanent result as a new jatropha which shorter than the original.

4.12. Mutagenesis of *J. curcas* by NMU

Experiment 3.5.1: Effect of seed coat to % seed germination after treated with NMU in buffer.

From Figure 1, % seed germination of all treatments of naked seeds was less than those from the normal seeds. So the seed coat has effect on % seed germination after treated with NMU buffer solution. Time of soaking also affected %seed germination, the longer soaking time, the less %seed germination. The naked seed treated with 0.3% NMU buffer solution for 4 hrs., 0.1% NMU buffer solution for 12hr, 0.3% NMU buffer solution for 12hrs., and the normal seed treated with 0.3% NMU buffer solution for 12hrs. gave the least % seed germination but not significantly different. The best %seed germination is treatment at normal seed is treated with 0.1% NMU buffer solution for 4hrs.

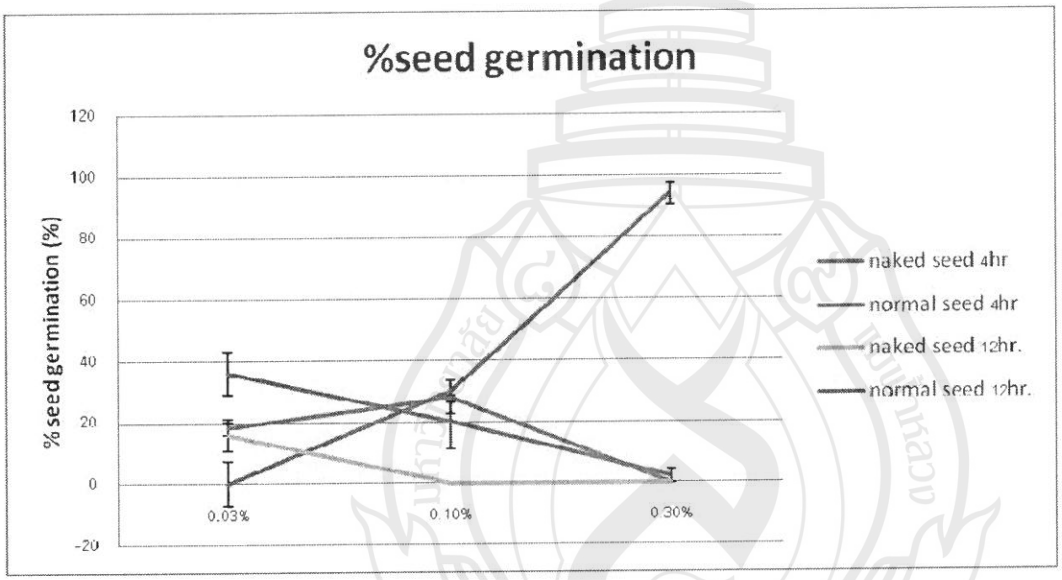

Figure 4.67 % seed germination of naked seed and normal seed after treated with NMU in buffer.

Experiment 3.5.2: Effect of concentration of Phosphate citrate buffer to % seed germination

Seeds from all treatments germinated differently (Figure 2). Seeds treated for 4 hrs. germinated less than those treated for 12 hrs. At 12 hr, treatment with 0.5x and 1.0x buffer concentration are significantly different from the water treated seeds, and the seed treated with 1.0x buffer germinated more than seed treated with 0.5x buffer at 12 hr. Comparing with the seed treated with water, %seed germination of the buffer treated seeds were lower, suggested that buffer has effect on reducing % germination of *J.*curcas seed.

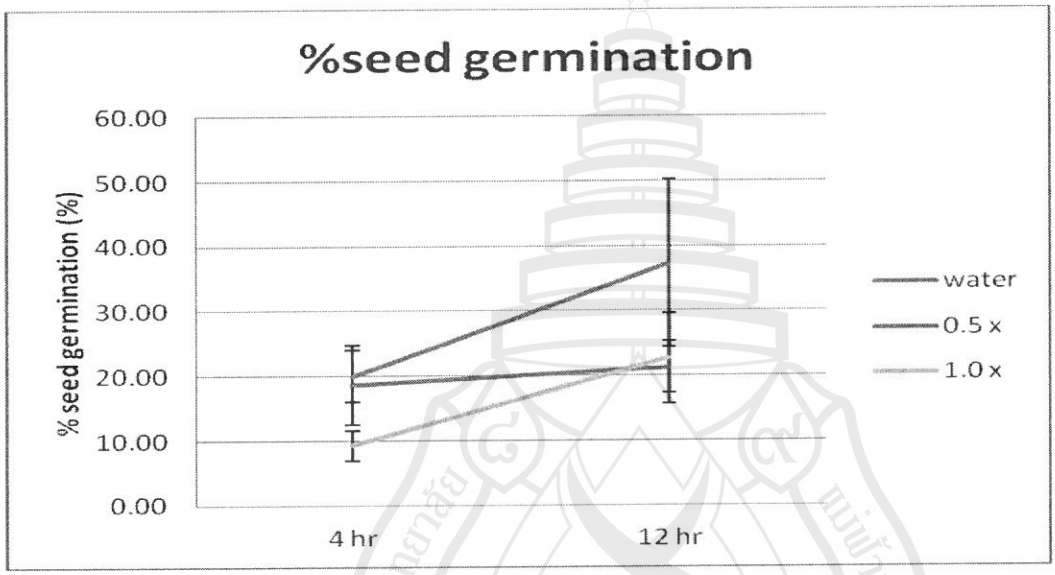

Figure 4.68 %seed germination of the *J. curcas* seeds are treated with 0.5x, 1.0x phosphate citrate buffer and water

Experiment 3.5.3: Effect of sterilize with Clorox to %seed germination

The % germination of the seeds sterilized with Clorox was less than those of the non-sterilized seed. This suggested that Clorox affected seed germination, and would not be used.

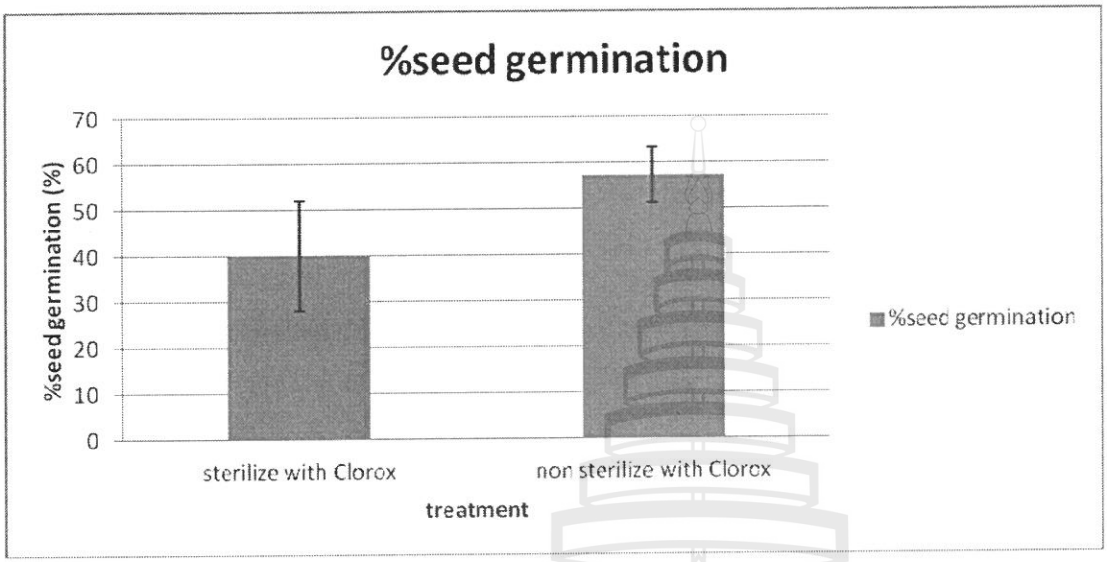

Figure 4.69 %seed germination of *J. curcas* seeds are sterilize and non sterilized with Clorox

Experiment 3.5.4: Effect of buffer and water to stability constant of pH of NMU solution.

From figure 4, all treatments of the NMU in water had pH lower than pH of pure water. The pH of all treatments with NMU in buffer was higher than the pure buffer. The pH of 0.5% and 1.0% NMU in buffer slightly increased from 10 to 14 hrs. and stable. This result suggested that NMU increased pH of buffer and but decreased pH of water. Therefore, we decided to treat *J. curcas* seeds with NMU in buffer solution for 14 hrs.

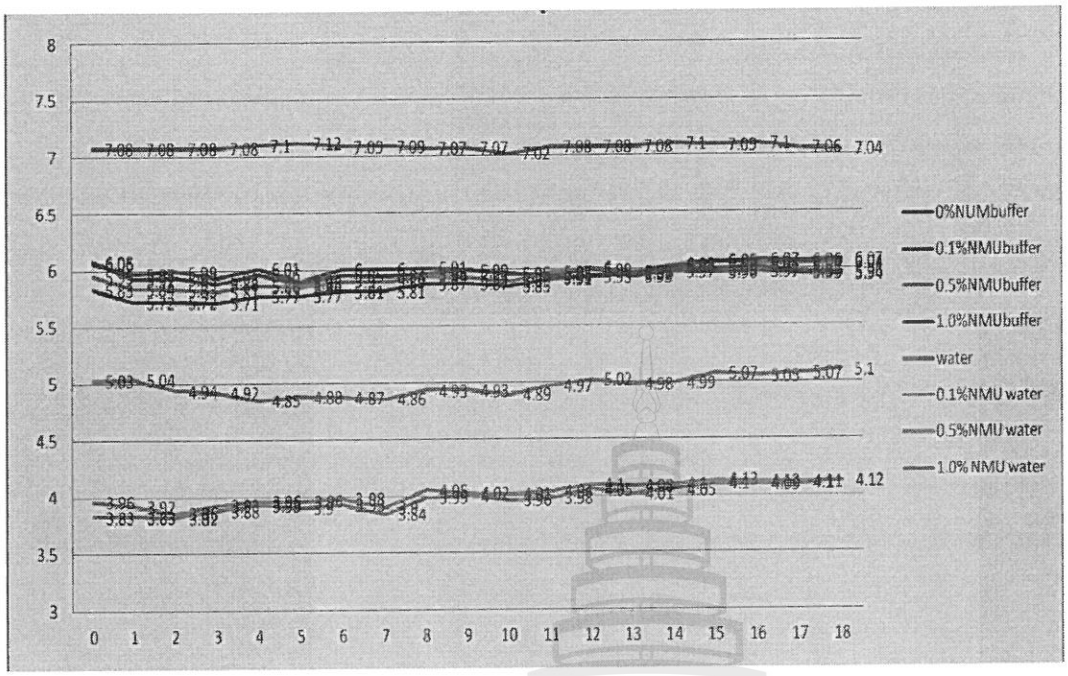

Figure 4.70 pH of all NMU solutions measured hourly for 18 hours.

Experiment 3.5.5: Effect of concentration of NMU to seed germination and growth.

% seed germination

Germination of NMU treated seeds were significantly different from controlled treatment, with the lowest in the pure buffer and 0.05%NMU (Figure 5.1). Seed germination at 0.1% NMU was the highest. All of the seedlings were not visually different, except only 1 abnormal *J. curcas* was found in the treatment. 0.8% NMU. This plant stem has 2 branch epicotyl (Figure 5.11).

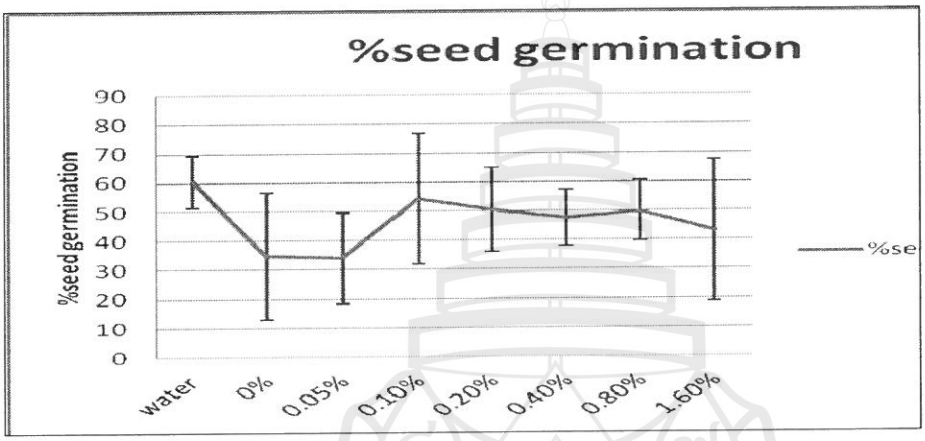

Figure 4.71 % seed germination of *J. curcas* seed is treated with NMU in phosphate citrate buffer.

% viability

Viability of the seedlings from all the NMU treated plants were lower than the controlled plants (Figure 5.2). NMU treated plants at 0.2, 0.4, 0.8, and 1.6% survived significantly less than the other treatments, but all the seedlings were not visually different. The abnormal plant from 0.8% NMU was growing normally, with one branch grew slower than the other.

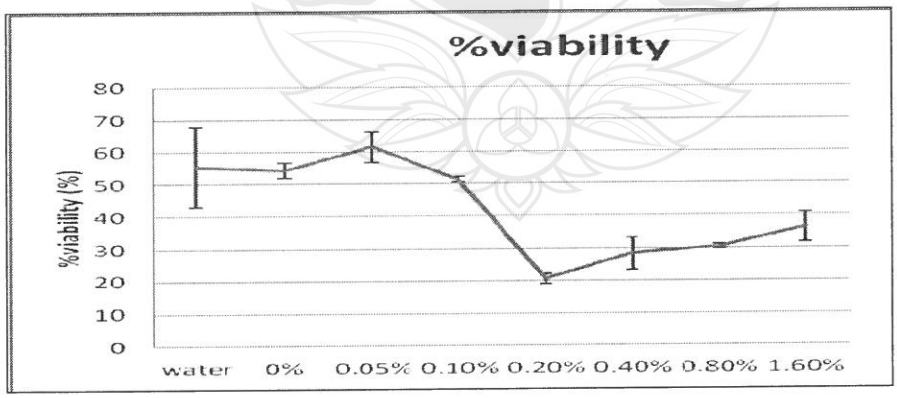

Figure 4.72 % viability of *J. curcas* seed is treated with NMU in phosphate citrate buffer

Plant height

Height of the plants from all treatments was not significantly different from the start until week 24 (Figure 5.3), when there was slightly height difference between the NMU treated and the controlled plants. Among the NMU treated, plants from 0.1 and 0.8% NMU were higher than the others. Difference in plant height were more obvious at week 39, when the plants treated with pure buffer were the shortest, followed by the medium group from treatments 0.4, 0.2 and 0.05% NMU and the tallest group from treatments 1.6, 0.8, 0.1 and controlled. At week 47, the taller group started to grow slower while the medium group started to grow faster. At the present, height of the plants in all treatments except those treated with the pure buffer, are not different.

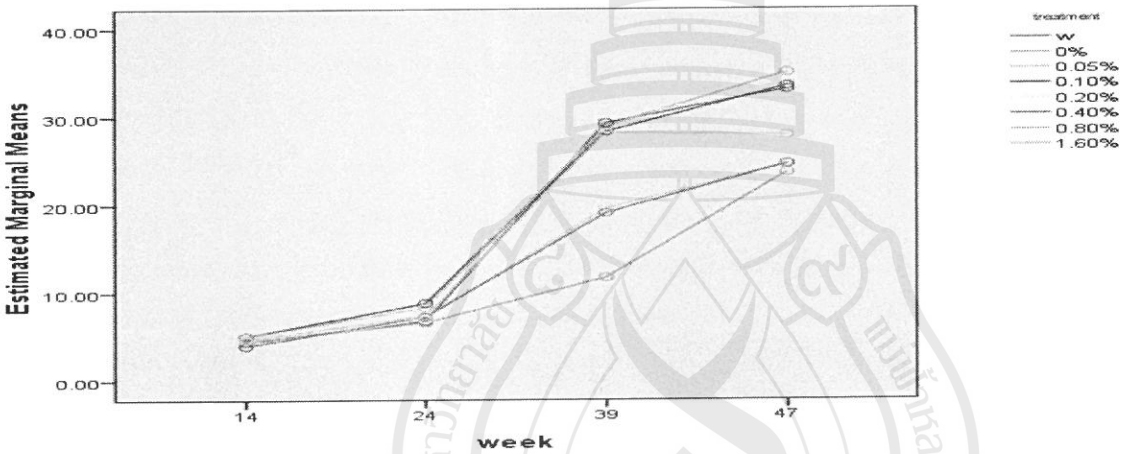

Figure 4.73 Plant height of *J. curcas* after application at 14, 24, 39 and 47 weeks

Average internode length

Although slightly different, internode length of the plants from all treatments was not significantly different from the start until week 39, except in the plant treated with pure buffer which has the shortest internode right thru the end (Figure 5.4). At 39 weeks internode length of the plant from treatment 0.8%, 1.6%, and 0.1% NMU is slightly longer than the others, but were significant only with the pure buffer treated plants.

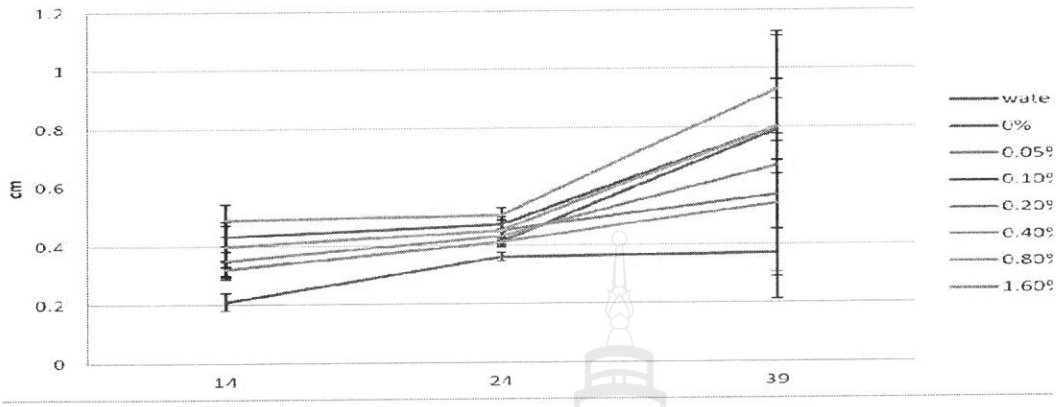

Figure 4.74 Inter node length of *J. curcas* after application at 14, 24 and 39 weeks

Number of node

Trend of node numbers (Figure 5.5) were similar to those of plant height, with not significantly different from the start until week 24, when number of node of 1.6% NMU was significantly more than other NMU treated plants and controlled. At 39 weeks, number of node of all the NMU treated plants were slightly less than controlled plants, except concentration at 1.6%, 0.1%, 0.8%, and 0.2% NMU in buffer.

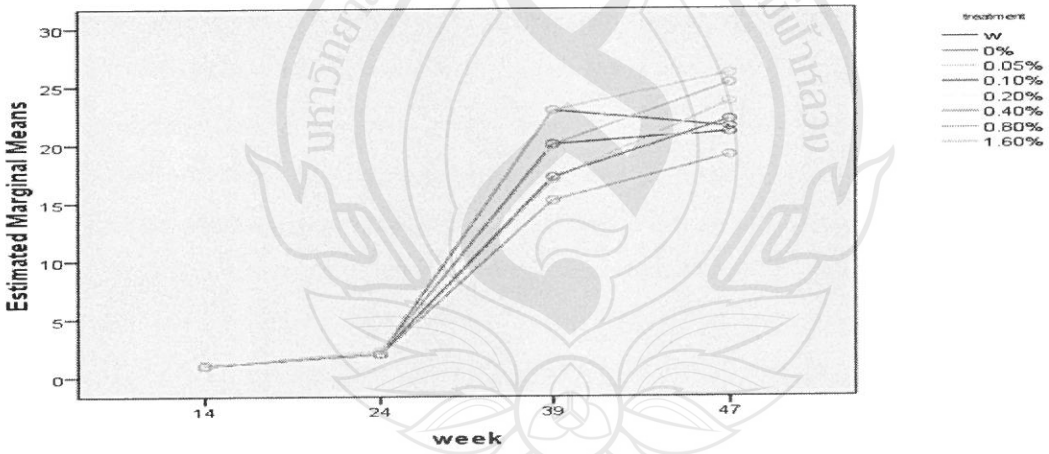

Figure 4.75 Number of node of *J. curcas* after application at 14, 24, 39 and 47 weeks

Stem diameters

From Figure 5.6, Stem diameters of plants treated with pure buffer was significantly the lowest at all times. All the NMU treated plants were significantly thinner than the controlled plants at the week 14 and 24, except at 39 weeks when stem diameter of only 0,4%NMU and pure buffer treated plants were significantly lower than all the others. However, they returned to the previous trend towards the week 47 until now.

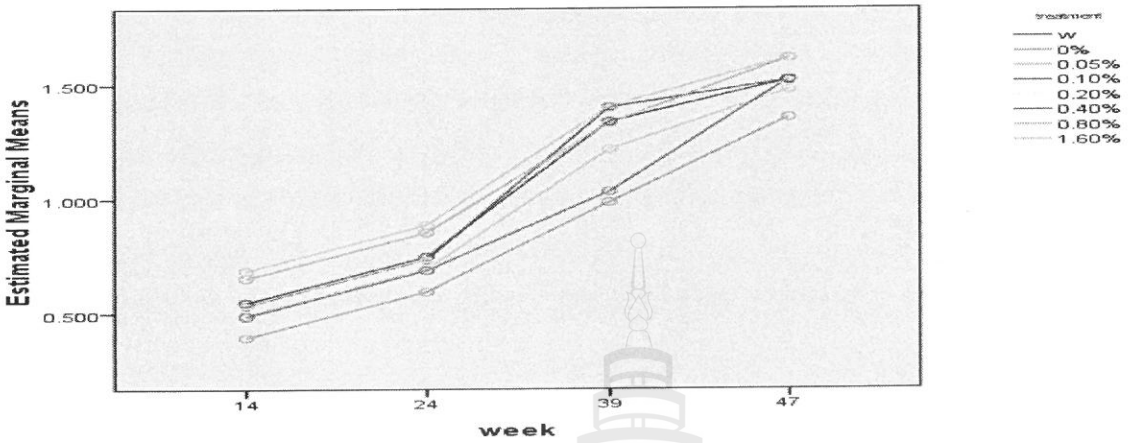

Figure 4.76 Stem diameters of *J. curcas* after application at 14, 24, 39 and 47 weeks

Number of branch

All the plants started branching from week 24(Figure 5.7). At 39 weeks, branching of the controlled and 0.1% NMU plants were slightly higher than the other treatments, and maintained this trend until week 47 up until now.

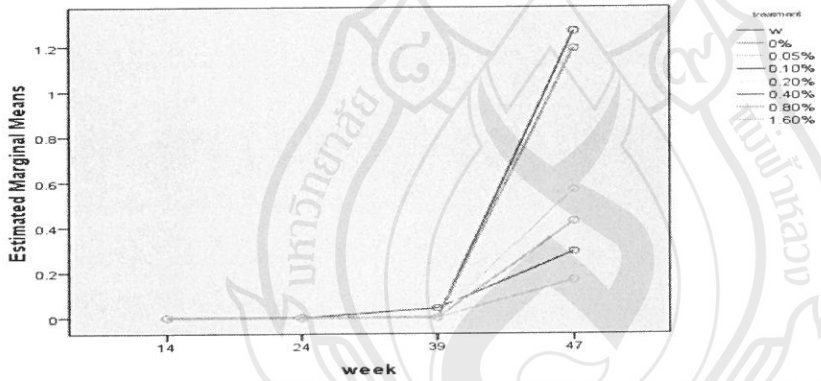

Figure 4.77 number of branch of *J. curcas* after application at 14, 24, 39 and 47 weeks

Discussion

The optimum techniques for NMU seed treatments were explored, and we could conclude from % viability that LD_{50} of NMU is at 0.2%. Unfortunately, as no NMU treated plants has been flowering at the time of this report, the M2 population could not be produced. From the result of %seed germination, % viability, and growth, all of these characters in the treated plants were not significantly abnormal, except the only one abnormal plants from the 0.8% NMU treatment with 2 branches epicotyl but growth of the extra branch was stopped since the early stage and not contributed to any further growth and production. Therefore, we could concluded that mutagenesis of *J.curcas* with NMU has not been successful in our study.

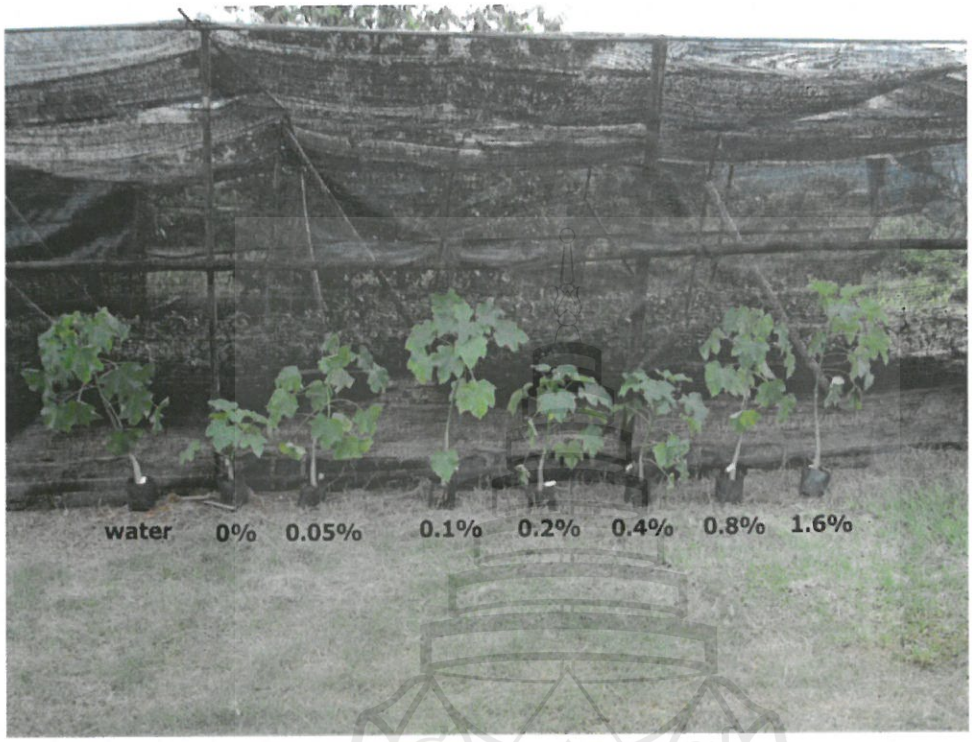

Figure 4.78 Picture of treatment NMU in buffer and water of J. curcas after application at 39 weeks.

Figure 4.79 Picture of abnormal *J. curcas* at 0.8% NMU in buffer at 39 weeks

Chapter 5

Conclusion & Suggestion

5.1. Research Objectives

1. To collect and study Jatropha germplasms
2. To study genetic diversity of the collected germplasm
3. To preliminary investigate mechanism for dwarfism using apical dominance hormone inhibitors (ADHI)

5.2. Scope of works

1. Germplasms collection
2. Establishing DNA fingerprints of at least 20 accessions in the existing germplasm using Amplified Fragment Length Polymorphism (AFLP) and karyology to identify genetic diversity
3. Investigating plant response, in terms of height, to plant-height-related hormones inhibitors
4. Investigate possibility of mutagenesis using NMU to increase genetic variation by testing optimum dose, LD_{50} and techniques to treat seeds with NMU, generating M1 and M2 population, and investigating genetic diversity

5.3. Conclusions and suggestings

- More than 60 accessions of Jatropha were collected.
- Although yield of all varieties were still low and genetic improvement is needed, managing environmental condition or cultural practices might be able to increase Jatropha yield.
- DNA fingerprints in terms of AFLP and karyology of 22 Jatropha accessions were successfully done and analyzed for their genetic diversity. AFLP analysis using 64 pairs of primers could be able to differentiate plant from different specie *J. podariga* from all the other *J. curcas* accessions, as well as successfully grouped the *J. curcas* accessions according to their geographical distribution and their toxicity. The non-toxic Mexico accession that has origin

from Mexico has the highest difference from all the toxic Thai and India accessions.

- High similarity index and percent polymorphism suggested that there is low genetic variation/ diversity among Thai Jatropha accessions, especially on plant height characteristic. Biotechnology techniques such as induce mutagenesis to create population genetic diversity, interspecific hybridization or various types of genetically modification techniques would be necessary to introduce new desirable traits in to *J. curcas* gene pools, which is necessary for varietal improvement. Work on induced mutagenesis is now on-going.
- Inhibiting apical dominant hormones (GA by paclobutrazol & IAA by cytokinin) significantly reduced Jatropha plant height. GA inhibitors at the concentrations of 250 and 500 µM could inhibit the plant height without reducing the total dry matter production The height reducion caused by internode length reduction, not the node numbers and branching, and should not effect flowering possibility as the plant will flowering at the branch. Therefore, genetically manipulating the involving genes could be useful for manipulating plant height. This finding might lead to the biochemical pathways important for genetically manipulating height of Jatropha plant, as genes related to GA biosynthesis have been studied /known. Confirming effect of GA inhibitor and investigating yield of the field grown plants is now on-going.
- There is an extra finding on carbon sequestration by *J. curcas*. The significant correlation between net CO_2 assimilation rate and accumulative CO_2 uptake with carbon sequestration suggests that both indices might be more suitable to non-destructive estimation of the carbon sequestration of *J. curcas* plant than the measurement of the dry matter production which is more destructive.
- The optimum techniques for NMU seed treatments were explored, and we could conclude from % viability that LD_{50} of NMU is at 0.2%. No significant abnormality found in all the treated plants and none of them flowering at the time of this report so no M2 population could be produced. Therefore, we could concluded that mutagenesis of *J.curcas* with NMU has not been successful in our study.

5.4. Other knowledge generation

- This project had been used as thesis/special problems of 4 students (1 graduates and 3 undergraduates)
- Publications in the International –full refereed- conferences, and won the Best Presentation Award in one of the publication
- Organized an international technical workshop with 50 international participants represented researchers, community, policy makers from academics industrial and business sectors, with published proceedings. (see Appendix 1)
- An Industrial partner has been interesting to establish collaborative research project based on the findings of this project.

5.5. Future/on-going research

- Using AFLP technique to investigate genetic diversity of new and unknown accession, as well as improve specific markers.
- Investigating yield potential of the existing accessions in a big plantation with high input cultural systems with optimum water and fertilizer management, as a basis for considering Jatropha in an industrial scale. (Collaborative project with a industry).
- Investigating genes involved in GA biosynthesis using inhibitors and/or gene silencing, in order to create dwarfism *J. curcas*

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APPENDIX A

Reaction composition of AFLP

Step 1; Restriction Digestion of Genomic DNA		
DNA (100 ng/µl)	2.50	µl
5X Reaction Buffer (10X Buffer of MseI)		2.50 µl
EcoRI (12 U/ µl)	0.21	µl
MseI (10U/ µl)	0.25	µl
SDW		19.54 µl
Step 2; Ligation of Adapters		
Digested DNA (from step 1)		25.00 µl
EcoRI adapter (5 pmole/ µl)		1.00 µl
MseI adapter (25 pmole/ µl)		2.00 µl
5X T4 ligase buffer (10X Buffer)	5.00	µl
T4 DNA ligase (20 U/ µl)	1.00	µl
SDW		16.00 µl
Total	50.00	µl
Step 3; Pre-selective amplification		
DNA (from Step 2)		2.00 µl
Primer E-A (5 pmole/ µl)	1.00	µl
Primer M-C (5 pmole/ µl)	1.00	µl
$dNTP_s$ (2mM)		2.50 µl
10X PCR buffer	2.50	µl
$MgCl_2$ (50 mM)	0.75	µl
Taq polymerase (5 U/ µl)	0.10	µl
SDW		15.15 µl
Total	25.00	µl
Step 4; Selective Amplification		
(Dilute DNA from Step 3 as 1:2 with TE buffer)		
Diluted DNA		5.00 µl
Primer E-ANN (5 pmole/ µl)		1.00 µl
Primer M-CNN (5 pmole/ µl)		1.00 µl
$dNTP_s$ (2mM)		2.00 µl
10X PCR buffer	2.00	µl
$MgCl_2$ (50 U/ µl)	0.60	µl
Taq polymerase (5 U/ µl)	0.10	µl
SDW		8.30 µl
Total	20.00	µl

APPENDIX B

Gels for AFLP Ananlysis

Figure B.1: Show the gel of AFLP DNA fingerprint bands of M-CAA/E-AAC

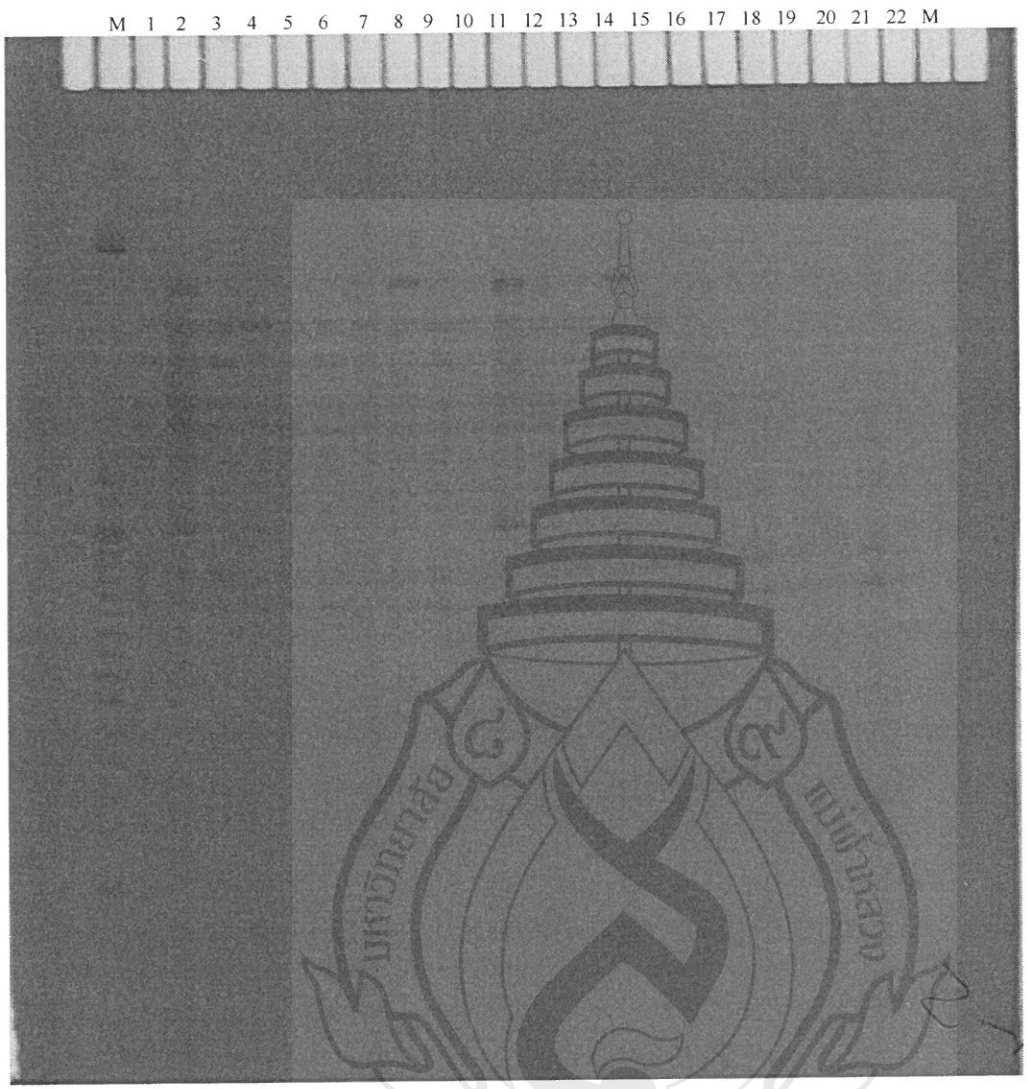

Figure B.2: Show the gel of AFLP DNA fingerprint bands of M-CAA/E-AAG

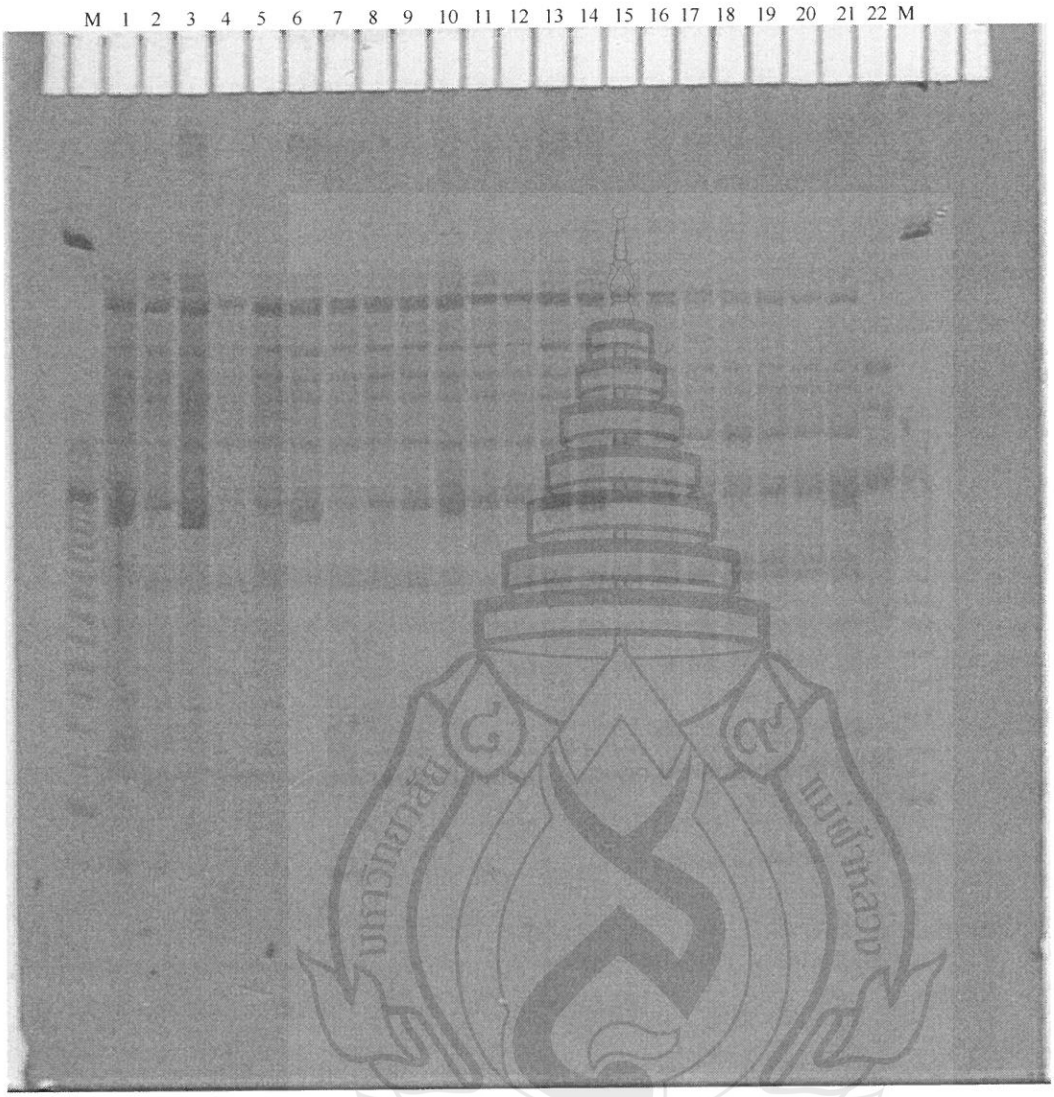

Figure B.3: Show the gel of AFLP DNA fingerprint bands of M-CAA/E-AAC

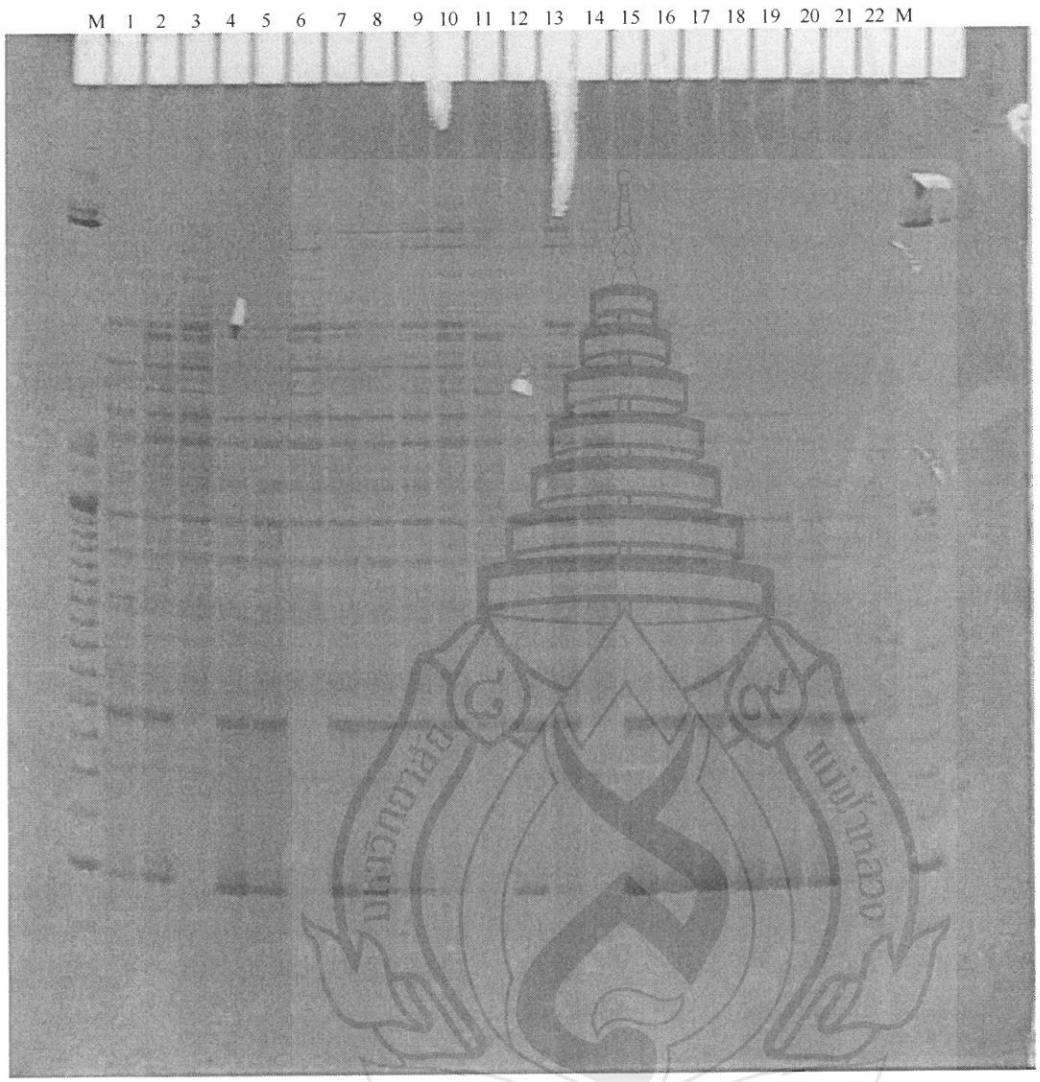

Figure B.4: Show the gel of AFLP DNA fingerprint bands of M-CAA/E-ACA

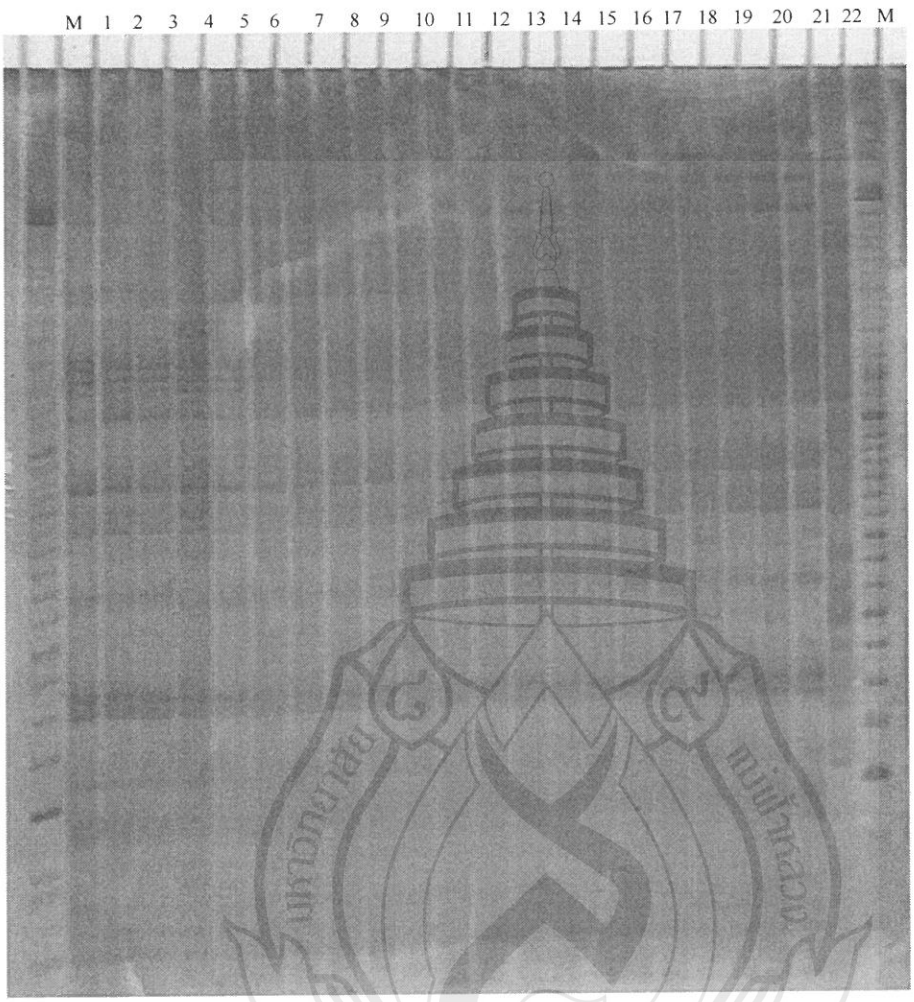

Figure B.5: Show the gel of AFLP DNA fingerprint bands of M-CAC/E-ACC

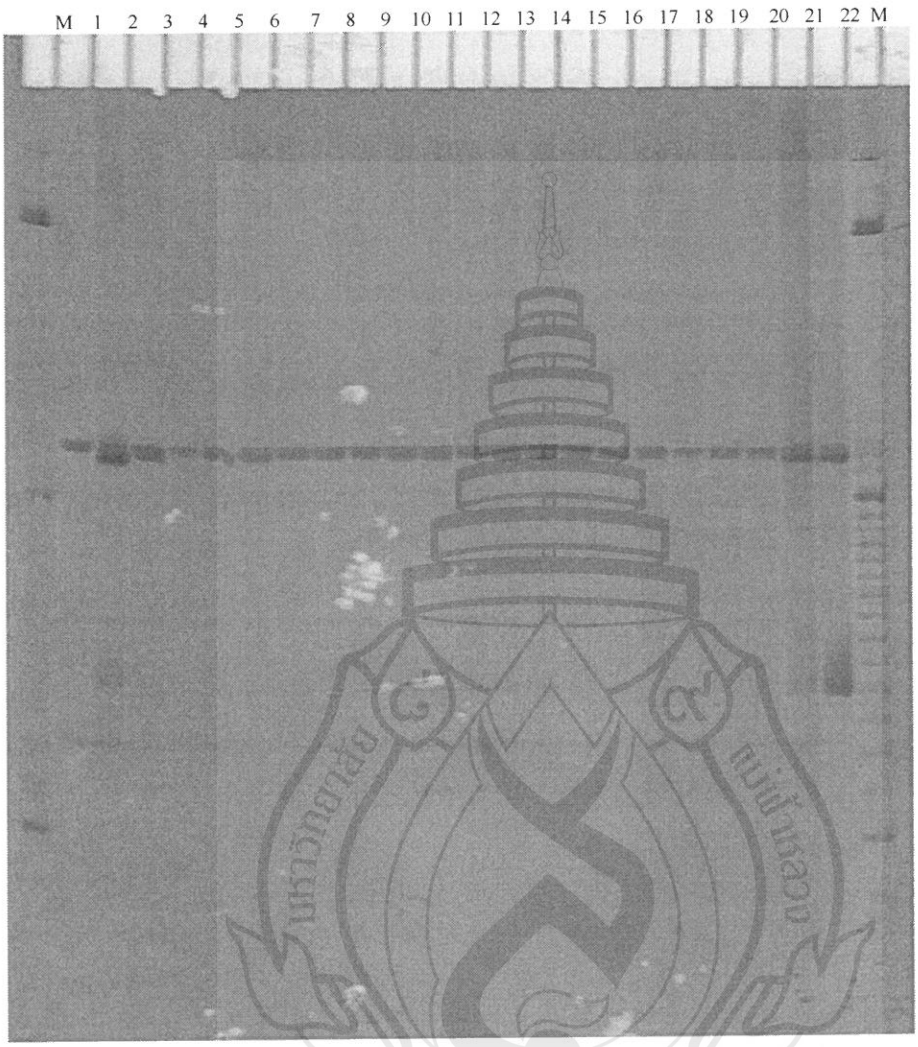

Figure B.6: Show the gel of AFLP DNA fingerprint bands of M-CAC/E-AAG

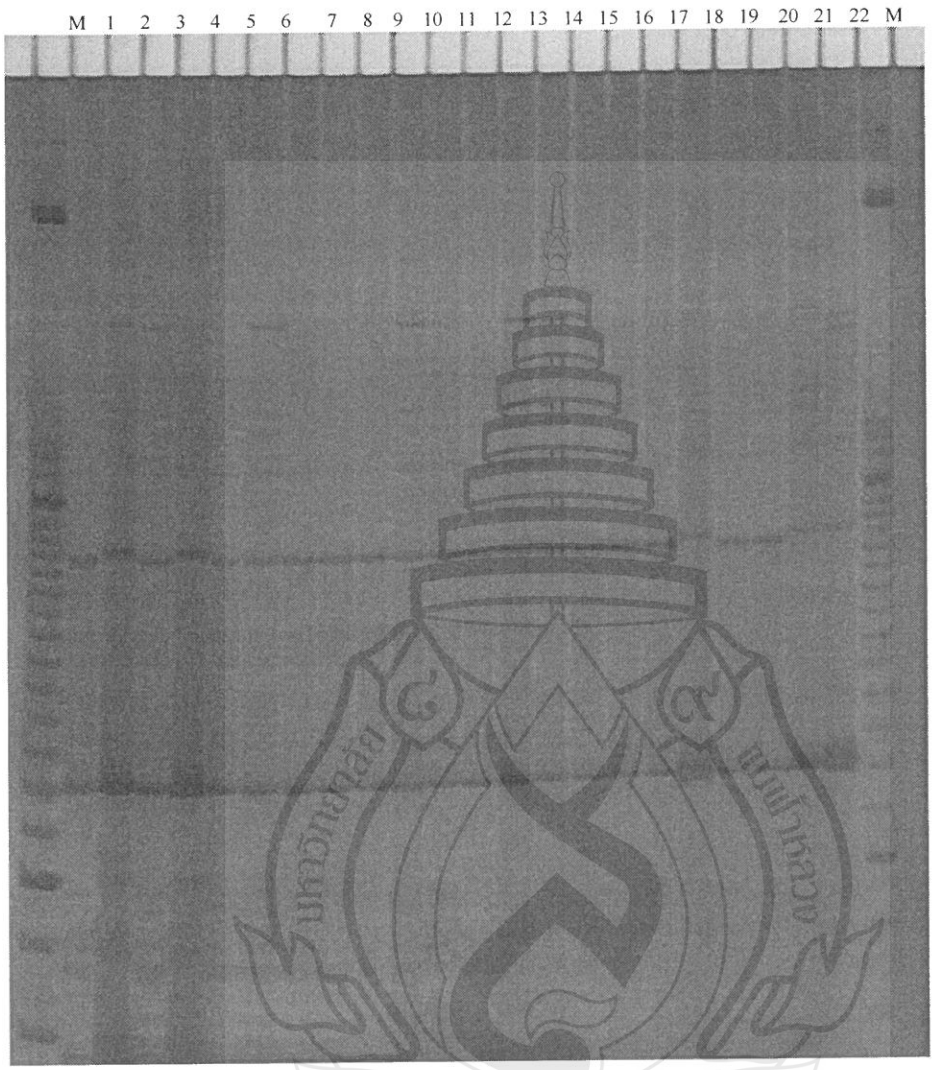

Figure B.7: Show the gel of AFLP DNA fingerprint bands of M-CAC/E-ACA

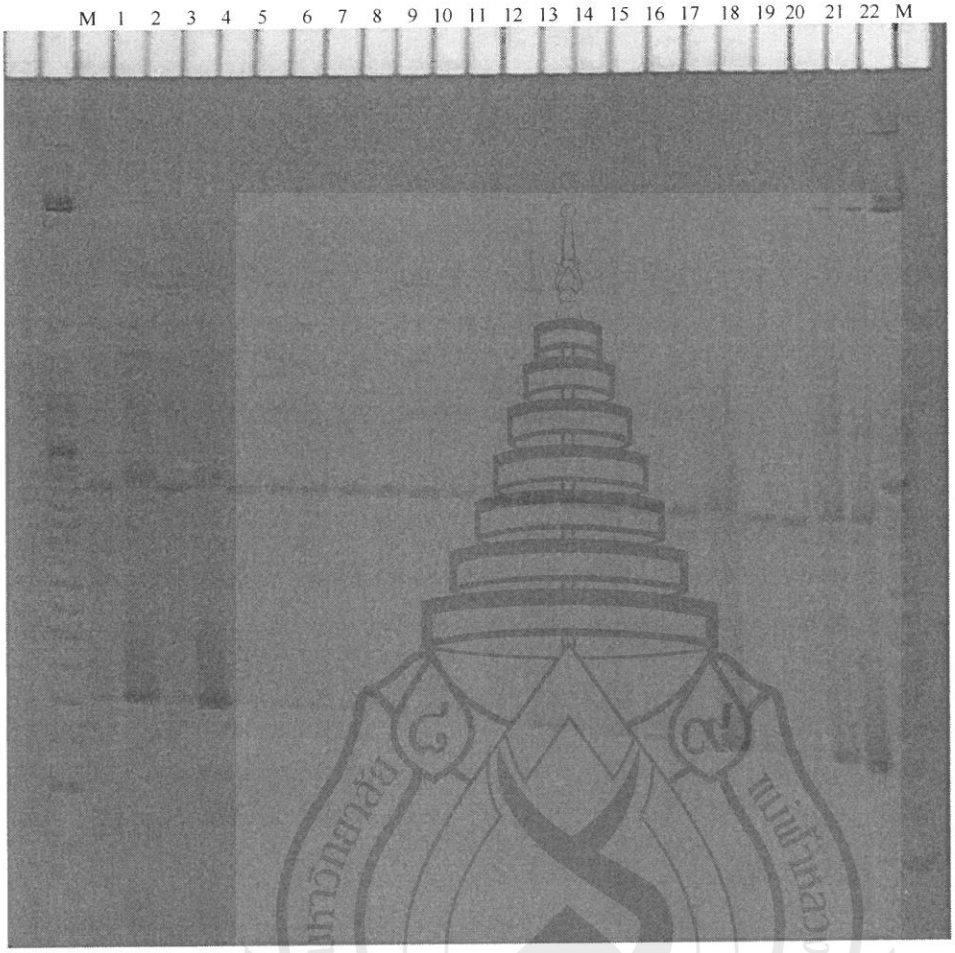

Figure B.8: Show the gel of AFLP DNA fingerprint bands of M-CAC/E-ACC

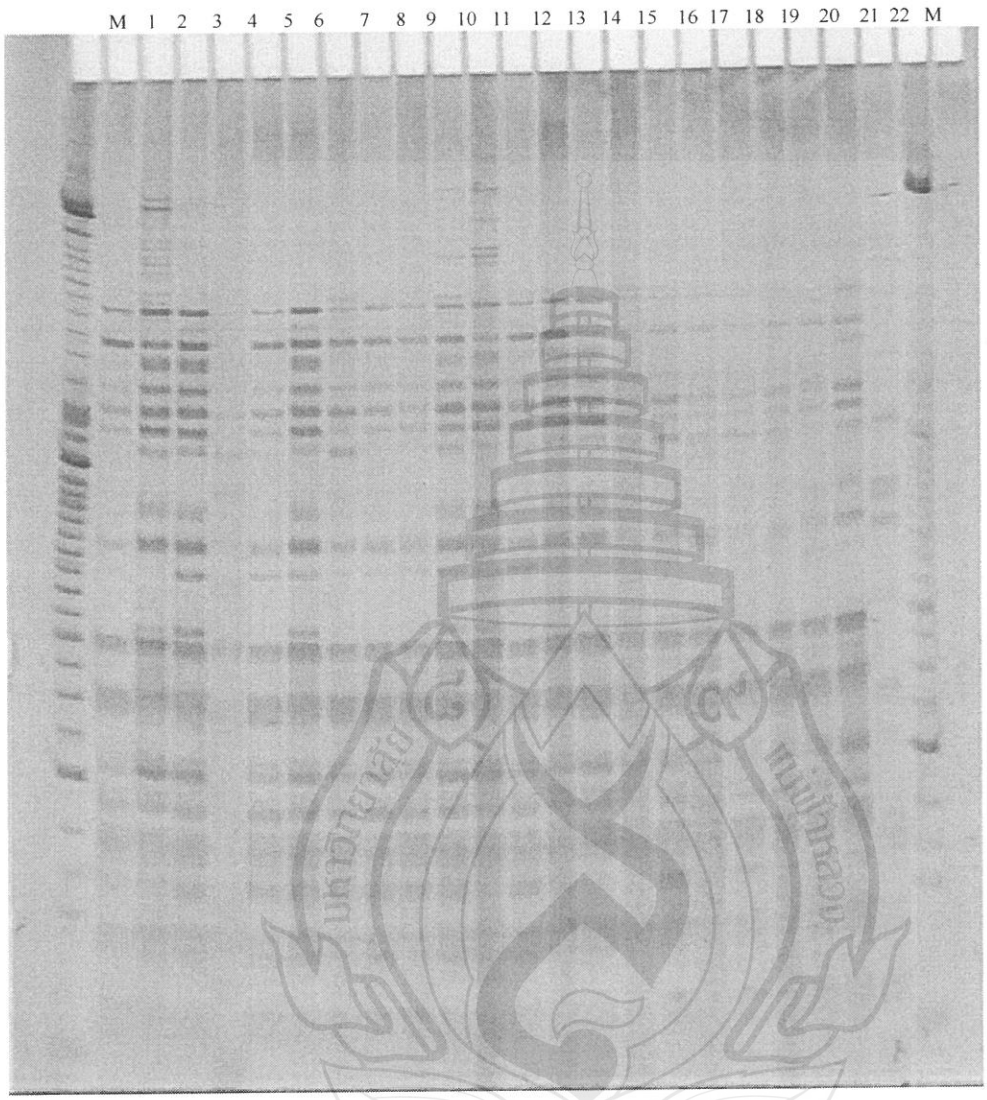

Figure B.9: Show the gel of AFLP DNA fingerprint bands of M-CAG/E-AAC

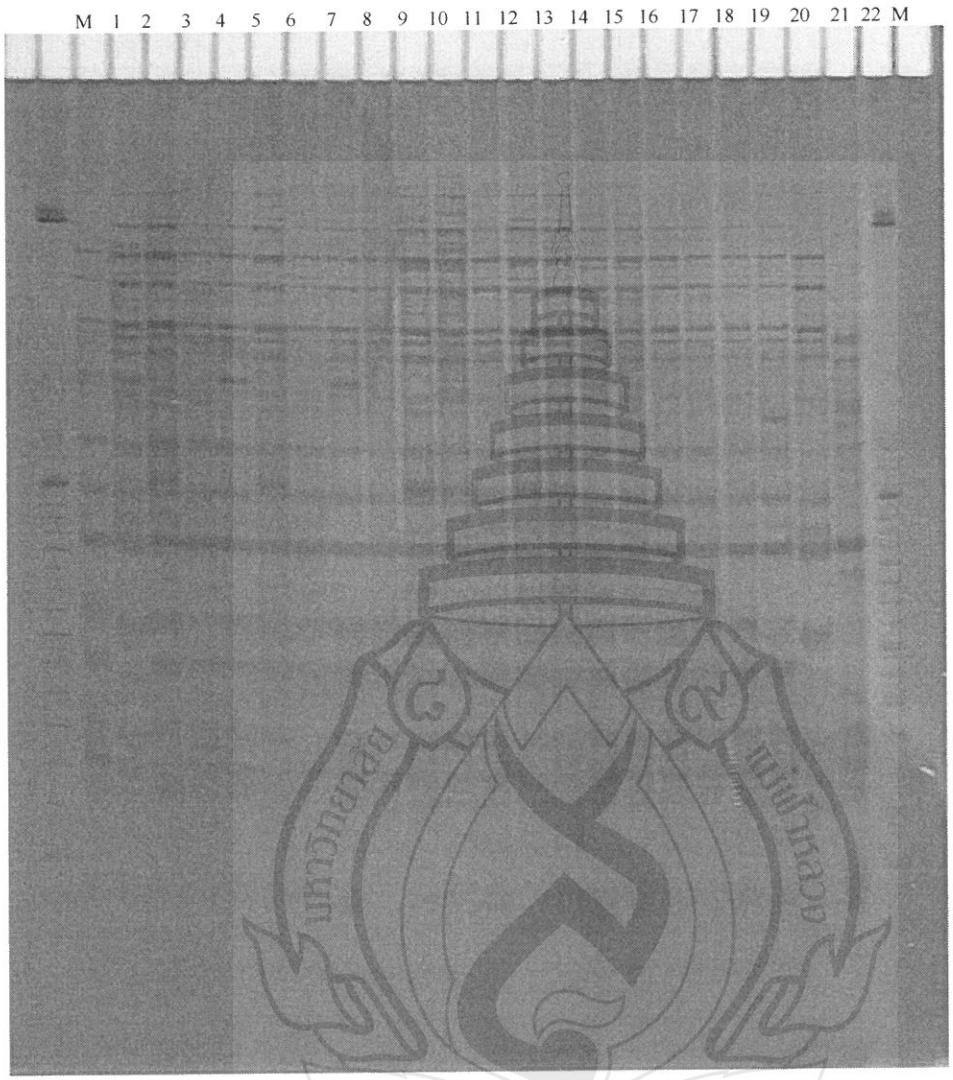

Figure B.10: Show the gel of AFLP DNA fingerprint bands of M-CAG/E-AAG

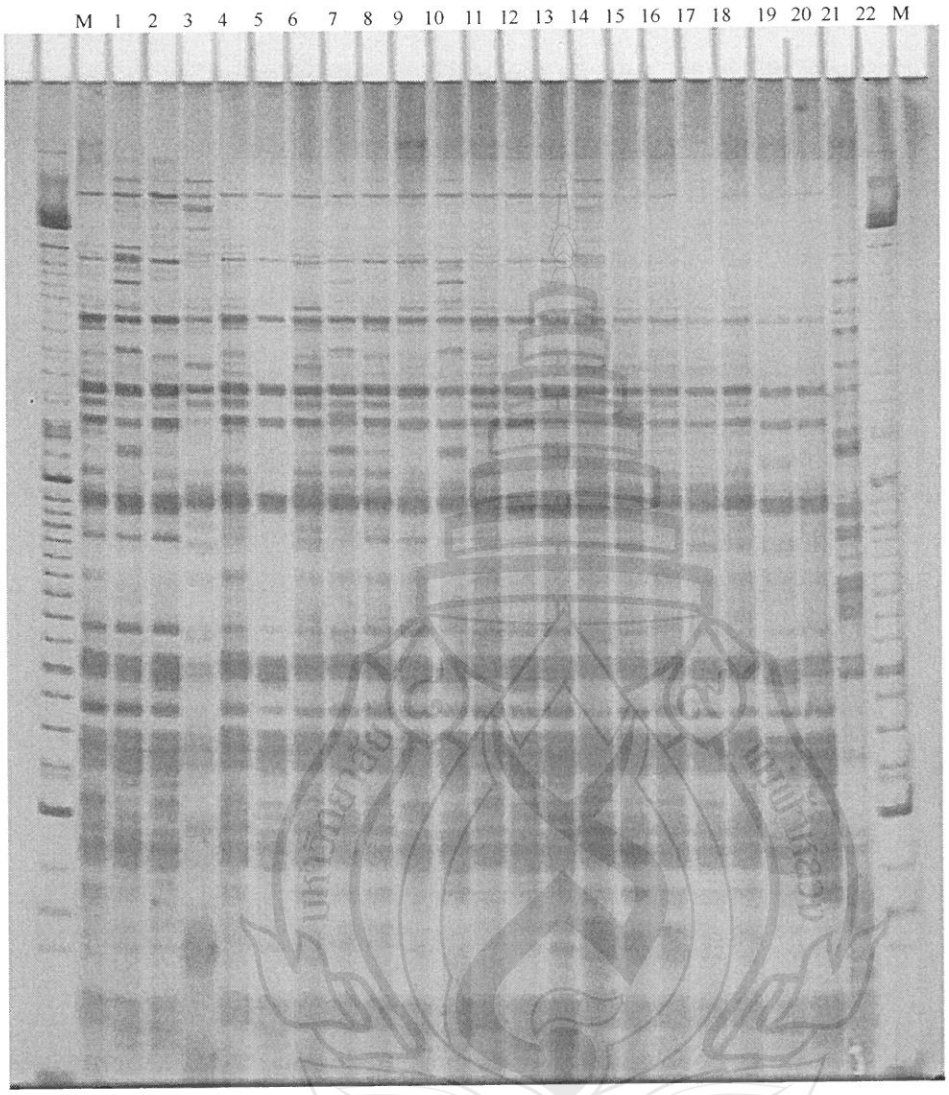

Figure B.11: Show the gel of AFLP DNA fingerprint bands of M-CAG/E-ACA

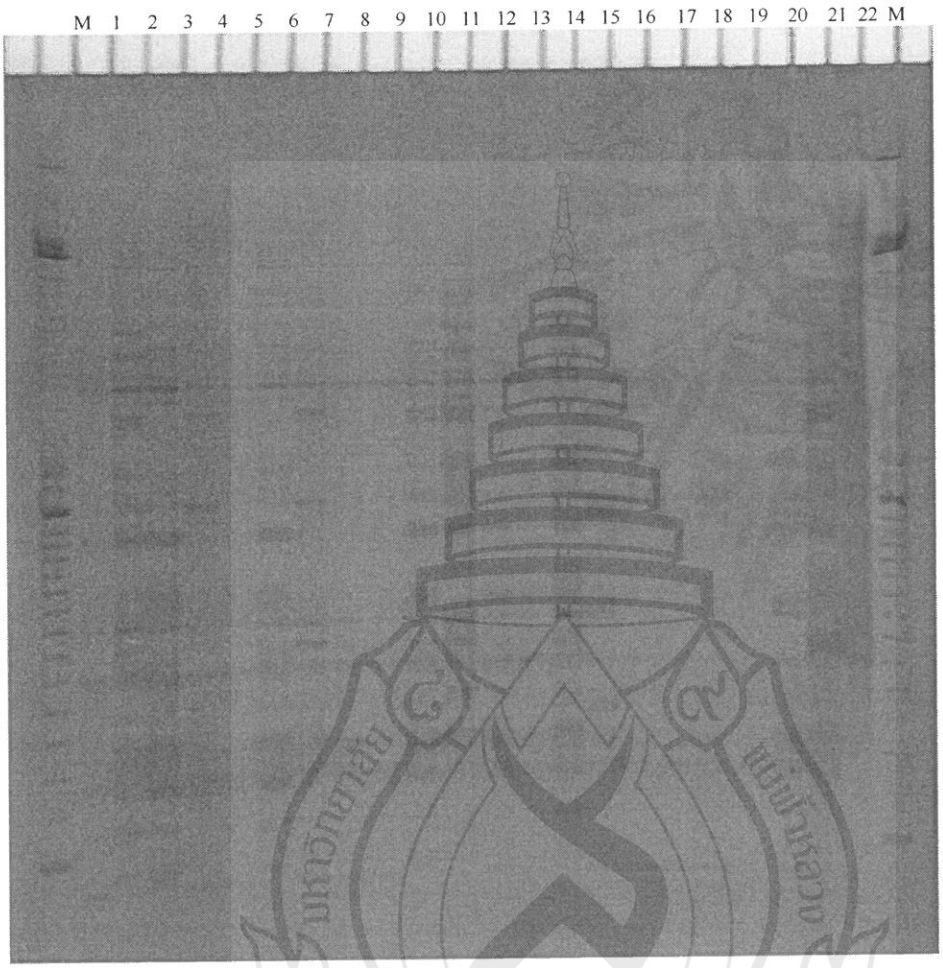

Figure B.12: Show the gel of AFLP DNA fingerprint bands of M-CAG/E-ACC

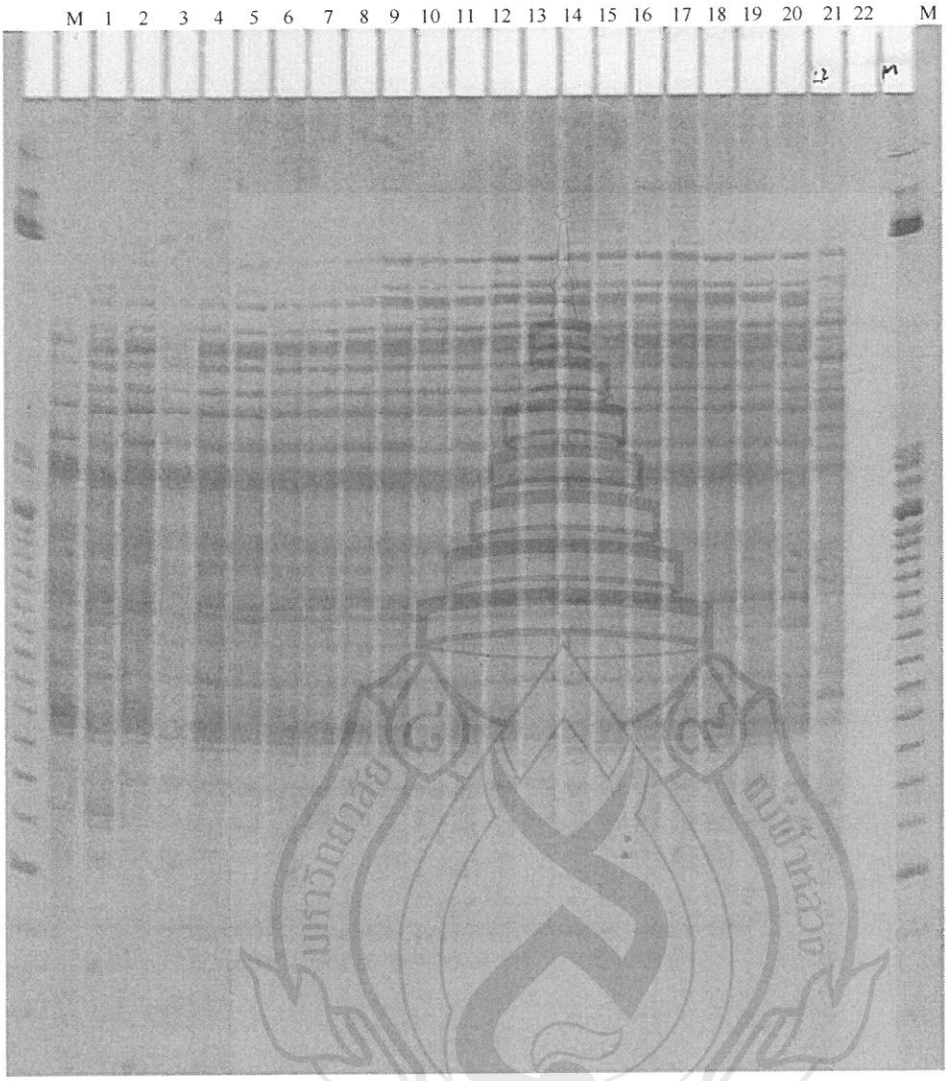

Figure B.13: Show the gel of AFLP DNA fingerprint bands of M-CAT/E-AAC

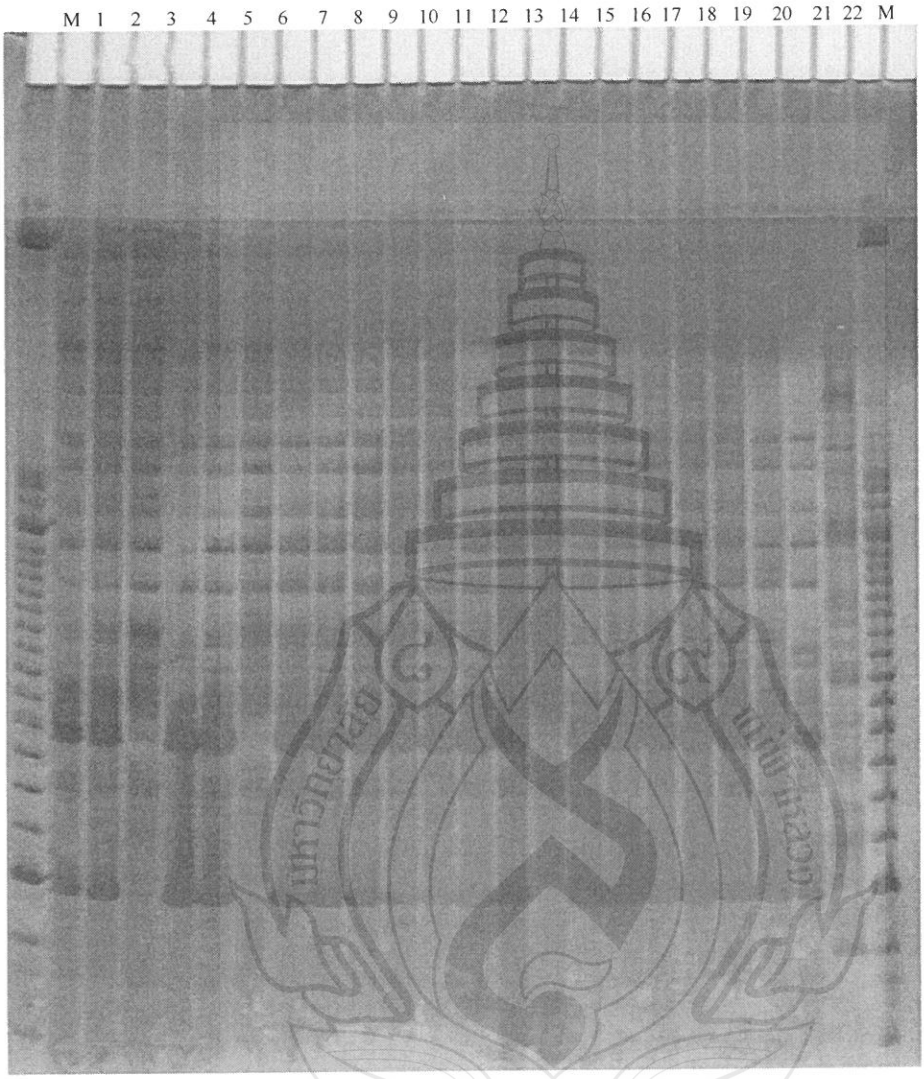

Figure B.14: Show the gel of AFLP DNA fingerprint bands of M-CAT/E-AAG

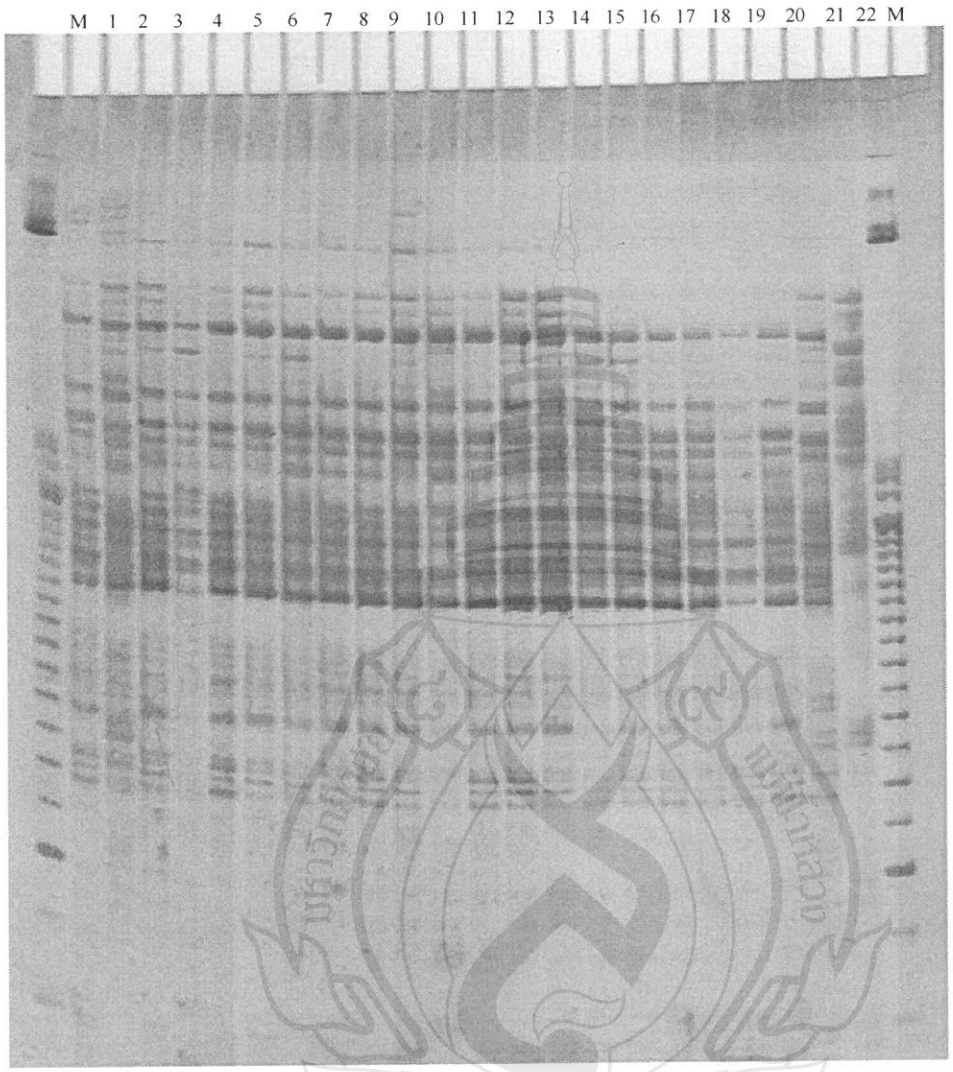

Figure B.15: Show the gel of AFLP DNA fingerprint bands of M-CAT/E-ACA

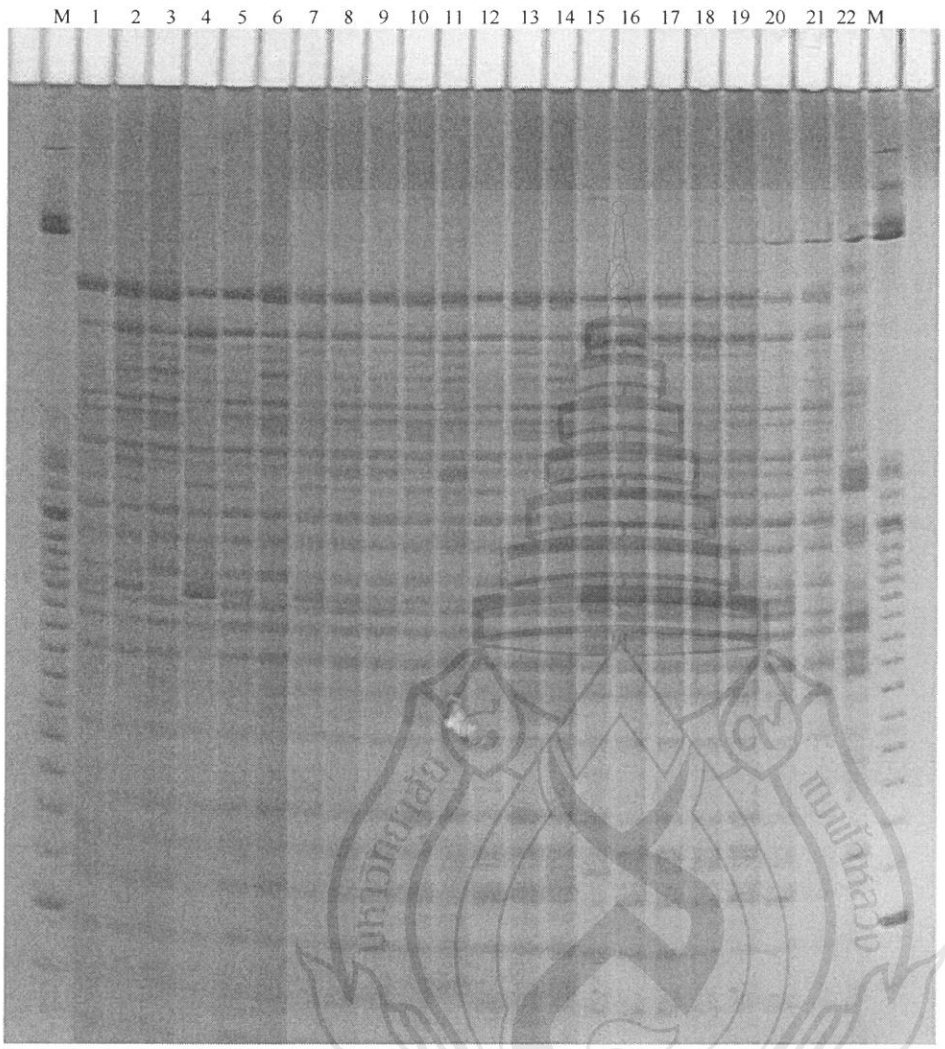

Figure B.16: Show the gel of AFLP DNA fingerprint bands of M-CAT/E-ACC

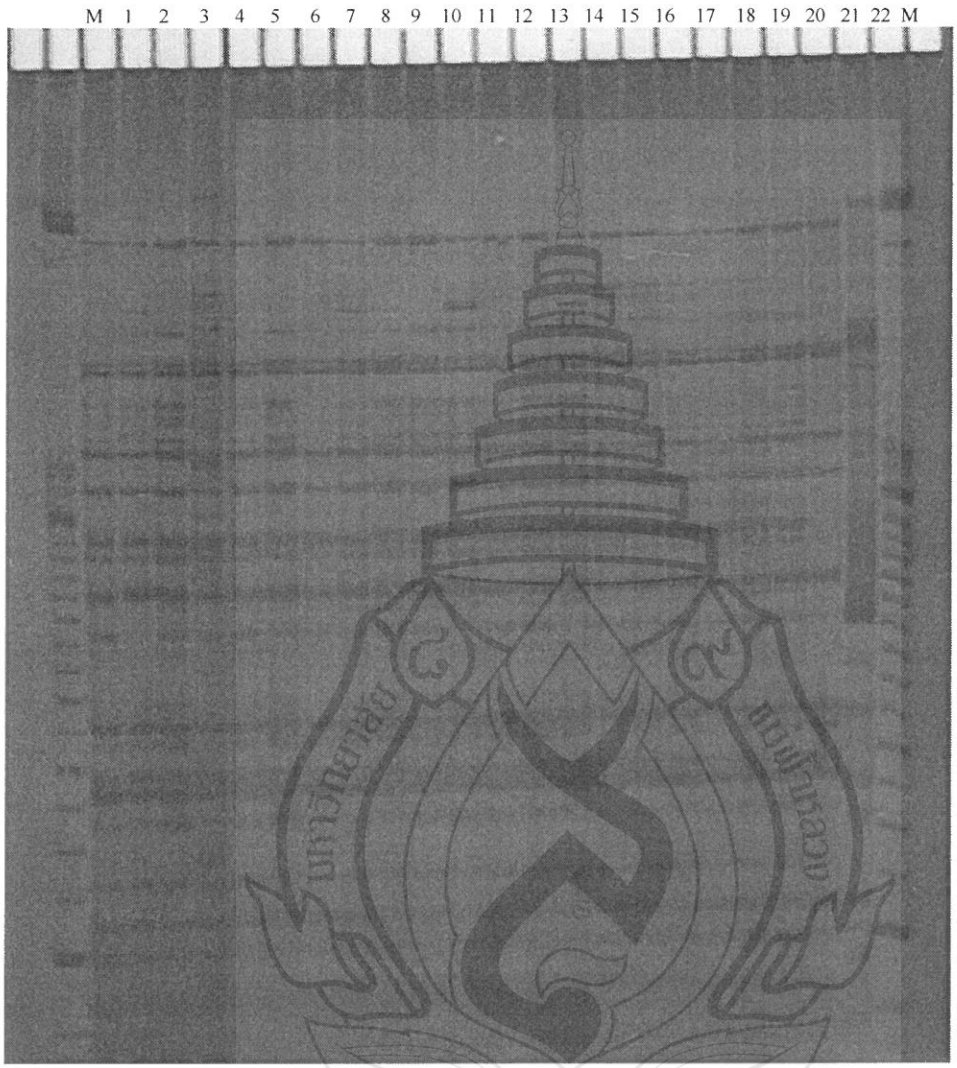

Figure B.17: Show the gel of AFLP DNA fingerprint bands of M-CTA/E-AAC

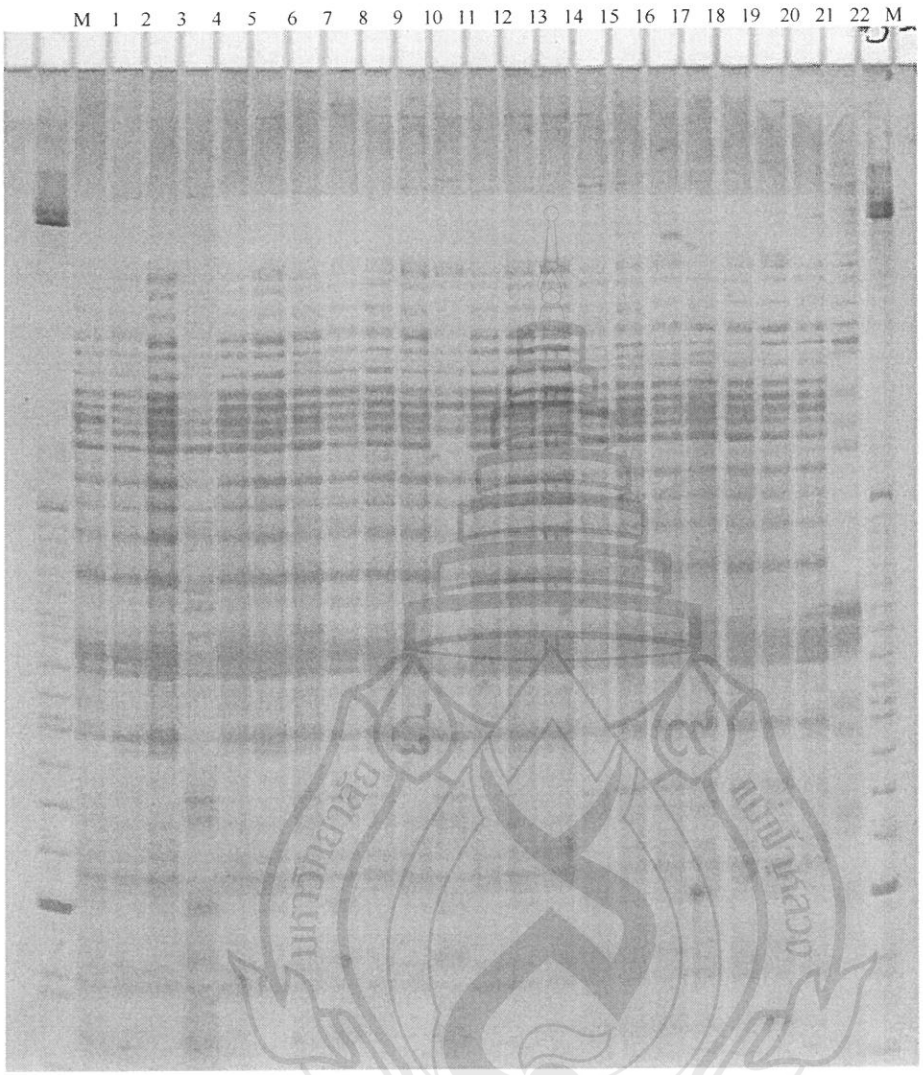

Figure B.18: Show the gel of AFLP DNA fingerprint bands of M-CTA/E-AAG

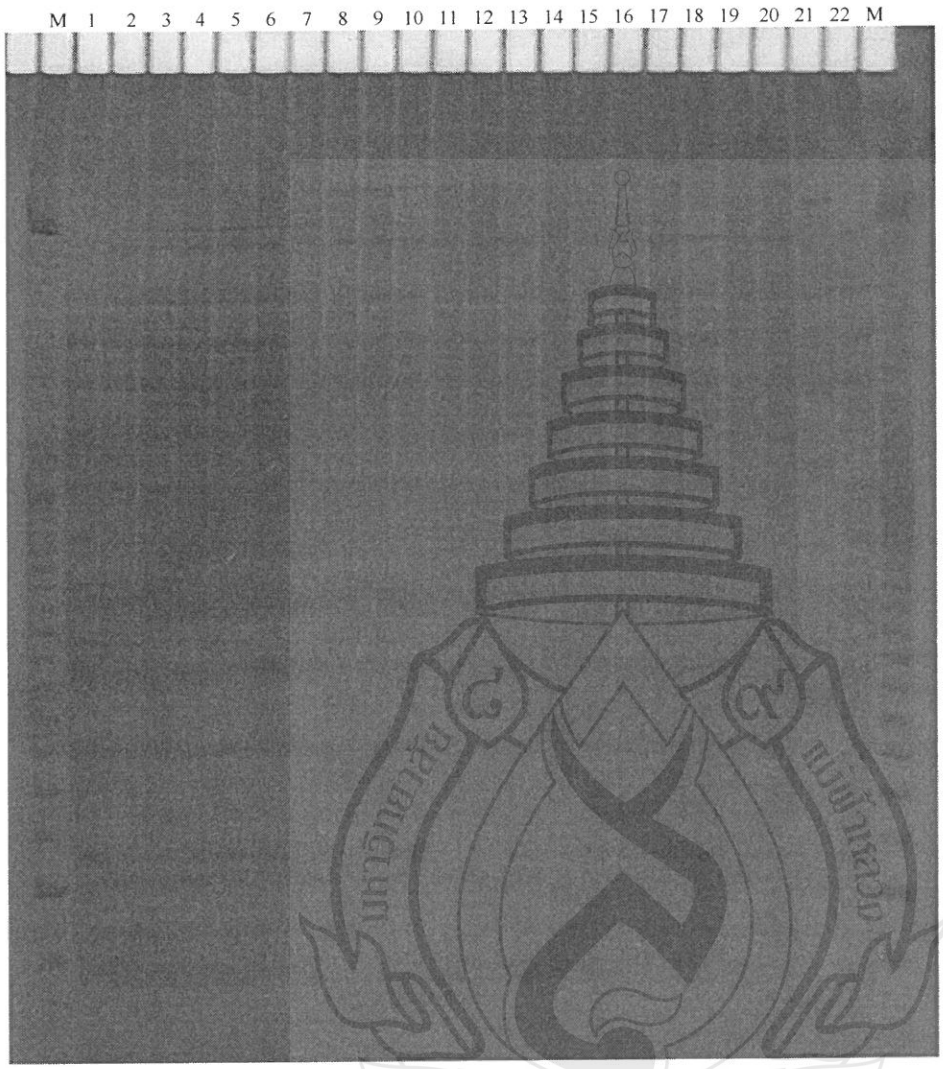

Figure B.19: Show the gel of AFLP DNA fingerprint bands of M-CTA/E-ACA

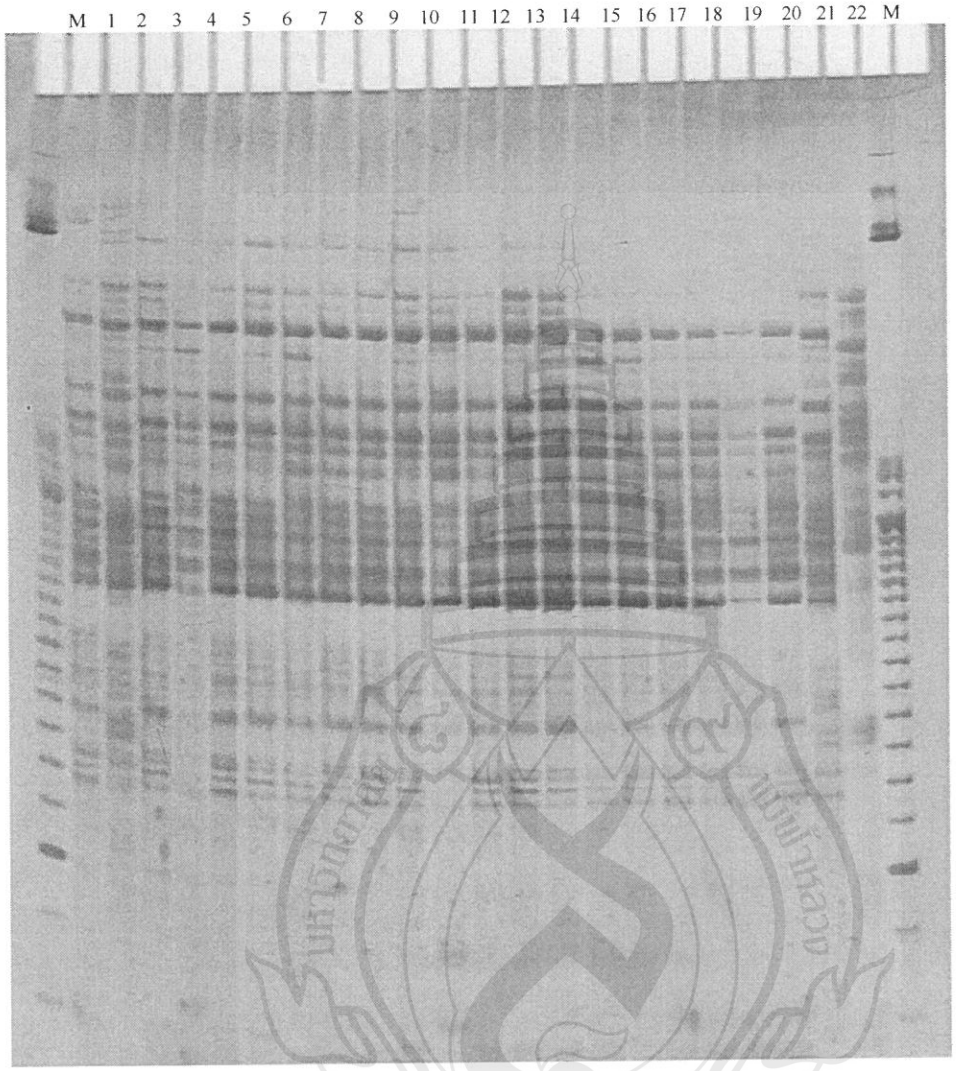

Figure B.20: Show the gel of AFLP DNA fingerprint bands of M-CTA/E-ACC

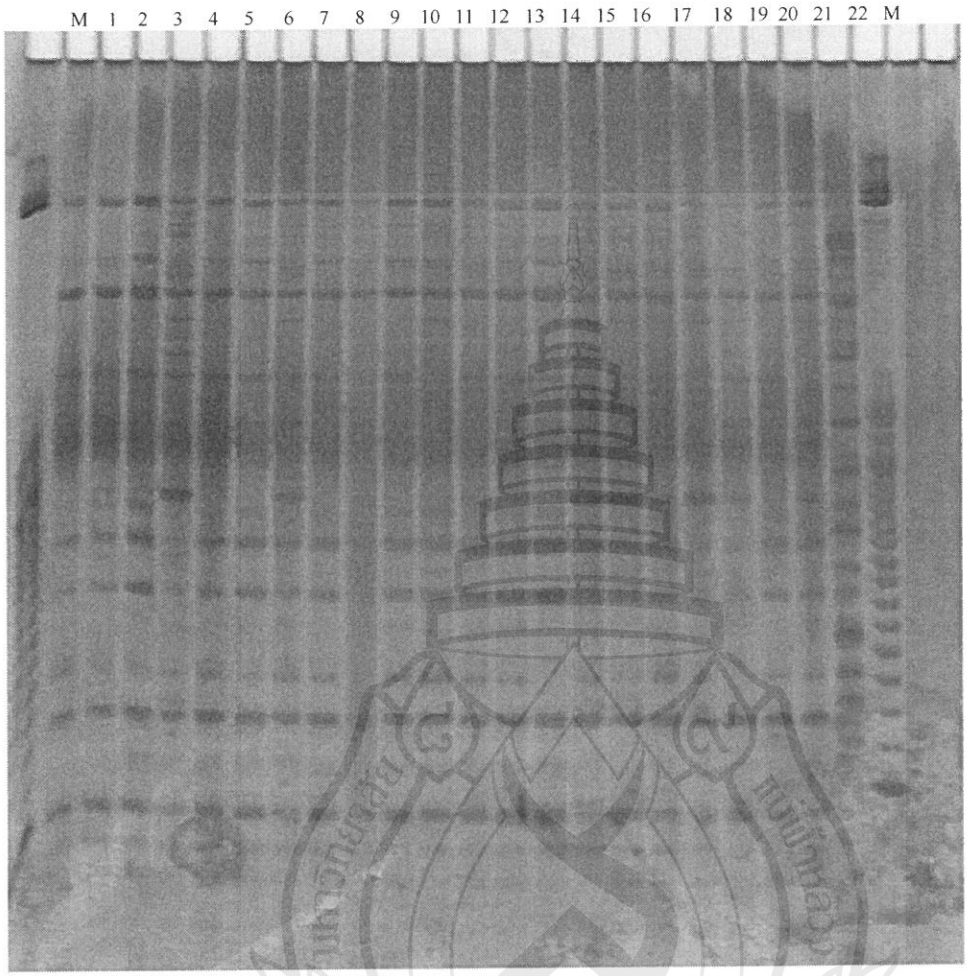

Figure B.21: Show the gel of AFLP DNA fingerprint bands of M-CTC/E-AAC

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Figure B.22: Show the gel of AFLP DNA fingerprint bands of M-CTC/E-AAG

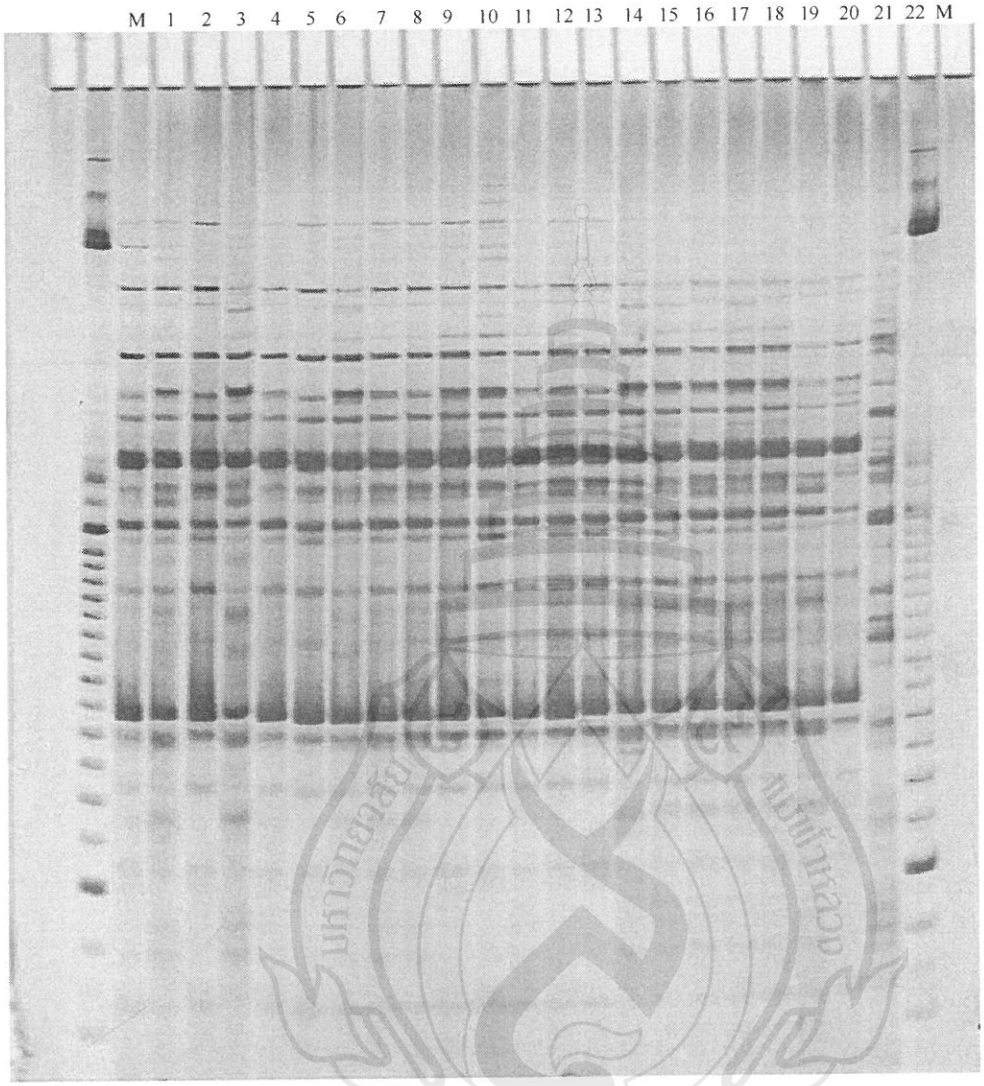

Figure B.23: Show the gel of AFLP DNA fingerprint bands of M-CTC/E-ACA

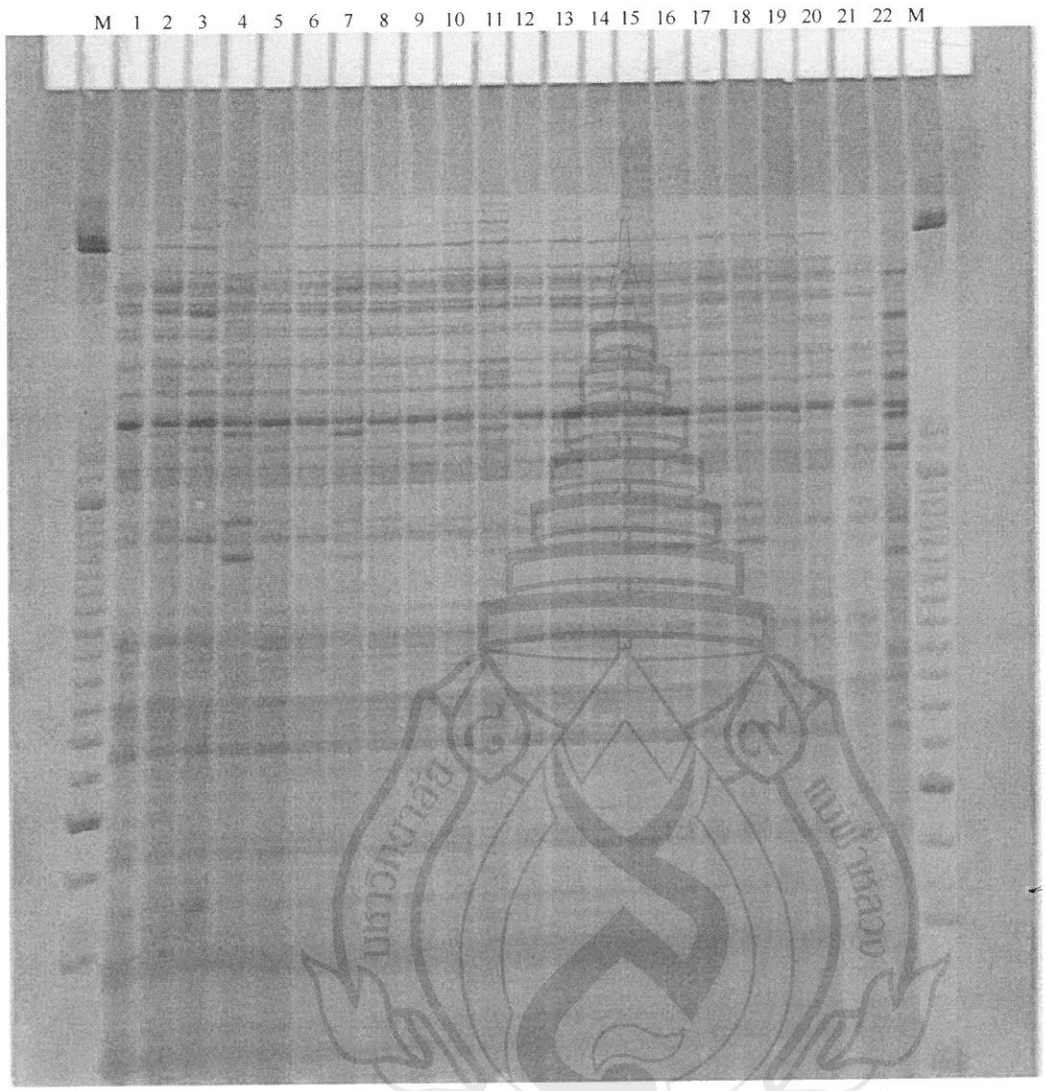

Figure B.24: Show the gel of AFLP DNA fingerprint bands of M-CTC/E-ACC

Figure B.25: Show the gel of AFLP DNA fingerprint bands of M-CTG/E-AAC

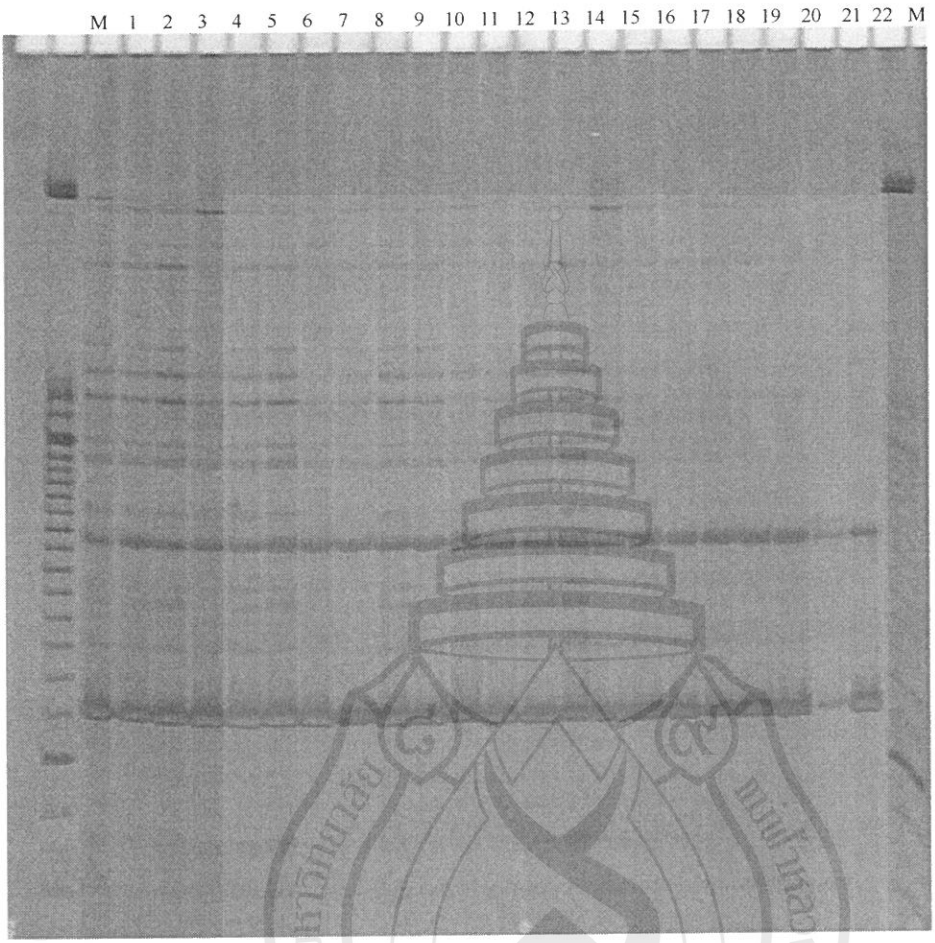

Figure B.26: Show the gel of AFLP DNA fingerprint bands of M-CTG/E-AAG

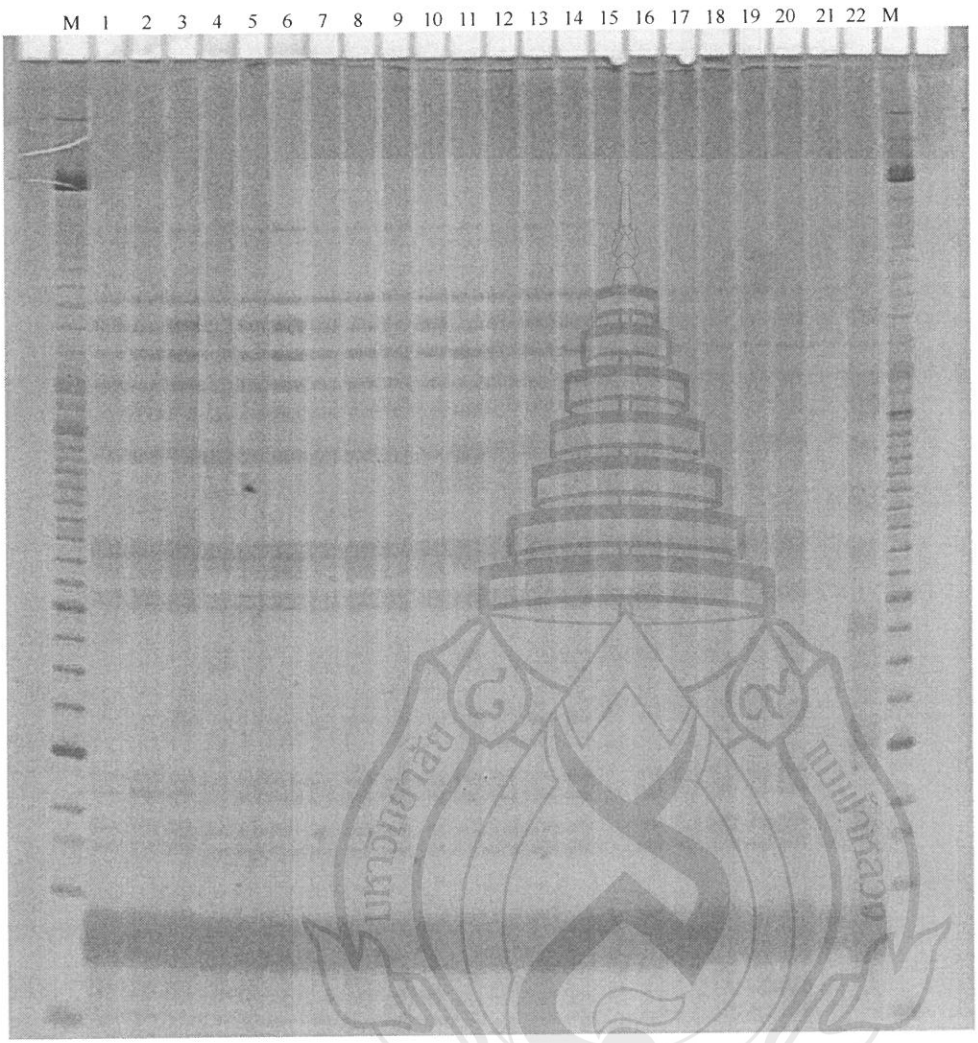

Figure B.27: Show the gel of AFLP DNA fingerprint bands of M-CTG/E-ACA

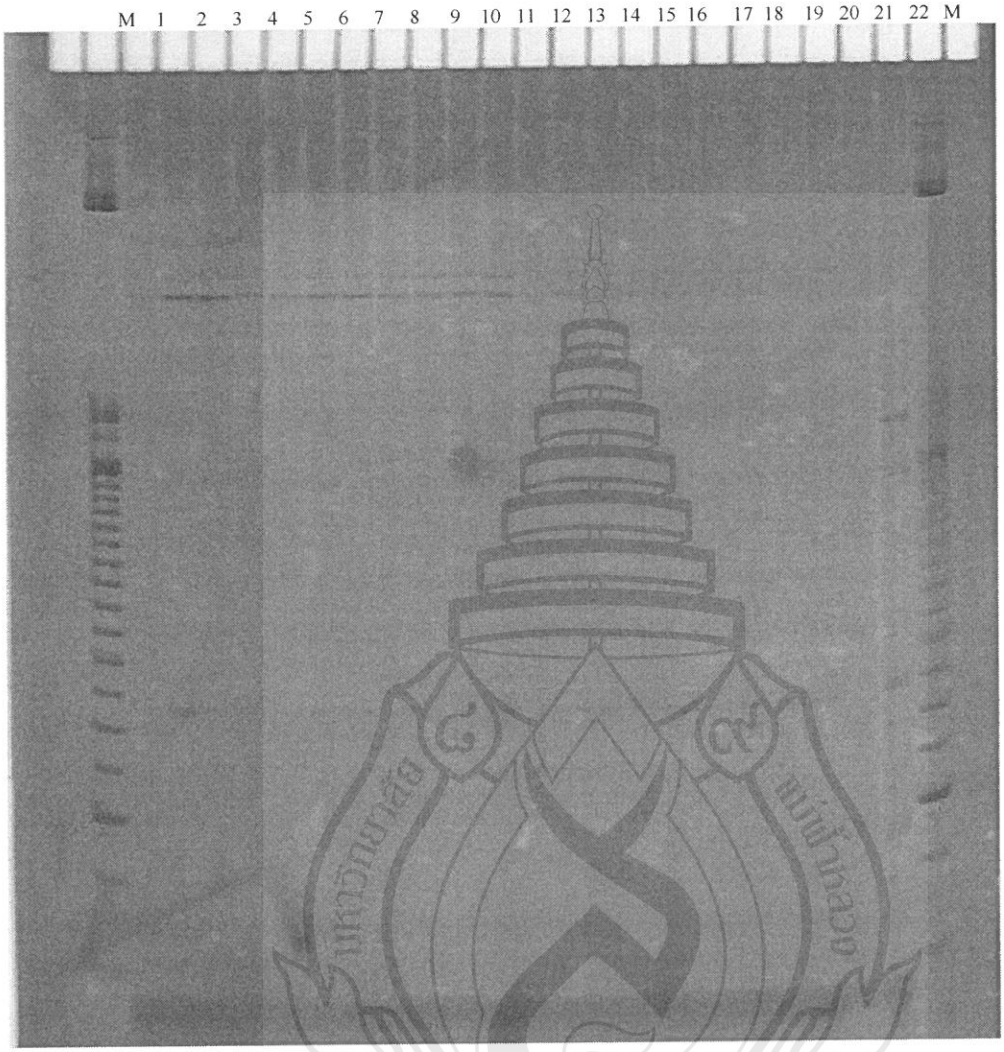

Figure B.28: Show the gel of AFLP DNA fingerprint bands of M-CTG/E-ACC

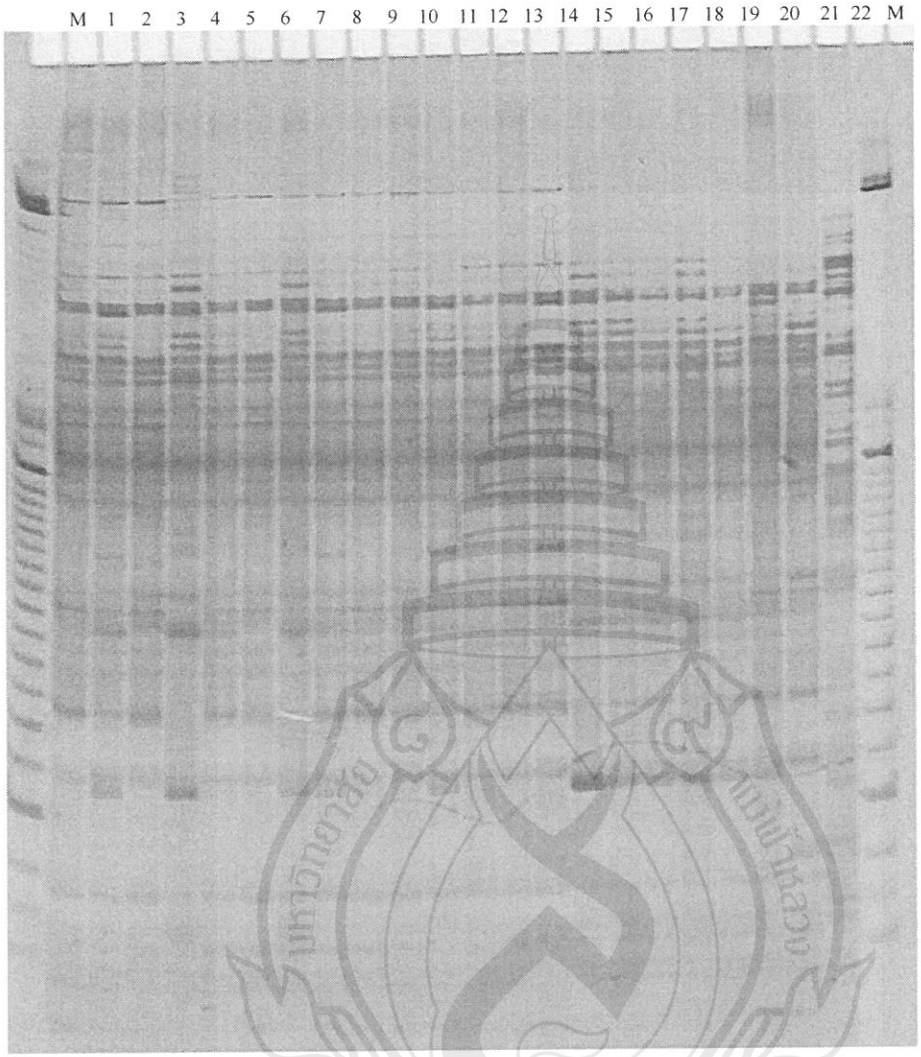

Figure B.29: Show the gel of AFLP DNA fingerprint bands of M-CTT/E-AAC

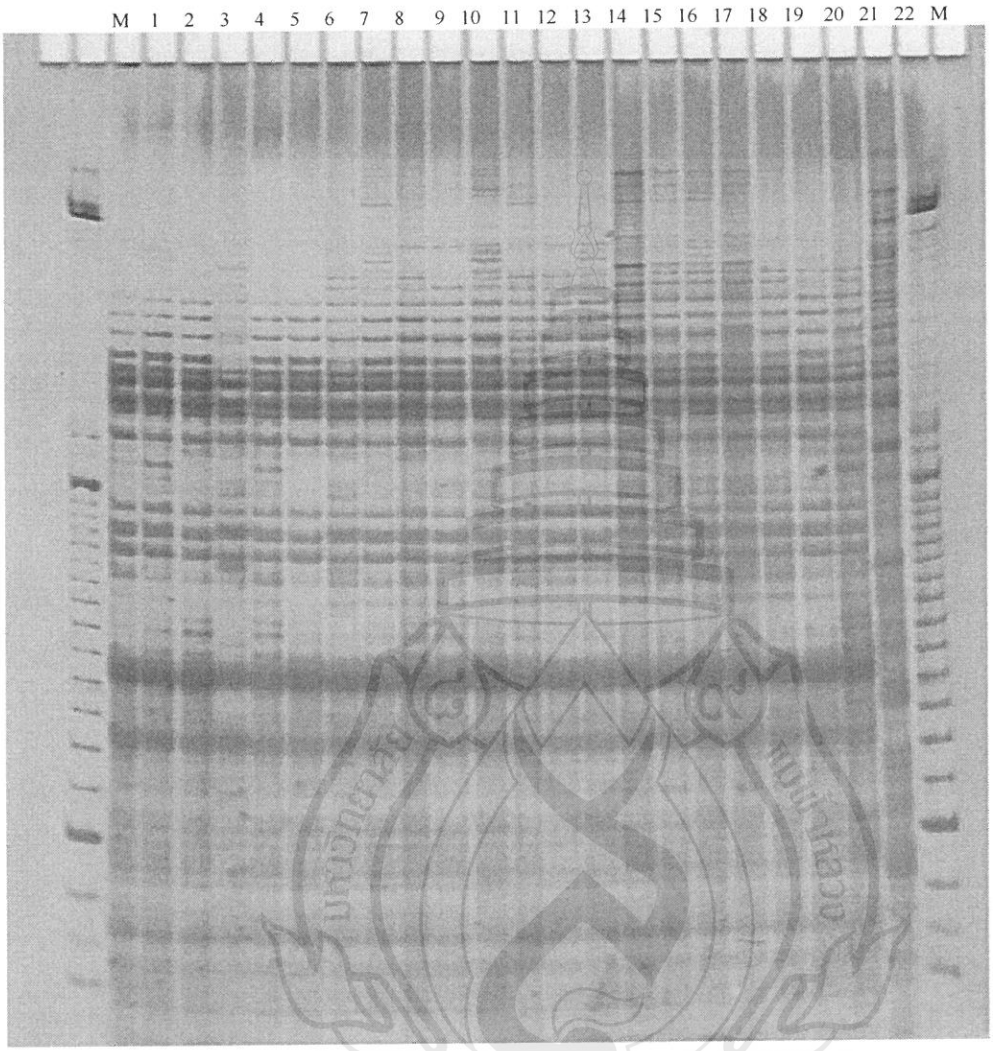

Figure B.30: Show the gel of AFLP DNA fingerprint bands of M-CTT/E-AAG

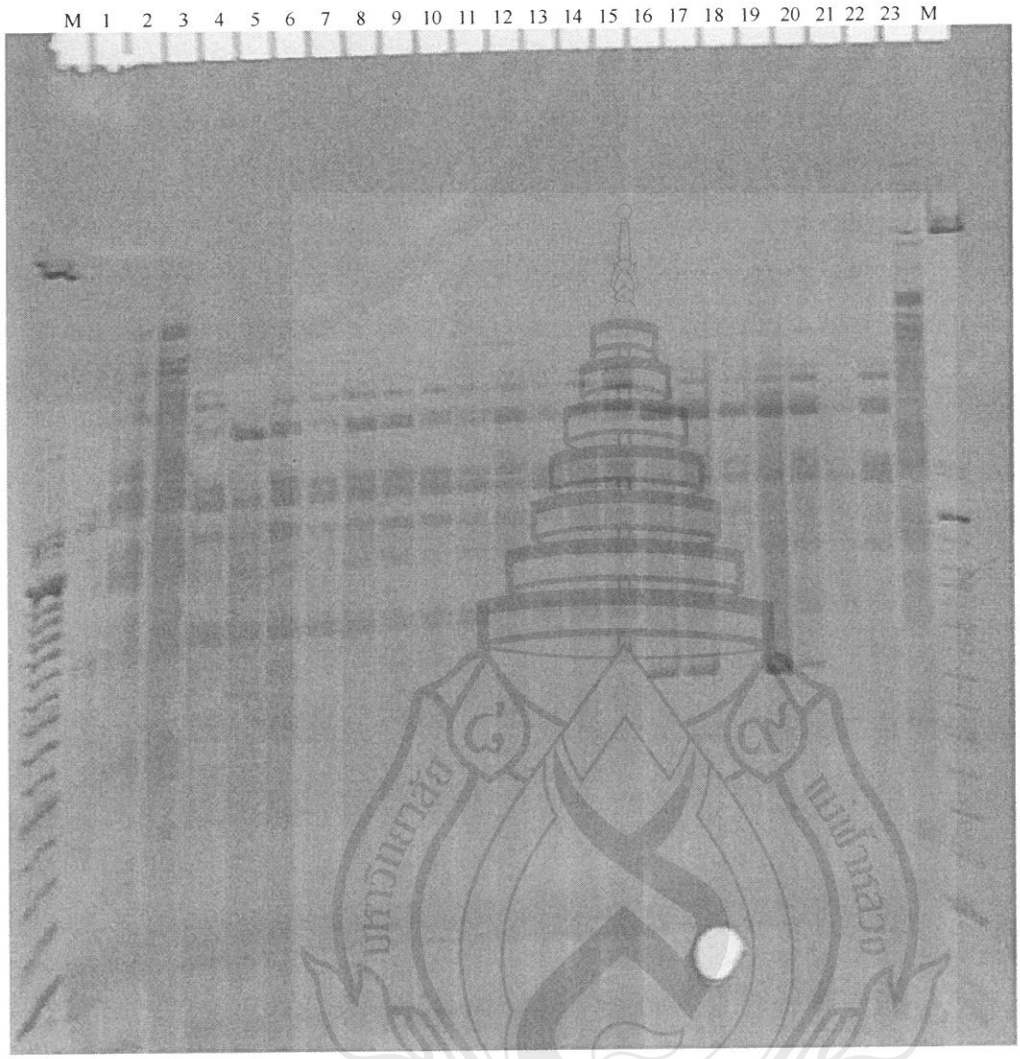

Figure B.31: Show the gel of AFLP DNA fingerprint bands of M-CTT/E-ACA

Figure B.32: Show the gel of AFLP DNA fingerprint bands of M-CTT/E-ACC

Appendix C
ANOVA of All Growth Measurements, Experiment 3.4.1

Plant Height

Between-Subjects Factors

		Value Label	N
Week	1	week 0	15
	2	week 1	15
	3	week 2	15
	4	week 3	15
	5	week 4	15
	6	week 5	15
	7	week 6	15
	8	week 7	15
	9	week 8	15
Treatment	1	Control	27
	2	IAA	27
	3	BA	27
	4	IAA/BA	27
	5	Paclobutazol	27
Replication	1	Replication 1	45
	2	Replication 2	45
	3	Replication 3	45

Tests of Between-Subjects Effects

Dependent Variable:Plant height (cm)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	11019.696[a]	46	239.559	39.389	.000
Intercept	252070.165	1	252070.165	4.145E4	.000
Time	7992.100	8	999.012	164.260	.000
Treatment	2288.409	4	572.102	94.066	.000
Replication	132.670	2	66.335	10.907	.000
Time * Treatment	606.518	32	18.954	3.116	.000
Error	535.207	88	6.082		
Total	263625.069	135			
Corrected Total	11554.904	134			

a. R Squared = .954 (Adjusted R Squared = .929)

Number of Leaves

Between-Subjects Factors

		Value Label	N
Week	1	week 0	15
	2	week 1	15
	3	week 2	15
	4	week 3	15
	5	week 4	15
	6	week 5	15
	7	week 6	15
	8	week 7	15
	9	week 8	15
Treatment	1	Control	27
	2	IAA	27
	3	BA	27
	4	IAA/BA	27
	5	Paclobutazol	27
Replication	1	Replication 1	45
	2	Replication 2	45
	3	Replication 3	45

Tests of Between-Subjects Effects

Dependent Variable: number of leaves per plant

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3437.204[a]	46	74.722	11.787	.000
Intercept	82710.435	1	82710.435	1.305E4	.000
Time	3143.625	8	392.953	61.987	.000
Treatment	107.905	4	26.976	4.255	.003
Replication	22.461	2	11.231	1.772	.176
Time * Treatment	163.213	32	5.100	.805	.753
Error	557.853	88	6.339		
Total	86705.492	135			
Corrected Total	3995.057	134			

a. R Squared = .860 (Adjusted R Squared = .787)

Leaf Area

Between-Subjects Factors

		Value Label	N
Replication	1	Replication 1	30
	2	Replication 2	30
	3	Replication 3	30
Treatment	1	Control	18
	2	IAA	18
	3	BA	18
	4	IAA/BA	18
	5	Paclobutazol	18
Week	1	week 0	15
	2	week 1	15
	3	week 2	15
	4	week 4	15
	5	week 6	15
	6	week 8	15

Tests of Between-Subjects Effects

Dependent Variable: Leaf area (cm^2/plant)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.136E8[a]	31	3664661.416	30.095	.000
Intercept	2.902E8	1	2.902E8	2.383E3	.000
Treatment	1869222.610	4	467305.653	3.838	.008
Time	1.037E8	5	2.074E7	170.332	.000
Replication	868527.386	2	434263.693	3.566	.035
Treatment * Time	7161997.373	20	358099.869	2.941	.001
Error	7062532.463	58	121767.801		
Total	4.109E8	90			
Corrected Total	1.207E8	89			

a. R Squared = .941 (Adjusted R Squared = .910)

Dry Weight

Between-Subjects Factors

		Value Label	N
Replication	1	Replication 1	30
	2	Replication 2	30
	3	Replication 3	30
Treatment	1	Control	18
	2	IAA	18
	3	BA	18
	4	IAA/BA	18
	5	Paclobutazol	18
Week	1	week 0	15
	2	week 1	15
	3	week 2	15
	4	week 4	15
	5	week 6	15
	6	week 8	15

Tests of Between-Subjects Effects

Dependent Variable: Total dry weight (g/plant)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	143157.291[a]	31	4617.977	89.885	.000
Intercept	198545.769	1	198545.769	3.865E3	.000
Treatment	1337.441	4	334.360	6.508	.000
Time	136662.634	5	27332.527	532.004	.000
Replication	1492.061	2	746.030	14.521	.000
Treatment * Time	3665.155	20	183.258	3.567	.000
Error	2979.840	58	51.377		
Total	344682.900	90			
Corrected Total	146137.131	89			

a. R Squared = .980 (Adjusted R Squared = .969)

Leaf Dry Weight

Between-Subjects Factors

		Value Label	N
Replication	1	Replication 1	30
	2	Replication 2	30
	3	Replication 3	30
Treatment	1	Control	18
	2	IAA	18
	3	BA	18
	4	IAA/BA	18
	5	Paclobutazol	18
Week	1	week 0	15
	2	week 1	15
	3	week 2	15
	4	week 4	15
	5	week 6	15
	6	week 8	15

Tests of Between-Subjects Effects

Dependent Variable:Leaf dry weight (g/plant)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	7555.061[a]	31	243.712	83.011	.000
Intercept	14020.601	1	14020.601	4.776E3	.000
Treatment	92.572	4	23.143	7.883	.000
Time	7142.965	5	1428.593	486.596	.000
Replication	52.612	2	26.306	8.960	.000
Treatment * Time	266.911	20	13.346	4.546	.000
Error	170.282	58	2.936		
Total	21745.944	90			
Corrected Total	7725.343	89			

a. R Squared = .978 (Adjusted R Squared = .966)

Stem Dry Weight

Between-Subjects Factors

		Value Label	N
Replication	1	Replication 1	30
	2	Replication 2	30
	3	Replication 3	30
Treatment	1	Control	18
	2	IAA	18
	3	BA	18
	4	IAA/BA	18
	5	Paclobutazol	18
Week	1	week 0	15
	2	week 1	15
	3	week 2	15
	4	week 4	15
	5	week 6	15
	6	week 8	15

Tests of Between-Subjects Effects

Dependent Variable: Stem dry weight (g/plant)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	32666.987[a]	31	1053.774	72.051	.000
Intercept	41297.130	1	41297.130	2.824E3	.000
Treatment	327.222	4	81.806	5.593	.001
Time	31213.106	5	6242.621	426.837	.000
Replication	359.445	2	179.722	12.288	.000
Treatment * Time	767.214	20	38.361	2.623	.002
Error	848.268	58	14.625		
Total	74812.386	90			
Corrected Total	33515.256	89			

a. R Squared = .975 (Adjusted R Squared = .961)

Root Dry Weight

Between-Subjects Factors

		Value Label	N
Replication	1	Replication 1	30
	2	Replication 2	30
	3	Replication 3	30
Treatment	1	Control	18
	2	IAA	18
	3	BA	18
	4	IAA/BA	18
	5	Paclobutazol	18
Week	1	week 0	15
	2	week 1	15
	3	week 2	15
	4	week 4	15
	5	week 6	15
	6	week 8	15

Tests of Between-Subjects Effects

Dependent Variable: Root dry weight (g/plant)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	12416.462[a]	31	400.531	32.878	.000
Intercept	15365.877	1	15365.877	1.261E3	.000
Treatment	106.953	4	26.738	2.195	.081
Time	11781.351	5	2356.270	193.417	.000
Replication	154.441	2	77.220	6.339	.003
Treatment * Time	373.717	20	18.686	1.534	.105
Error	706.576	58	12.182		
Total	28488.915	90			
Corrected Total	13123.038	89			

a. R Squared = .946 (Adjusted R Squared = .917)

Crop Growth Rate

Between-Subjects Factors

		Value Label	N
Treatment	1	Control	15
	2	IAA	15
	3	BA	15
	4	IAA/BA	15
	5	Paclobutazol	15
Week	2	week 1	15
	3	week 2	15
	4	week 4	15
	5	week 6	15
	6	week 8	15
Replication	1	Replication 1	25
	2	Replication 2	25
	3	Replication 3	25

Tests of Between-Subjects Effects

Dependent Variable:Crop growth rate (g/week)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	999.515[a]	24	41.646	12.703	.000
Intercept	5023.697	1	5023.697	1.532E3	.000
Treatment	130.683	4	32.671	9.965	.000
Time	751.270	4	187.818	57.289	.000
Treatment * Time	117.562	16	7.348	2.241	.015
Error	163.920	50	3.278		
Total	6187.132	75			
Corrected Total	1163.435	74			

a. R Squared = .859 (Adjusted R Squared = .791)

Net Assimilation Rate

Between-Subjects Factors

		Value Label	N
Treatment	1	Control	15
	2	IAA	15
	3	BA	15
	4	IAA/BA	15
	5	Paclobutazol	15
Week	2	week 1	15
	3	week 2	15
	4	week 4	15
	5	week 6	15
	6	week 8	15
Replication	1	Replication 1	25
	2	Replication 2	25
	3	Replication 3	25

Tests of Between-Subjects Effects

Dependent Variable:Net assimilation rate (g/week)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.936[a]	24	.081	47.294	.000
Intercept	1.726	1	1.726	1.012E3	.000
Treatment	.016	4	.004	2.388	.063
Time	1.903	4	.476	278.994	.000
Treatment * Time	.016	16	.001	.595	.872
Error	.085	50	.002		
Total	3.747	75			
Corrected Total	2.021	74			

a. R Squared = .958 (Adjusted R Squared = .938)

Concentration of GA in the plant tissue

Between-Subjects Factors

		Value Label	N
Replication	1	Replication 1	5
	2	Replication 2	5
	3	Replication 3	5
Treatment	1	Control	3
	2	IAA	3
	3	BA	3
	4	IAA/BA	3
	5	Paclobutazol	3

Tests of Between-Subjects Effects

Dependent Variable:Amount of GA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.137E6[a]	6	189543.084	3.153	.068
Intercept	429889.270	1	429889.270	7.151	.028
Replication	287898.797	2	143949.398	2.394	.153
Treatment	849359.710	4	212339.927	3.532	.061
Error	480936.823	8	60117.103		
Total	2048084.600	15			
Corrected Total	1618195.330	14			

a. R Squared = .703 (Adjusted R Squared = .480)

Table D.1 Summary of the ANOVA of plant height (cm) from the whole experimental period of experiment 1

Source of variation	df	Mean Square	F	Significance (P)
Time	11	309404.9	1138.9	0
Type of inhibitors	1	583.396	58.82	0
Concentration	3	5485.172	553.01	0
Replication	2	243.692	24.57	0
Type of inhibitors x concentration	3	109.009	10.99	0
Error	270	9.919		

Table D.2 Summary of the ANOVA of plant height (cm) of each individual sampling week of experiment 1

Source of variation	df	F of Each Sampling Week											
		0	1	2	3	4	6	9	12	15	20	26	32
Types of inhibitor	1	1.61^{ns}	1.87^{ns}	14.39**	3.41^{ns}	0.27^{ns}	41.62**	11.30**	36.08**	3.10^{ns}	5.23*	6.64*	6.36*
Concentrations	3	0.31^{ns}	7.13**	16.74**	59.44**	152.70**	223.81**	81.43**	146.60**	59.20**	85.40**	101.34**	94.29**
Replication	2	1.49	4.32	3.90	3.90	18.51	23.04	10.16	10.75	2.02	4.40	1.054	0.84
Types of inhibitor x Concentrations	3	0.2^{ns}	0.32^{ns}	1.94^{ns}	0.40^{ns}	0.47^{ns}	11.23**	4.31*	7.40**	0.73^{ns}	1.87^{ns}	2.10^{ns}	1.86^{ns}
Error	14												
Total	23												

Note: * = significant at 0.05; ** = significant at 0.01; ns = non significant

Table D.3 Summary of the ANOVA of number of nodes from the whole experimental period of experiment 1

Source of variation	df	Mean Square	F	Significance (P)
Time	11	572.3	126.1	0
Type of inhibitors	1	641.9	7.2	0.018
Concentration	3	616.9	8.1	0
Replication	2	151.7	7.8	0.005
Type of inhibitors x concentration	3	79.3	1.9	0.174
Error	270			

Table D.4 Summary of the ANOVA of number of nodes of individual each sampling week of experiment 1

Source of variation	df	F of Each Sampling Week										
	0	1	2	3	4	6	9	12	15	20	26	32
Types of inhibitor	1 25^{ns}	.84^{ns}	75^{ns}	.05**	97**	.28**	.04^{ns}	.99^{ns}	.40^{ns}	1^{ns}	24^{ns}	.14^{ns}
Concentrations	3 51^{ns}	.17**	81*	.37**	63*	.03**	.58*	.72**	.13^{ns}	6**	99**	.53*
Replication	2 06	.01	01	.52	94	.59	.69	.91	.05	2	.41	.57
Types of inhibitor x Concentrations	3.59^{ns}	4.35*	0.89^{ns}	16.39**	2.53^{ns}	1.55^{ns}	0.59^{ns}	0.42^{ns}	0.17^{ns}	0.69^{ns}	1.58^{ns}	0.84^{ns}
Error	14											
Total	23											

Note: * = significant at 0.05; ** = significant at 0.01; ns = non significant

Table D.5 Summary of the ANOVA of average internodes length (cm) from the whole experimental period of experiment 1

Source of variation	df	Mean Square	F	Significance (P)
Time	11	1.7	51.9	0
Type of inhibitors	1	0.95	23	0
Concentration	3	1.987	48.406	0
Replication	2	0.185	4.518	0.031
Type of inhibitors x concentration	3	0.115	2.809	0.078
Error	270	0.041		

Table D.6 Summary of the ANOVA of average internodes length (cm) of each individual sampling week of experiment 1.

Source of variation	df	F of Each Sampling Week										
		0	1	2	3	4	6	9	12	15	20	26	32
Types of inhibitor	1	.7^{ns}	1^{ns}	.85**	.95**	.70**	.39**	6^{ns}	.31^{ns}	.24^{ns}	.50^{ns}	.13^{ns}	.01^{ns}
Concentrations	3	6^{ns}	.2*	.62**	.46**	.65**	.76**	7**	.98**	.28**	.93**	.76**	.82**
Replication	2	0	0	.38	.82	1.06	.54	6	.52	.94	.22	.64	.00
x	3	.07^{ns}	0.11^{ns}	5.72^{ns}	5.23*	1.47^{ns}	2.73^{ns}	0.67^{ns}	0.33^{ns}	0.84^{ns}	0.50^{ns}	0.22^{ns}	1.43^{ns}
Error	14												
Total	23												

Note: * = significant at 0.05; ** = significant at 0.01; ns = non significant

Table D.7 Summary of the ANOVA of number of branches from the whole experimental period of experiment 1

Source of variation	df	Mean Square	F	Significance (P)
Time	11	6.9	27.8	0
Type of inhibitors	1	2.2	7.5	0.02
Concentration	3	32.8	113.4	0
Replication	2	1.7	5.9	0.014
Type of inhibitors x concentration	3	0.6	2.0	0.161
Error	270	0.3		

Table D.8 Summary of the ANOVA of number of branches of each individual sampling week of experiment 1.

Source of variation	df	F of Each Sampling Week											
		0	1	2	3	4	6	9	12	15	20	26	32
Types of inhibitor	1	0.00	0.00	0.00	4.00^{ns}	7.60*	1.47^{ns}	1.80^{ns}	1.80^{ns}	0.84^{ns}	1.21^{ns}	3.31^{ns}	2.03^{ns}
Concentrations	3	0.00	0.00	0.00	32.00**	23.02**	43.71**	32.20**	32.20**	34.22**	47.47**	45.56**	27.92**
Replication	2	0.00	0.00	0.00	.00	0.30	.78	.60	.60	.48	2.73	7.73	2.03
Types of inhibitor x Concentrations	3	0.00	0.00	0.00	4.00*	1.11^{ns}	0.49^{ns}	0.73^{ns}	0.73^{ns}	0.28^{ns}	0.40^{ns}	1.35^{ns}	0.82^{ns}
Error	14												
Total	23												

Note: * = significant at 0.05; ** = significant at 0.01; ns = non significant

Table D.9 Summary of the ANOVA of total dry weight (g) from the whole experimental period of experiment 1

Source of variation	df	Mean Square	F	Significance (P)
Time	11	190316.06	987.1	0
Type of inhibitors	1	913.283	17.917	0.001
Concentration	3	4559.477	89.449	0
Replication	2	757.436	14.86	0
Type of inhibitors x concentration	3	858.64	16.845	0
Error	270	50.973		

Table D.10 Summary of the ANOVA of total dry weight (g) of each individual sampling week of experiment 1

Source of variation	df	F of Each Sampling Week											
		0	1	2	3	4	6	9	12	15	20	26	32
Types of inhibitor	1	2.37^{ns}	0.36^{ns}	0.43^{ns}	21.86**	103.29**	76.62**	3.04^{ns}	3.70^{ns}	0.02^{ns}	0.84^{ns}	1.20^{ns}	0.01^{ns}
Concentrations	3	2.65^{ns}	11.17**	9.50**	40.17**	103.61**	98.67**	22.11**	21.07**	25.85**	31.06**	8.72**	4.97*
Replication	2	.2	.89	.75	8.15	8.56	12.30	1.96	.89	.05	4.06	7.71	37
Types of inhibitor x Concentrations	3	1.36^{ns}	13.49**	0.43^{ns}	8.83**	32.52**	15.44**	2.39^{ns}	4.96*	2.08^{ns}	1.04^{ns}	1.03^{ns}	0.51^{ns}
Error	14												
Total	23												

Note: * = significant at 0.05; ** = significant at 0.01; ns = non significant

Table D.11 Summary of the ANOVA of stem diameter (cm) from the whole experimental period of experiment 1

Source of variation	df	Mean Square	F	Significance (P)
Time	11	36.5	641.7	0
Type of inhibitors	1	807.6	16.3	0.001
Concentration	3	2579.7	52.0	0
Replication	2	511.9	10.3	0.002
Type of inhibitors x concentration	3	413.0	8.3	0.002
Error	270	49.6		

Table D.12 Summary of the ANOVA of stem diameter (cm) of each individual sampling week of experiment 1

Source of variation	df	F of Each Sampling Week											
		0	1	2	3	4	6	9	12	15	20	26	32
Types of inhibitor	1	0.79^{ns}	66.57**	81.91**	49.21**	137.84**	17.91**	74.65**	74.65**	26.67**	7.64*	8.19*	40.04**
Concentrations	3	0.22^{ns}	24.89**	32.29**	19.92**	45.95**	9.39**	16.82**	70.06**	47.66**	28.21**	31.48**	137.34**
Replication	2	.68	31	'49	13	86	8.98	10	10	.46	'.52	'.52	2.09
Types of inhibitor x Concentrations	3	0.18^{ns}	10.27**	12.10**	7.62**	20.35**	2.84^{ns}	10.59**	10.59**	3.78*	0.96^{ns}	1.02^{ns}	4.65*
Error	14												
Total	23												

Note: * = significant at 0.05; ** = significant at 0.01; ns = non significant

Table D.13 Summary of the ANOVA of shoot dry weight (g) from the whole experimental period of experiment 1

Source of variation	df	Mean Square	F	Significance (P)
Time	11	111917	737.4	0
Type of inhibitors	1	826	16.015	0.001
Concentration	3	3358.884	65.142	0
Replication	2	442.703	8.586	0.004
Type of inhibitors x concentration	3	518.516	10.056	0.001
Error	270	51.563		

Table D.14 Summary of the ANOVA of shoot dry weight (g) of each individual sampling week of experiment 1

Source of variation	df	F of Each Sampling Week											
		0	1	2	3	4	6	9	12	15	20	26	32
Types of inhibitor	1	2.09^{ns}	0.32^{ns}	0.13^{ns}	19.65**	87.37**	70.29**	1.82^{ns}	1.22^{ns}	0.47^{ns}	2.06^{ns}	2.72^{ns}	0.05^{ns}
Concentrations	3	2.52^{ns}	11.69**	7.93**	31.45**	83.53**	79.30**	14.26**	6.62**	16.14**	17.84**	3.52*	1.81^{ns}
Replication	2	.69	.24	6.27	6.58	6.90	2.37	1.93	.35	.18	2.73	.59	.36
Types of inhibitor x Concentrations	3	1.27^{ns}	13.49**	0.38^{ns}	7.24**	25.74**	14.41**	0.58^{ns}	0.30^{ns}	1.40^{ns}	0.92^{ns}	1.07^{ns}	0.30^{ns}
Error	14												
Total	23												

Note: * = significant at 0.05; ** = significant at 0.01; ns = non significant

Table D.15 Summary of the ANOVA of stem dry weight (g) from the whole experimental period of experiment 1

Source of variation	df	Mean Square	F	Significance (P)
Time	11	66827.8	517.7	0
Type of inhibitors	1	808	16.269	0.001
Concentration	3	2579.713	51.97	0
Replication	2	511.928	10.313	0.002
Type of inhibitors x concentration	3	412.978	8.32	0.002
Error	270			

Table D.16 Summary of the ANOVA of stem dry weight (g) of each individual sampling week of experiment 1

Source of variation	df	F of Each Sampling Week											
		0	1	2	3	4	6	9	12	15	20	26	32
Types of inhibitor	1	2.28^{ns}	0.42^{ns}	0.03^{ns}	21.95**	91.24**	70.26**	1.65^{ns}	1.80^{ns}	0.92^{ns}	3.78^{ns}	4.20^{ns}	0.07^{ns}
Concentrations	3	2.53^{ns}	11.44**	7.39**	28.84**	80.48**	73.31**	11.75**	.35*	10.00**	34.72**	6.51**	2.50^{ns}
Replication	2	.86	.33	.23	8.23	.23	2.89	.46	.02	0.16	0.01	.66	.53
Types of inhibitor x Concentrations	3	1.31^{ns}	13.00**	0.60^{ns}	7.38**	24.66**	12.16**	1.26^{ns}	0.21^{ns}	0.87^{ns}	0.93^{ns}	1.83^{ns}	0.53^{ns}
Error	14												
Total	23												

Note: * = significant at 0.05; ** = significant at 0.01; ns = non significant

Table D.17 Summary of the ANOVA of leaf dry weight (g) from the whole experimental period of experiment 1

Source of variation	df	Mean Square	F	Significance (P)
Time	11	5865.7	1222.9	0
Type of inhibitors	1	0.099	0.032	0.86
Concentration	3	74.593	24.412	0
Replication	2	4.397	1.439	0.27
Type of inhibitors x concentration	3	7.475	2.446	0.107
Error	270	3.056		

Table D.18 Summary of the ANOVA of leaf dry weight (g) of each individual sampling week of experiment 1

Source of variation	df	F of Each Sampling Week											
		0	1	2	3	4	6	9	12	15	20	26	32
Types of inhibitor	1	0.22^{ns}	0.16^{ns}	1.28^{ns}	0.35^{ns}	5.59*	.89*	0.39^{ns}	0.08^{ns}	0.40^{ns}	0.17^{ns}	0.40^{ns}	0.68^{ns}
Concentrations	3	1.21^{ns}	7.67**	9*	28.57**	92.75**	17.88**	5.38*	.55*	.80*	1.25^{ns}	0.74^{ns}	0.15^{ns}
Replication	2	.53	.27	7	.55	0.44	.45	0.24	.30	.60	.26	.26	.69
Types of inhibitor x Concentrations	3	0.43^{ns}	0.48^{ns}	0.55^{ns}	1.62^{ns}	10.31**	10.72**	3.06^{ns}	1.18^{ns}	0.83^{ns}	0.68^{ns}	0.12^{ns}	0.11^{ns}
Error	14												
Total	23												

Note: * = significant at 0.05; ** = significant at 0.01; ns = non significant

Table D.19 Summary of the ANOVA of number of leaves from the whole experimental period of experiment 1

Source of variation	df	Mean Square	F	Significance (P)
Time	11	572.3	126.1	0
Type of inhibitors	1	641.9	7.2	0.018
Concentration	3	616.9	8.1	0
Replication	2	151.7	7.8	0.005
Type of inhibitors x concentration	3	79.3	1.9	0.174
Error	270			

Table D.20 Summary of the ANOVA of number of leaves of each individual sampling week of experiment 1

Source of	df	F of Each Sampling Week											
		0	1	2	3	4	6	9	12	15	20	26	32
Types of inhibitor	1	0.25^{ns}	3.84^{ns}	.75^{ns}	49.05**	9.97**	11.28**	3.04^{ns}	1.99^{ns}	0.40^{ns}	0.01^{ns}	0.24^{ns}	1.14^{ns}
Concentrations	3	0.61^{ns}	11.17**	.81*	66.37**	5.63*	6.03**	3.58*	5.72**	1.13^{ns}	9.76**	7.99**	4.53*
Replication	2	.06	1.01	.01	0.52	.94	1.59	3.69	5.91	9.05	.92	.41	2.57
x Concentration	3	1.59^{ns}	4.35*	0.89^{ns}	16.39**	2.53^{ns}	1.55^{ns}	0.59^{ns}	0.42^{ns}	0.17^{ns}	0.69^{ns}	1.58^{ns}	0.84^{ns}
Error	14												
Total	23												

Note: * = significant at 0.05; ** = significant at 0.01; ns = non significant

Table D.21 Summary of the ANOVA of total leaf area (cm^2) from the whole experimental period of experiment 1

Source of variation	df	Mean Square	F	Significance (P)
Time	11	1.2	217.1	0
Type of inhibitors	1	2130534.788	2.627	0.127
Concentration	3	3227173.514	3.978	0.03
Replication	2	2650785.373	3.268	0.068
Type of inhibitors x concentration	3	1141614.753	1.407	0.282
Error	270	811158		

Table D.22 Summary of the ANOVA of total leaf area (cm^2) of each individual sampling week of experiment 1

Source of variation	df	F of Each Sampling Week											
		0	1	2	3	4	6	9	12	15	20	26	32
Types of inhibitor	1	0.96^{ns}	0.86^{ns}	0.08^{ns}	0.01^{**}	0.01^{**}	0.47^{ns}	0.86^{ns}	0.83^{ns}	0.45^{ns}	0.60^{ns}	0.86^{ns}	0.78^{ns}
Concentrations	3	0.02^{*}	0.04^{*}	0.02^{*}	0.01^{**}	0.01^{**}	0.01^{**}	0.08^{ns}	0.19^{ns}	0.69^{ns}	0.98^{ns}	0.88^{ns}	0.97^{ns}
Replication	2	0.01	0.96	0.43	0.13	0.09	0.02	0.15	0.05	0.01	0.01	0.16	0.03
Types of inhibitor x Concentrations	3	0.88^{ns}	0.92^{ns}	0.39^{ns}	0.01^{*}	0.01^{**}	0.24^{ns}	0.36^{ns}	0.55^{ns}	0.86^{ns}	0.62^{ns}	0.44^{ns}	0.53^{ns}
Error	14												
Total	23												

Note: * = significant at 0.05; ** = significant at 0.01; ns = non significant

Table D.23 Summary of the ANOVA of root dry weight (g) from the whole experimental period of experiment 1

Source of variation	df	Mean Square	F	Significance (P)
Time	11	10615.9	1508.7	0
Type of inhibitors	1	11.508	3.317	0.09
Concentration	3	61.455	17.713	0
Replication	2	41.772	12.04	0.001
Type of inhibitors x concentration	3	28.265	8.147	0.002
Error	270	3.469		

Table D.24 Summary of the ANOVA of root dry weight (g) of each individual sampling week of experiment 1

Source of variation	df	F of Each Sampling Week											
		0	1	2	3	4	6	9	12	15	20	26	32
Types of inhibitor	1	2.12^{ns}	0.61^{ns}	4.49^{ns}	1.78^{ns}	23.07^{**}	23.87^{**}	22.20^{**}	5.13^{*}	3.61^{ns}	2.96^{ns}	0.43^{ns}	0.25^{ns}
Concentrations	3	2.12^{ns}	2.15^{ns}	22.73^{**}	28.43^{**}	50.68^{**}	96.18^{**}	144.97^{**}	11.22^{**}	20.49^{**}	18.04^{**}	3.31^{ns}	2.11^{ns}
Replication	2	.16	.08	3.87	3.87	3.87	.88	3.88	.98	2.34	0.30	.69	5.65
Types of inhibitor x Concentrations	3	1.42^{ns}	6.41^{**}	0.71^{ns}	3.74^{*}	17.04^{**}	4.00^{*}	127.84^{**}	3.97^{*}	2.19^{ns}	0.77^{ns}	0.15^{ns}	0.12^{ns}
Error	14												
Total	23												

Note: * = significant at 0.05; ** = significant at 0.01; ns = non significant

Table D.25 Summary of the ANOVA of net assimilation rate (g/week) from the whole experimental period of experiment 1

Source of variation	df	Mean Square	F	Significance (P)
Time	11	0.009	33.41	0
Type of inhibitors	1	0.019	8.916	0.01
Concentration	3	0.005	2.191	0.135
Replication	2	0.022	10.394	0.002
Type of inhibitors x concentration	3	0.01	4.476	0.021
Error	270	0.002		

Table D.26 Summary of the ANOVA of net assimilation rate (g/week) of each individual sampling week

Source of variation	df	F of Each Sampling Week											
		0	1	2	3	4	6	9	12	15	20	26	32
Types of inhibitor	1	0	0.36^{ns}	0.56^{ns}	6.49*	15.16**	14.01**	11.39**	11.25**	11.27**	11.18**	11.14**	11.25**
Concentrations	3	0	.72*	2.64^{ns}	2.97^{ns}	2.90^{ns}	1.92^{ns}	1.98^{ns}	1.89^{ns}	1.89^{ns}	1.74^{ns}	1.74^{ns}	1.75^{ns}
Replication	2	0	.29	.05	.17	.34	.05	0.77	.25	.82	9.33	9.26	9.31
Types of inhibitor x Concentrations	3	0	5.18*	1.91^{ns}	4.16*	6.21**	5.04*	4.94*	4.80*	4.68*	4.61*	4.60*	4.62*
Error	14												
Total	23												

Note: * = significant at 0.05; ** = significant at 0.01; ns = non significant

Table D.27 Summary of the ANOVA of crop growth rate (g/week) from the whole experimental period of experiment 1

Source of variation	df	Mean Square	F	Significance (P)
Time	11	372.1	206.9	0
Type of inhibitors	1	153.875	62.957	0
Concentration	3	146.359	59.882	0
Replication	2	64.002	26.186	0
Type of inhibitors x concentration	3	52.852	21.624	0
Error	270	2.444		

Table D.28 Summary of the ANOVA of crop growth rate (g/week) of each individual sampling week of experiment 1

Source of variation	df	F of Each Sampling Week											
		0	1	2	3	4	6	9	12	15	20	26	32
Types of inhibitor	1	0	0.04^{ns}	0.01^{ns}	25.01**	102.64**	96.78**	58.88**	110.64**	102.60**	89.52**	85.84**	102.73**
Concentrations	3	0	8.78**	.24*	28.87**	80.70**	81.36**	94.54**	124.75**	89.66**	60.78**	60.80**	72.53**
Replication	2	0	5.95	.61	1.96	2.28	5.91	3.75	2.17	9.09	.97	.07	1.77
Types of inhibitor x Concentrations	3	0	12.92**	0.53^{ns}	9.64**	32.13**	24.96**	25.14**	41.11**	31.47**	26.01**	25.07**	29.32**
Error	14												
Total	23												

Note: * = significant at 0.05; ** = significant at 0.01; ns = non significant

Table D.29 Summary of the ANOVA of relative growth rate (g/week) from the whole experimental period of experiment 1

Source of variation	df	Mean Square	F	Significance (P)
Time	11	18.97	67.53	0
Type of inhibitors	1	14.514	10.450	0.006
Concentration	3	0.875	0.63	0.608
Replication	2	122.491	88.191	0
Type of inhibitors x concentration	3	7.059	5.083	0.014
Error	270	1.389		

Table D.30 Summary of the ANOVA of relative growth rate (g/week) of each individual sampling week of experiment 1

Source of variation	df	F of Each Sampling Week											
		0	1	2	3	4	6	9	12	15	20	26	32
Types of inhibitor	1	0	1.39^{ns}	1.75^{ns}	11.48**	21.06**	16.99**	11.62**	11.49**	12.09**	11.99**	11.91**	11.95**
Concentrations	3	0	1.35^{ns}	0.57^{ns}	1.53^{ns}	2.73^{ns}	1.66^{ns}	0.96^{ns}	0.68^{ns}	0.61^{ns}	0.55^{ns}	0.55^{ns}	0.56^{ns}
Replication	2	0	.66	.82	.22	9.95	6.02	.4	9.93	0.75	9.70	.08	8.97
Types of inhibitor x Concentrations	3	0	4.44*	2.31^{ns}	5.62*	8.39**	6.17**	5.44*	5.04*	5.03*	4.93*	4.90*	4.90*
Error	14												
Total	23												

Note: * = significant at 0.05; ** = significant at 0.01; ns = non significant

Table D.31 Summary of the ANOVA of leaf area ratio (cm^2/week) from the whole experimental period of experiment 1

Source of variation	df	Mean Square	F	Significance (P)
Time	11	3006.01	97.98	0
Type of inhibitors	1	1374.726	26.495	0
Concentration	3	241.717	4.659	0.018
Replication	2	92.938	1.791	0.203
Type of inhibitors x concentration	3	355.275	6.847	0.005
Error	270	51.886		

Table D.32 Summary of the ANOVA of leaf area ratio (cm^2/week) of each individual sampling week of experiment 1

Source of variation	df	F of Each Sampling Week										
	0	1	2	3	4	6	9	12	15	20	26	32
Types of inhibitor	1 0	0.11^{ns}	2.23^{ns}	35.82**	76.31**	45.43**	15.11**	2.70^{ns}	2.46^{ns}	2.65^{ns}	2.01^{ns}	2.12^{ns}
Concentrations	3 0	9.88**	12.27**	8.43**	.82*	.65*	6.91**	5*	3.69*	1.62^{ns}	0.55^{ns}	0.07^{ns}
Replication	2 0	0.75	.01	.66	.41	.93	.10	6	5.57	6	2	5
Types of inhibitor x Concentrations	3 0	1.30^{ns}	4.07*	5.81**	8.68**	6.39**	7.37**	4.34*	2.68^{ns}	2.29^{ns}	2.09^{ns}	1.54^{ns}
Error	14											
Total	23											

Note: * = significant at 0.05; ** = significant at 0.01; ns = non significant

Table D.33 Summary of the ANOVA of leaf area duration (cm^2/week) from the whole experimental period of experiment 1

Source of variation	df	Mean Square	F	Significance (P)
Time	11	5.283546062	1952.6	0
Type of inhibitors	1	6283459.325	1.252	0.282
Concentration	3	6523530.427	1.299	0.314
Replication	2	19750000	3.933	0.044
Type of inhibitors x concentration	3	7548226	1.503	0.257
Error	270	5020730		

Table D.34 Summary of the ANOVA of leaf area duration (cm^2/week) of each individual sampling week of experiment 1

Source of variation	df	F of Each Sampling Week											
		0	1	2	3	4	6	9	12	15	20	26	32
Types of inhibitor	1	0	0.02^{ns}	1.13^{ns}	.96*	14.88**	3.92^{ns}	0.25^{ns}	0.01^{ns}	0.34^{ns}	0.89^{ns}	0.18^{ns}	0.04^{ns}
Concentrations	3	0	69*	3.12^{ns}	11.09**	24.85**	18.22**	1.79^{ns}	3.50*	1.34^{ns}	0.39^{ns}	0.08^{ns}	0.12^{ns}
Replication	2	0	.52	.27	1.74	2.67	3.88	4.79	3.69	7.22	2.99	5.46	2.80
Types of inhibitor x Concentrations	3	0	0.06^{ns}	0.20^{ns}	2.15^{ns}	7.88**	1.50^{ns}	1.28^{ns}	1.49^{ns}	0.63^{ns}	0.46^{ns}	1.21^{ns}	0.94^{ns}
Error	14												
Total	23												

Note: * = significant at 0.05; ** = significant at 0.01; ns = non significant

Table D.35 Summary of the ANOVA of GA concentration (µg/g dry matter) from the whole experimental period of experiment 1

Source of variation	df	Mean Square	F	Significance (P)
Time	11	623311.3	205.1	0
Type of inhibitors	1	5375.609	169.578	0
Concentration	3	320606.53	10110	0
Replication	2	46.536	1.468	0.264
Type of inhibitors x concentration	3	837.767	26.428	0
Error	270	31.7		

Table D.36 Summary of the ANOVA of GA concentration (µg/g dry matter) of each individual sampling week of experiment 1

Source of variation	df	F of Each Sampling Week											
		0	1	2	3	4	6	9	12	15	20	26	32
Types of inhibitor	1	34.93**	4456.00**	772.49**	39.04**	6.59*	0.37ns	0.42ns	0.37ns	536.67**	24.18**	33.26**	11.67**
Concentrations	3	165.67**	12110.00**	12150.00**	541.37**	85260.00**	2041.00**	411.12**	1062.00**	5391.00**	2781.00**	979.27**	766.79**
Replication	2	0.61	1.67	0.91	0.65	1.86	0.96	0.39	2.14	1.71	0.53	2.98	1.38
Types of inhibitor x Concentrations	3	10.13**	24670.00**	97.13**	4.64*	207.07**	53.70**	0.22ns	0.34ns	82.15**	166.69**	7.68**	2.20**
Error	14												
Total	23												

Note: * = significant at 0.05; ** = significant at 0.01; ns = non significant

Table D.37 Summary of the ANOVA of GA content (µg) per plant from the whole experimental period of experiment 1

Source of variation	df	Mean Square	F	Significance (P)
Time	11	46830873076	295.517	0
Type of inhibitors	1	70160000	41.027	0
Concentration	3	12670000000	7408	0
Replication	2	5198292.061	3.04	0.08
Type of inhibitors x concentration	3	69230000	40.484	0
Error	270	1710127.859		

Table D.38 Summary of the ANOVA of GA content per plant (µg) of each individual sampling week of experiment 1

Source of variation	df	F of Each Sampling Week											
		0	1	2	3	4	6	9	12	15	20	26	32
Types of inhibitor	1	48.62**	4.45ns	17.01**	3.55ns	116.23**	78.98**	0.97ns	2.96ns	121.30**	15.97**	49.79**	11.36**
Concentrations	3	28.36**	17.85**	316.21**	318.43**	482.58**	526.18**	164.11**	302.92**	1376.00**	2229.00**	1021.00**	667.68**
Replication	2	09.44	14.25	10.31	5.30	7.28	14.70	0.91	3.02	2.20	2.42	0.97	2.22
Types of inhibitor x Concentrations	3	34.39**	14.23**	2.67ns	3.69*	38.37**	27.49**	1.62ns	1.93ns	21.08**	157.11**	13.55**	2.37ns
Error	14												
Total	23												

Note: * = significant at 0.05; ** = significant at 0.01; ns = non significant

Table D.39 Summary of the ANOVA of net CO_2 assimilation rate (μmol/s) from the whole experimental period of experiment 1

Source of variation	df	Mean Square	F	Significance (P)
Time	10	3235295749	78.85	0
Type of inhibitors	1	30910000	1.647	0.22
Concentration	3	109500000	5.837	0.008
Replication	2	273800000	14.589	0
Type of inhibitors x concentration	3	105700000	5.634	0.01
Error	247	18770000		

Table D.40 Summary of the ANOVA of net CO_2 assimilation rate (μmol/s) of each individual sampling week of experiment 1

Source of variation	df	F of Each Sampling Week										
		0	1	2	3	4	6	9	15	20	26	32
Types of inhibitor	1	0.15^{ns}	2.85^{ns}	6.06^{*}	9.82^{**}	8.39^{*}	1.28^{ns}	0.02^{ns}	3.96^{ns}	1.59^{ns}	0.18^{ns}	0.01^{ns}
Concentrations	3	1.74^{ns}	6.56^{**}	5.85^{**}	11.53^{**}	13.03^{**}	22.86^{**}	2.52^{ns}	4.51^{*}	1.30^{ns}	0.47^{ns}	0.64^{ns}
Replication	2	7.77	7.96	7.97	1.57	7.98	4.81	6.04	7.83	0.30	0.29	2.73
Types of inhibitor x Concentrations	3	1.11^{ns}	0.43^{ns}	1.14^{ns}	2.84^{ns}	3.89^{ns}	15.34^{**}	5.17^{*}	1.04^{ns}	0.97^{ns}	0.92^{ns}	0.76^{ns}
Error	14											
Total	23											

Note: * = significant at 0.05; ** = significant at 0.01; ns = non significant

Table D.41 Summary of the ANOVA of accumulative CO_2 uptake (mol) from the whole experimental period of experiment 1

Source of variation	df	Mean Square	F	Significance (P)
Time	10	6405872744	427.9	0
Type of inhibitors	1	1436600.485	0.132	0.722
Concentration	3	16130000	1.485	0.262
Replication	2	88560000	8.153	0.004
Type of inhibitors x concentration	3	84650000	7.793	0.003
Error	247	10860000		

Table D.42 Summary of the ANOVA of accumulative CO_2 uptake (mol) of each individual sampling week of experiment 1

Source of variation	df	F of Each Sampling Week										
		0	1	2	3	4	6	9	15	20	26	32
Types of inhibitor	1	0	1.58^{ns}	$.06^{*}$	9.09^{**}	9.03^{**}	2.35^{ns}	0.60^{ns}	2.50^{ns}	1.19^{ns}	0.30^{ns}	0.08^{ns}
Concentrations	3	0	4.78^{*}	5.80^{**}	9.37^{**}	12.67^{**}	25.20^{**}	9.45^{**}	5.00^{*}	9.54^{**}	1.34^{ns}	0.33^{ns}
Replication	2	0	0.08	.76	.11	9.33	10.61	6.29	18.15	7.47	0.39	1.26
Types of inhibitor x Concentrations	3	0	0.19^{ns}	0.68^{ns}	1.76^{ns}	3.54^{*}	1.50^{ns}	17.94^{**}	5.97^{**}	2.18^{ns}	1.07^{ns}	0.99^{ns}
Error	14	0										
Total	23											

Note: * = significant at 0.05; ** = significant at 0.01; ns = non significant

Table D.43 Summary of the ANOVA of carbon sequestration (g C) from the whole experimental period of experiment 1

Source of variation	df	Mean Square	F	Significance (P)
Time	11	74342.47	987.1	0
Type of inhibitors	1	356.675	17.904	0.001
Concentration	3	1781.153	89.407	0
Replication	2	295.912	14.854	0
Type of inhibitors x concentration	3	335.434	16.837	0
Error	270	19.922		

Table D.44 Summary of the ANOVA of carbon sequestration (g C) of each individual sampling week of experiment 1

Source of variation	df	F of Each Sampling Week											
		0	1	2	3	4	6	9	12	15	20	26	32
Types of inhibitor	1	2.37^{ns}	0.36^{ns}	0.43^{ns}	21.80**	103.27**	76.60**	3.04^{ns}	3.70^{ns}	0.02^{ns}	0.84^{ns}	1.20^{ns}	0.01^{ns}
Concentrations	3	2.64^{ns}	11.17**	9.49**	40.18**	103.60**	98.65**	22.10**	21.05**	25.85**	31.06**	8.73**	4.96*
Replication	2	1.13	15.89	7.74	8.15	8.56	12.30	1.96	2.89	2.05	4.06	7.73	6.37
Types of inhibitor x Concentrations	3	1.36^{ns}	13.49**	0.43^{ns}	8.84**	32.51**	15.44**	2.39^{ns}	4.95*	2.08^{ns}	1.04^{ns}	1.03^{ns}	0.51^{ns}
Error	14												
Total	23												

Note: * = significant at 0.05; ** = significant at 0.01; ns = non significant

'able E.1 Mean of (a) plant height (cm) and (b) total dry weight (g) at each sampling week from experiment 1.

Treatment	Week after application											
	0	1	2	3	4	6	9	12	15	20	26	32
a. Plant Height (cm)												
'B 0 µM	43	70^{a}	13^{a}	47^{a}	33^{a}	73^{a}	33^{a}	43^{a}	03^{a}	.70^{a}	.67^{a}	.17^{a}
'B 250 µM	53	03^{ab}	50^{bc}	00^{ab}	20^{a}	30^{b}	73^{b}	30^{bc}	73^{abc}	.30^{b}	.00^{b}	.20^{b}
'B 500 µM	50	20^{ab}	70^{c}	30^{cd}	23^{bc}	53^{c}	37^{c}	13^{cd}	70^{c}	.17^{c}	.83^{cd}	.17^{cd}
'B 750 µM	77	23^{b}	33^{c}	07^{e}	30^{e}	70^{d}	10^{c}	70^{e}	10^{d}	.53^{d}	.87^{e}	.07^{e}
X 0 µM	43	70^{a}	13^{a}	47^{a}	33^{a}	73^{a}	33^{a}	43^{a}	03^{a}	.70^{a}	.67^{a}	.17^{a}
X 250 µM	23	33^{a}	50^{a}	40^{a}	30^{a}	30^{b}	10^{b}	10^{ab}	57^{ab}	.63^{b}	.33^{b}	.83^{b}
X 500 µM	03	73^{ab}	70^{ab}	70^{bc}	03^{b}	03^{b}	50^{b}	33^{bc}	30^{bc}	.07^{bc}	.77^{c}	.17^{c}
X 750 µM	17	30^{ab}	30^{c}	10de	17^{bc}	07^{cd}	10^{c}	37^{d}	00^{d}	.73^{c}	.10^{d}	.27^{d}
Total Dry Weight (g)												
'B 0 µM	34	25^{bc}	32^{a}	36^{a}	.41^{a}	.75^{a}	.81^{a}	.79^{a}	.40^{a}	.80^{d}	.01^{b}	.69
'B 250 µM	17	29^{c}	23^{ab}	17^{a}	.14^{a}	.82^{b}	.31^{bc}	.68^{bc}	.84^{a}	.87^{cd}	.30^{ab}	.80
'B 500 µM	46	12^{a}	03^{a}	18^{a}	.34^{a}	.80^{b}	.88^{ab}	.35^{a}	.86^{a}	.16^{ab}	.63^{ab}	.44
'B 750 µM	20	20^{c}	12^{b}	51^{b}	.91^{bc}	.33^{c}	.94^{d}	.20^{d}	.68^{b}	.18^{a}	.63^{a}	.47
X 0 µM	34	25^{bc}	32^{a}	36^{a}	.41^{a}	.75^{a}	.81^{a}	.79^{a}	.40^{a}	.80^{d}	.01^{b}	.69
X 250 µM	52	73^{ab}	30^{a}	09^{b}	.20^{b}	.15^{c}	.82^{bc}	.12^{ab}	.35^{a}	.93^{bc}	.80^{ab}	.19
X 500 µM	00	30^{c}	33^{ab}	25^{b}	.67^{c}	.52^{c}	.68^{abc}	.43^{abc}	.59^{a}	.10^{ab}	.58^{ab}	.41
X 750 µM	06	59^{c}	31^{ab}	26^{b}	.73^{bc}	.34^{c}	.21^{c}	.46^{c}	.22^{b}	.13^{a}	.15^{a}	.37

:ans comparison within a column by DMRT at 5% level.

ble E.2 Mean of (a) no of nodes and (b) average internodes length (cm) at each sampling week from experiment 1.

Treatment	Week after application											
	0	1	2	3	4	6	9	12	15	20	26	32
Number of Nodes												
ıM	10.7	20.0	36.3	42.7	50.0	57.0	59.7	62.0	61.3	63.0	62.3	61.3
) µM	10.8	13.0*	31.8*	37.0*	43.0*	51.3*	58.3	60.2	61.0	65.3*	66.2*	64.5*
) µM	9.5*	21.8*	29.5*	35.0*	43.7*	53.3	55.3*	58.8*	59.8	67.3*	68.0*	66.8*
) µM	0.8	17.3*	19.8*	23.5*	35.2*	41.3*	48.3*	53.2*	57.3*	65.3*	66.3*	65.3*
D 0.05	1.2	1.6	4.5	1.4	3.6	3.9	3.8	2.3	2.4	0.8	1.2	1.5
Average Internodes Length (cm)												
ıM	).67	0.93	0.97	1.07	1.10	1.10	0.90	0.97	1.00	1.43	1.77	1.90
) µM	).73	1.15*	0.87*	0.97*	0.98*	0.88*	0.70*	0.85*	0.92*	1.25*	1.48*	1.60*
) µM	1.02*	0.83	0.93	0.82*	0.72*	0.67*	0.65*	0.75*	0.82*	1.10*	1.35*	1.48*
) µM	0.85*	0.73*	0.72*	0.62*	0.48*	0.62*	0.50*	0.58*	0.68*	0.93*	1.17*	1.22*
D 0.05	).16	0.12	0.06	0.06	0.09	0.08	0.08	0.05	0.05	0.05	0.06	0.04

ımber of Nodes and Average Internodes Length * = different from the control treatment of each week which is significant at
SD $_{(concentration)}$ 0.05; the interaction between types of inhibitor and concentrations was not significant.

ble E.3 Mean of (a) no of branches and (b) total leaf area (cm^2) at each sampling week from experiment 1.

Treatment	Week after application											
	0	1	2	3	4	6	9	12	15	20	26	32
Number of Branches												
ıM	0	0	0	0	0	0	0	0	0	0	0.3	0.3
) µM	0	0	0	0	0.3*	0.7*	0.7*	0.7*	0.7*	1.0*	1.0*	1.0*
) µM	0	0	0	0.3*	0.8*	0.8*	1.0*	1.0*	1.2*	1.5*	1.7*	1.7*
) µM	0	0	0	1*	1.7*	2.2*	2.5*	2.5*	2.5*	2.5*	2.5*	2.5*
D 0.05	0	0	0	0.1	0.2	0.2	0.3	0.3	0.3	0.2	0.2	0.2
Total Leaf Area (cm^2)												
ıM	09.7	56.4	55.9	93.6	85.5	46.2	93.3	25.3	83.0	6415.9	6644.7	6812.6
) µM	411.0*	942.7*	1321.1*	2650.2*	3925.2*	00.1	4182.1*	90.9	97.9	6391.8	6519.5	6779.3
) µM	364.4*	13.3	1319.3*	1573.3*	1741.8*	3694.1*	5303.8*	5633.1*	5763.8*	6373.2	6588.5	6761.7
) µM	267.9*	1062.3*	1203.9*	1520.9*	1856.8*	3399.8*	97.3	04.7	69.7	6345.4	6676.3	6777.4
D 0.05	8.4	75.2	71.0	31.2	70.6	97.5	01.7	23.2	10.3	185.5	209.1	118.7

ımber of Branches and Total Leaf Area * = different from the control treatment of each week which is significant at $LSD_{(concentration)}$ 0.05; the interaction between types of inhibitor and concentrations was not significant.

'able E.4 Mean of (a) stem diameter (cm) and (b) stem dry weight (g) at each sampling week from experiment 1

Treatment	Week after application											
	0	1	2	3	4	6	9	12	15	20	26	32
a. Stem Diameter (cm)												
'B 0 µM	:0^{a}	:4	'5	3	8	:2	:7	:7	1^{b}	:4^{c}	:7^{c}	:6^{d}
'B 250 µM	'2^{a}	'6	'7	:5	:0	:3	'2	:7	:5^{ab}	'9^{bc}	'4^{bc}	'9^{c}
'B 500 µM	:9^{a}	3	:4	:2	:7	:0	7	'7	:9^{ab}	'5^{ab}	1^{ab}	7^{b}
'B 750 µM	:7^{a}	1	:2	:0	:4	:1	3	:3	'1^{a}	:4^{a}	:3^{a}	:6^{a}
X 0 µM	:0^{a}	:4	'5	3	8	:2	:7	:7	1^{b}	:4^{c}	:7^{c}	:6^{d}
X 250 µM	'9^{a}	:4	'4	2	6	:3	:3	:8	6^{b}	:7^{c}	:1^{c}	:0^{d}
X 500 µM	:2^{a}	:6	'8	5	9	:6	:7	:7	:8^{ab}	'5^{bc}	:0^{bc}	:2^{c}
X 750 µM	:4^{a}	'4	'6	:3	:7	:4	'5	:5	:3^{ab}	1^{ab}	9^{ab}	'9^{b}
Stem Dry Weight (g)												
'B 0 µM	5	80^{b}	49^{ab}	69^{a}	39^{a}	56^{a}	93^{a}	43^{a}	.08^{a}	2.68^{a}	1.01^{b}	5.92
'B 250 µM	8	13^{b}	68^{ab}	38^{a}	59^{a}	53^{b}	03^{abc}	97^{ab}	.51^{ab}	4.55^{a}	1.48^{b}	7.43
'B 500 µM	2	49^{a}	15^{a}	10^{a}	55^{a}	65^{b}	42^{ab}	18^{ab}	.46^{a}	0.43^{ab}	4.96^{ab}	7.65
'B 750 µM	7	13^{b}	66^{b}	40^{b}	64^{b}	80^{c}	15^{c}	98^{b}	.19^{c}	0.68^{a}	5.05^{ab}	8.03
X 0 µM	5	80^{b}	49^{ab}	69^{a}	39^{a}	56^{a}	93^{a}	43^{a}	.08^{a}	2.68^{a}	1.01^{b}	5.92
X 250 µM	2	53^{a}	52^{ab}	98^{b}	95^{b}	90^{c}	76^{bc}	70^{ab}	0.20^{a}	7.55^{b}	7.20^{a}	8.53
X 500 µM	2	71^{b}	57^{ab}	40^{b}	74^{b}	66^{c}	35^{ab}	93^{a}	.82^{ab}	0.16^{ab}	5.73^{ab}	8.24
X 750 µM	4	51^{b}	21^{ab}	20^{b}	69^{b}	73^{c}	61^{bc}	31^{ab}	.40^{bc}	2.11^{a}	7.34^{a}	0.00

Means comparison within a column by DMRT at 5% level.

ole E.5 Mean of (a) root dry weight (g) and (b) shoot dry weight (g) at each sampling week from experiment 1.

Treatment	Week after application											
	0	1	2	3	4	6	9	12	15	20	26	32
a. Root Dry Weight (g)												
M	'3	;2	·6	;4	.82	.50	.20	.55	.55	.74	.65	.19
) µM	;9*	;7	·3*	7*	.42*	.92*	.13*	.14	.47	.38*	.51	.07
) µM	'0	09*	;3*	;3*	.54*	.94*	.05*	.95	.29*	.32*	.91*	.35*
) µM	;1*	·7	0*	'2*	.85*	.12*	.48*	.42*	.92*	.05*	.19*	.79*
D 0.05	1	;7	;2	;2	.52	.52	.52	.60	.53	.48	.54	53
b. Shoot Dry Weight (g)												
B 0 µM	;1	;4bc	06a	02a	;9a	.24a	.60a	.58a	.85a	.05c	.36	.50
B 250 µM	;7	01c	;6ab	;9a	;3a	.30b	.28bc	.65ab	.45a	.22bc	.55	.83
B 500 µM	04	;6a	20a	;6a	03a	.46b	.16ab	.62a	.44a	.65ab	.52	.54
B 750 µM	;5	22c	11b	10b	70b	.61c	.51c	.77b	.40b	.42a	.46	.05
; 0 µM	;1	;4bc	06a	02a	;9a	.24a	.60a	.58a	.85a	.05c	.36	.50
; 250 µM	;4	38ab	;1a	13b	17b	.82c	.58bc	.88a	.81a	.82ab	.54	.02
; 500 µM	13	38c	19ab	10b	01b	.98c	.31ab	.06a	.44a	.97a	.88	.62
; 750 µM	19	34c	12ab	03b	24b	.83c	.69bc	.94ab	.67b	.79a	.95	.22

ot Dry Weight * = different from the control treatment of each week which is significant at LSD $_{(concentrations)}$ 0.05; the types of inhibitors was not significant.

oot Dry Weight = Means comparison within a column by DMRT at 5% level.

ıble E.6 Mean of (a) net CO_2 assimilation rate (µmol/s) and (b) accumulative CO_2 uptake (mol) at each sampling week from experiment 1

Treatment	Week after application										
	0	1	2	3	4	6	9	15	20	26	32
a. Net CO_2 Assimilation Rate (µmol/s)											
ıM	34	72	23	328	461	547	586	427	209	236	209
) µM	32	93*	26*	547*	755*	579*	982*	316*	527*	541*	570
) µM	71*	70	34*	788*	881*	448*	133*	380*	400*	396	074
) µM	53*	12*	72*	731*	536*	431*	527	730*	451*	539	997
D 0.05	59	31	09	595	121	303	116	272	478	50	359
b. Accumulative CO_2 Uptake (mol)											
B 0 µM	0	08^{a}	3^{a}	6^{a}	2^{a}	13^{a}	75^{ab}	68^{a}	12^{b}	47	76
B 250 µM	0	7^{b}	7^{b}	5^{b}	7^{b}	41^{b}	09^{bcd}	82^{a}	17^{a}	46	48
B 500 µM	0	1^{a}	6^{ab}	0^{b}	7^{b}	96^{b}	37^{cd}	67^{a}	10^{ab}	42	89
B 750 µM	0	2^{ab}	6^{ab}	4^{b}	3^{b}	12^{b}	14^{d}	30^{a}	31^{ab}	12	43
X 0 µM	0	08^{a}	3^{a}	6^{a}	2^{a}	13^{a}	75^{ab}	79^{a}	12^{b}	47	76
X 250 µM	0	7^{ab}	2^{ab}	7^{ab}	0^{a}	46^{b}	64^{d}	63^{a}	25^{ab}	72	59
X 500 µM	0	1^{a}	6^{ab}	7^{ab}	2^{b}	27^{b}	54^{b}	44^{a}	76^{a}	34	13
X 750 µM	0	3^{ab}	9^{ab}	9^{ab}	8^{ab}	48^{b}	48^{bc}	00^{a}	31^{ab}	00	30

t CO_2 Assimilation Rate * = different from the control treatment of each week which is significant at LSD $_{(concentrations)}$ 0.05; the types of inhibitors was not significant.

cumulative CO_2 uptake = Means comparison within a column by DMRT at 5% level.

'able E.7 Mean of (a) GA concentration (µg/g dry matter) and (b) GA content (µg) at each sampling week from experiment 1.

Treatment	Week after application											
	0	1	2	3	4	6	9	12	15	20	26	32
b. GA Concentration (µg/g dry matter)												
'B 0 µM	5[a]	5[a]	1[a]	3[a]	)7[a]	)9[a]	35[a]	)0[a]	77[a]	34[a]	53[a]	54[a]
'B 250 µM	)[b]	4[b]	5[c]	5[d]	24[b]	18[b]	79[b]	12[b]	77[d]	49[b]	14[c]	43[b]
'B 500 µM	2[a]	5[d]	5[c]	)[d]	37[c]	46[c]	)9[c]	52[c]	77[e]	34[c]	71[c]	77[d]
'B 750 µM	7[a]	5[c]	1[f]	2[f]	25[g]	19[f]	)1[d]	30[d]	37[g]	)4[f]	34[f]	38[e]
X 0 µM	5[a]	5[a]	1[a]	3[a]	)7[a]	)9[a]	35[a]	)0[a]	77[a]	34[a]	53[a]	54[a]
X 250 µM	5[a]	2[b]	4[b]	7[b]	48[c]	43[b]	60[b]	21[b]	22[b]	32[c]	73[b]	75[b]
X 500 µM	5[a]	5[c]	3[c]	)[c]	15[d]	73[e]	38[c]	98[c]	14[c]	30[d]	38[d]	43[c]
X 750 µM	1[a]	4[d]	2[d]	3[e]	54[f]	24[d]	)4[d]	35[d]	78[f]	20[e]	76[f]	)8[e]
GA Content per Plant (μg^2)												
'B 0 µM	)[b]	.0[ab]	.6[a]	.8[a]	4.2[a]	4.2[a]	2.5[a]	2.1[a]	8.6[a]	46.4[a]	85.3[a]	31.5[a]
'B 250 µM	)[b]	.2[bc]	.2[bc]	.2[b]	3.7[b]	7.4[b]	1.7[bc]	4.5[b]	8.7[c]	29.5[ab]	65.5[ab]	93.3[ab]
'B 500 µM	4[b]	.7[ab]	.8[c]	.0[bc]	8.1[bc]	1.9[b]	9.9[b]	7.7[bc]	9.4[cd]	17.2[ab]	51.1[bc]	73.9[bc]
'B 750 µM	)[b]	.2[d]	.4[c]	.2[d]	4.3[d]	5.6[d]	7.1[d]	9.4[d]	2.1[d]	62.6[d]	75.2[c]	19.1[c]
X 0 µM	)[b]	.0[ab]	.6[a]	.8[a]	4.2[a]	4.2[a]	2.5[a]	2.1[a]	8.6[a]	46.4[a]	85.3[a]	31.5[a]
X 250 µM	)[a]	.1[a]	.2[b]	.8[bc]	0.1[c]	1.3[c]	8.6[b]	7.7[b]	1.4[b]	74.8[b]	64.1[a]	10.0[ab]
X 500 µM	4[a]	.4[bc]	.4[bc]	.5[c]	6.3[d]	5.3[d]	7.2[bc]	0.6[bc]	0.4[c]	18.2[c]	26.8[bc]	10.4[bc]
X 750 µM	3[b]	.1[cd]	.2[d]	.1[cd]	7.2[d]	1.2[d]	9.9[c]	0.9[d]	5.3[d]	25.8[c]	56.1[c]	)51.1[c]

Mean comparison within a column by DMRT at 5% level.

'able E.8 Mean of carbon sequestration (g C) at each sampling week from experiment 1.

Treatment	Week after application											
	0	1	2	3	4	6	9	12	15	20	26	32
rbon Sequestration (g C)												
'B 0 µM	5[a]	01[bc]	05[a]	11[a]	76[a]	59[a]	50[a]	52[a]	.25[a]	.50[d]	.13[b]	.55
'B 250 µM	5[b]	06[c]	14[ab]	23[a]	21[a]	54[b]	07[bc]	12[bc]	.90[a]	.42[cd]	.94[ab]	.25
'B 500 µM	0[a]	45[a]	14[a]	09[a]	71[a]	25[b]	58[ab]	72[a]	.16[a]	.35[ab]	.65[ab]	.28
'B 750 µM	7[b]	00[c]	70[b]	57[b]	32[bc]	46[c]	71[d]	75[d]	.42[b]	.49[a]	.39[a]	.67
X 0 µM	5[a]	01[bc]	05[a]	11[a]	76[a]	59[a]	50[a]	52[a]	.25[a]	.50[d]	.13[b]	.55
X 250 µM	0[a]	06[ab]	17[a]	09[b]	50[b]	59[c]	53[bc]	53[ab]	.47[a]	.95[bc]	.00[ab]	.75
X 500 µM	5[a]	59[c]	77[ab]	28[b]	57[c]	32[c]	43[abc]	40[abc]	.87[a]	.94[ab]	.24[ab]	.26
X 750 µM	0[b]	09[c]	26[ab]	56[b]	05[bc]	59[c]	38[c]	04[c]	.64[b]	.83[a]	.09[a]	.73

Mean comparison within a column by DMRT at 5% level.

APPENDIX F

Some of Mean Comparisons : Experiment 3.5

Table F.1: % seed germination of naked seed and normal seed after treat with NMU in buffer

Tests of Between-Subjects Effects
Dependent Variable: % seed germination

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	22181.667(a)	13	1706.282	72.561	.000
Intercept	17689.000	1	17689.000	752.238	.000
treat	22163.000	11	2014.818	85.682	.000
rep	18.667	2	9.333	.397	.677
Error	517.333	22	23.515		
Total	40388.000	36			
Corrected Total	22699.000	35			

a R Squared = .977 (Adjusted R Squared = .964)

treatment	Mean% seed germination
naked seed 4hr 0.03%NMU	18^{b}
naked seed 4hr 0.1%NMU	28^{cde}
naked seed 4hr 0.3%NMU	0^{a}
naked seed 12 hr 0.03% NMU	16^{b}
naked seed 12 hr 0.1% NMU	0^{a}
naked seed 12 hr 0.3% NMU	0^{a}
normal seed 4 hr 0.03% NMU	30^{de}
normal seed 4 hr 0.1% NMU	94^{f}
normal seed 4 hr 0.3% NMU	22^{bcd}
normal seed 12 hr 0.03% NMU	36^{e}
normal seed 12 hr 0.1% NMU	20^{bc}
normal seed 12 hr 0.3% NMU	2^{a}

Table F.2: % seed germination of the *J. curcas* seeds treated with 0.5x, 1.0x phosphate citrate buffer and water

Tests of Between-Subjects Effects
Dependent Variable: %seed germination

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5030.222(a)	7	718.603	20.518	.000
Intercept	15723.556	1	15723.556	448.959	.000
treat	4804.444	5	960.889	27.437	.000
rep	225.778	2	112.889	3.223	.083
Error	350.222	10	35.022		
Total	21104.000	18			
Corrected Total	5380.444	17			

a R Squared = .935 (Adjusted R Squared = .889)

treatment	Mean% seed germination
water 4hr.	20[ab]
water 12hr.	57.33[d]
0.5x buffer 4hr.	18.66[ab]
1.0x buffer 4hr.	9.33[a]
0.5x buffer 12hr.	28[b]
1.0x buffer 12hr.	44[c]

Table F.3: %seed germination of *J. curcas* seeds sterilized and non sterilized with Clorox.

Case Processing Summary

	Cases					
	Included		Excluded		Total	
	N	Percent	N	Percent	N	Percent
% seed germination * treatment	6	100.0%	0	.0%	6	100.0%

treatment	Mean	N	Std. Deviation
sterilize with Clorox	40.0000	3	12.00000
non sterilize with Clorox	57.3333	3	6.11010
Total	48.6667	6	12.75408

Table F. 4: The pH value of each % concentration of NMU with phosphate citrate buffer and water at every an hour for 18 hours.

treatment	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Buffer	6.06	5.97	5.99	5.93	6.01	5.9	6	6	6.01	5.99	5.96	5.93	5.99	5.96	6	5.99	6.01	5.97	5.96
0.1%NMUbuffer	6.08	5.92	5.93	5.88	5.96	5.88	5.95	5.94	5.96	5.99	5.95	5.95	5.99	5.93	5.97	5.96	5.97	5.95	5.94
0.5%NMUbuffer	5.95	5.84	5.83	5.81	5.86	5.85	5.9	5.89	5.94	5.92	5.89	5.97	6	5.97	6.03	6.06	6.04	6.04	6.04
1.0%NMUbuffer	5.83	5.72	5.72	5.71	5.77	5.77	5.81	5.81	5.87	5.87	5.85	5.91	5.93	5.95	6.02	6.05	6.07	6.06	6.07
water	7.08	7.08	7.08	7.08	7.1	7.12	7.09	7.09	7.07	7.07	7.02	7.08	7.08	7.08	7.1	7.09	7.1	7.06	7.04
0.1%NMU water	5.03	5.04	4.94	4.92	4.85	4.88	4.87	4.86	4.93	4.93	4.89	4.97	5.02	4.98	4.99	5.07	5.05	5.07	5.1
0.5%NMU water	3.96	3.92	3.86	3.93	3.96	3.96	3.98	3.9	4.05	4.02	4.01	4.04	4.1	4.08	4.1	4.13	4.12	4.11	4.12
1.0%NMU water	3.83	3.83	3.82	3.88	3.93	3.9	3.93	3.84	3.99	4	3.96	3.98	4.05	4.01	4.05	4.1	4.09	4.1	4.12

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
0 hr.	Between Groups	3.784	3	1.261	.999	.479
	Within Groups	5.052	4	1.263		
	Total	8.835	7			
1 hr.	Between Groups	3.864	3	1.288	1.112	.442
	Within Groups	4.633	4	1.158		
	Total	8.497	7			
2 hr.	Between Groups	3.989	3	1.330	1.101	.446
	Within Groups	4.830	4	1.207		
	Total	8.818	7			
3 hr.	Between Groups	3.735	3	1.245	1.091	.449
	Within Groups	4.564	4	1.141		
	Total	8.299	7			
4 hr.	Between Groups	3.746	3	1.249	1.061	.459
	Within Groups	4.708	4	1.177		
	Total	8.454	7			
5 hr.	Between Groups	3.593	3	1.198	1.003	.478
	Within Groups	4.779	4	1.195		
	Total	8.372	7			
6 hr.	Between Groups	3.594	3	1.198	1.001	.479
	Within Groups	4.788	4	1.197		
	Total	8.381	7			
7 hr.	Between Groups	3.791	3	1.264	.992	.482
	Within Groups	5.098	4	1.274		
	Total	8.889	7			
8 hr.	Between Groups	3.325	3	1.108	.954	.495
	Within Groups	4.646	4	1.161		
	Total	7.971	7			
9 hr.	Between Groups	3.320	3	1.107	.942	.499
	Within Groups	4.698	4	1.175		
	Total	8.018	7			
10 hr.	Between Groups	3.258	3	1.086	.929	.504

	Within Groups	4.677	4	1.169		
	Total	7.935	7			
11 hr.	Between Groups	3.126	3	1.042	.856	.532
	Within Groups	4.866	4	1.217		
	Total	7.992	7			
12 hr.	Between Groups	3.065	3	1.022	.881	.522
	Within Groups	4.637	4	1.159		
	Total	7.701	7			
13 hr.	Between Groups	3.077	3	1.026	.864	.529
	Within Groups	4.746	4	1.187		
	Total	7.823	7			
14 hr.	Between Groups	3.008	3	1.003	.821	.547
	Within Groups	4.888	4	1.222		
	Total	7.896	7			
15 hr.	Between Groups	2.828	3	.943	.791	.559
	Within Groups	4.765	4	1.191		
	Total	7.592	7			
16 hr	Between Groups	2.907	3	.969	.804	.553
	Within Groups	4.821	4	1.205		
	Total	7.727	7			
17 hr.	Between Groups	2.759	3	.920	.772	.567
	Within Groups	4.765	4	1.191		
	Total	7.523	7			
18 hr.	Between Groups	2.663	3	.888	.759	.573
	Within Groups	4.680	4	1.170		
	Total	7.343	7			

Table F. 5.1: % seed germination of *J. curcas* seed is treated with NMU in phosphate citrate buffer

Tests of Between-Subjects Effects
Dependent Variable: % seed germination

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	7750.300(a)	11	704.573	4.475	.001
Intercept	87796.900	1	87796.900	557.592	.000
con	2936.700	7	419.529	2.664	.030
rep	4813.600	4	1203.400	7.643	.000
Error	4408.800	28	157.457		
Total	99956.000	40			
Corrected Total	12159.100	39			

a R Squared = .637 (Adjusted R Squared = .495)

treatment	means
water	60.40[b]
0%	34.80[a]
0.05%	34.00[a]
0.10%	54.40[b]
0.20%	50.40[ab]
0.40%	47.60[ab]
0.80%	50.00[ab]
1.60%	43.20[ab]

Table F. 5.2: % viability of *J. curcas* seed is treated with NMU in phosphate citrate buffer

Tests of Between-Subjects Effects
Dependent Variable: % viability

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	8200.895(a)	11	745.536	19.770	.000
Intercept	71472.652	1	71472.652	1895.288	.000
con	8091.590	7	1155.941	30.653	.000
rep	109.305	4	27.326	.725	.583
Error	1055.900	28	37.711		
Total	80729.448	40			
Corrected Total	9256.795	39			

a R Squared = .886 (Adjusted R Squared = .841)

treatment	%viability
water	55.46^{cd}
0%	54.36^{cd}
0.05%	61.48^{d}
0.10%	51.39^{c}
0.20%	20.51^{a}
0.40%	28.24^{ab}
0.80%	30.45^{b}
1.60%	36.29^{b}

Table F. 5.3: Stem height of *J. curcas* after application at 14, 24, 39 and 47 weeks.

Tests of Between-Subjects Effects
Dependent Variable: stem diameters at 14 weeks

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.857(a)	11	.169	9.117	.000
Intercept	72.967	1	72.967	3940.640	.000
con	1.760	7	.251	13.580	.000
rep	.037	4	.009	.498	.738
Error	5.777	312	.019		
Total	98.138	324			
Corrected Total	7.634	323			

a R Squared = .243 (Adjusted R Squared = .217)

Tests of Between-Subjects Effects
Dependent Variable: stem diameters at 24 weeks

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.857(a)	11	.169	9.117	.000
Intercept	136.745	1	136.745	7385.031	.000
con	1.760	7	.251	13.580	.000
rep	.037	4	.009	.498	.738
Error	5.777	312	.019		
Total	179.594	324			
Corrected Total	7.634	323			

a R Squared = .243 (Adjusted R Squared = .217)

Tests of Between-Subjects Effects
Dependent Variable: stem diameters at 39 weeks

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5.885(a)	11	.535	2.675	.003
Intercept	386.640	1	386.640	1933.028	.000
con	5.353	7	.765	3.823	.001
rep	.322	4	.080	.402	.807
Error	62.406	312	.200		
Total	564.516	324			
Corrected Total	68.291	323			

a R Squared = .086 (Adjusted R Squared = .054)

Tests of Between-Subjects Effects
Dependent Variable: stem diameters at 47 weeks

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	146477.81	31	4725.09	26.96	0.000
Intercept	264507.37	1	264507.37	1509.28	0.000
con	8907.90	7	1272.56	7.26	0.000
rep	107654.67	3	35884.89	204.76	0.000
Error	7656.90	21	364.61	2.08	0.003
Total	221170.28	1262	175.25		
Corrected Total	688927.68	1294			
	367648.09	1293			

a R Squared = .086 (Adjusted R Squared = .054)

Treatment	Means			
	14	24	39	47
water	4.43^{abc}	6.77^{a}	29.27^{b}	1.05^{cd}
0%	4.45^{abc}	6.61^{a}	11.71^{a}	0.83^{a}
0.05%	4.77^{cd}	7.29^{ab}	19.57^{ab}	0.97^{bc}
0.10%	5.06^{d}	8.82^{c}	28.29^{b}	1.04^{cd}
0.20%	4.10^{ab}	7.04^{ab}	20.32^{ab}	1.00^{cd}
0.40%	3.97^{a}	7.26^{ab}	19.05^{ab}	0.92^{b}
0.80%	5.07^{d}	8.36^{bc}	28.90^{b}	1.11^{dc}
1.60%	4.63^{bcd}	7.39^{ab}	28.16^{b}	1.14^{d}

Table F. 5.4: inter node length of *J. curcas* after application at 14, 24 and 39 weeks

Tests of Between-Subjects Effects
Dependent Variable: inter node length at 14 weeks

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.144(a)	11	.195	10.364	.000
Intercept	32.701	1	32.701	1738.609	.000
con	1.955	7	.279	14.852	.000
rep	.151	4	.038	2.007	.093
Error	5.868	312	.019		
Total	52.235	324			
Corrected Total	8.013	323			

a R Squared = .268 (Adjusted R Squared = .242)

Tests of Between-Subjects Effects
Dependent Variable: inter node length at 24 weeks

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.536(a)	11	.049	10.364	.000
Intercept	46.225	1	46.225	9830.569	.000
con	.489	7	.070	14.852	.000
rep	.038	4	.009	2.007	.093
Error	1.467	312	.005		
Total	63.234	324			
Corrected Total	2.003	323			

a R Squared = .268 (Adjusted R Squared = .242)

Tests of Between-Subjects Effects
Dependent Variable: inter node length at 39 weeks

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	9.217(a)	11	.838	4.604	.000
Intercept	117.412	1	117.412	645.169	.000
con	8.634	7	1.233	6.778	.000
rep	.263	4	.066	.361	.837
Error	56.780	312	.182		
Total	204.556	324			
Corrected Total	65.997	323			

a R Squared = .140 (Adjusted R Squared = .109)

treatment	means		
	14	24	39
water	0.32^{b}	0.41^{b}	7.9^{cd}
0%	0.21^{a}	0.36^{a}	0.37^{a}
0.05%	0.32^{b}	0.41^{b}	0.54^{ab}
0.10%	0.433^{de}	0.47^{de}	0.80^{cd}
0.20%	0.40^{c}	0.45^{cd}	0.57^{abc}
0.40%	0.35^{bc}	0.43^{bc}	0.67^{bc}
0.80%	0.49^{e}	0.50^{e}	0.93^{d}
1.60%	0.40^{cd}	0.45^{cd}	0.80^{cd}

Table F. 5.5: number of node of *J. curcas* after application at 14, 24, 39 and 47 weeks

Tests of Between-Subjects Effects
Dependent Variable: number of node at 14 weeks

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.037(a)	11	.003	1.108	.354
Intercept	250.350	1	250.350	81412.559	.000
con	.017	7	.002	.779	.605
rep	.022	4	.005	1.765	.136
Error	.959	312	.003		
Total	327.000	324			
Corrected Total	.997	323			

a R Squared = .038 (Adjusted R Squared = .004)

Tests of Between-Subjects Effects
Dependent Variable: number of node at 24 weeks

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.302(a)	11	.118	10.200	.000
Intercept	1028.229	1	1028.229	88601.984	.000
con	1.272	7	.182	15.654	.000
rep	.063	4	.016	1.367	.245
Error	3.621	312	.012		
Total	1321.000	324			
Corrected Total	4.923	323			

a R Squared = .264 (Adjusted R Squared = .239)

Tests of Between-Subjects Effects
Dependent Variable: number of node at 39 weeks

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1901.156(a)	11	172.832	1.710	.070
Intercept	94326.759	1	94326.759	933.381	.000
con	1652.700	7	236.100	2.336	.025
rep	273.447	4	68.362	.676	.609
Error	31530.483	312	101.059		
Total	149599.000	324			
Corrected Total	33431.639	323			

a R Squared = .057 (Adjusted R Squared = .024)

Tests of Between-Subjects Effects
Dependent Variable: number of node at 47 weeks

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	121592.67	31	3922.34	91.21	0.000
Intercept	130699.07	1	130699.07	3039.38	0.000
con	1007.02	7	143.86	3.35	0.002
rep	99042.85	3	33014.28	767.74	0.000
Error	1855.04	21	88.34	2.05	0.003
Total	54268.33	1262	43.00		
Corrected Total	332457.00	1294			
	175861.00	1293			

a R Squared = .140 (Adjusted R Squared = .109)

Treatment	Means			
	14	24	39	47
water	1.00[a]	2.00[a]	22.92[b]	11.85[bc]
0%	1.00[a]	2.00[a]	15.18[a]	9.31[a]
0.05%	1.02[a]	2.00[a]	16.96[a]	10.92[ab]
0.10%	1.00[a]	2.00[a]	20.00[ab]	11.02[ab]
0.20%	1.00[a]	2.00[a]	19.30[ab]	10.91[ab]
0.40%	1.00[a]	2.00[a]	17.18[a]	10.45[ab]
0.80%	1.00[a]	2.00[a]	19.93[ab]	12.05[bc]
1.60%	1.00[a]	2.26[b]	22.89[b]	12.89[c]

Table F. 5.6: The stem diameters of *J. curcas* after application at 14, 24, 39 and 47 weeks

Tests of Between-Subjects Effects
Dependent Variable: stem diameters at 14 weeks

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.857(a)	11	.169	9.117	.000
Intercept	72.967	1	72.967	3940.640	.000
con	1.760	7	.251	13.580	.000
rep	.037	4	.009	.498	.738
Error	5.777	312	.019		
Total	98.138	324			
Corrected Total	7.634	323			

a R Squared = .243 (Adjusted R Squared = .217)

Tests of Between-Subjects Effects
Dependent Variable: stem diameters at 24 weeks

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.857(a)	11	.169	9.117	.000
Intercept	136.745	1	136.745	7385.031	.000
con	1.760	7	.251	13.580	.000
rep	.037	4	.009	.498	.738
Error	5.777	312	.019		
Total	179.594	324			
Corrected Total	7.634	323			

a R Squared = .243 (Adjusted R Squared = .217)

Tests of Between-Subjects Effects
Dependent Variable: stem diameters at 39 weeks

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5.885(a)	11	.535	2.675	.003
Intercept	386.640	1	386.640	1933.028	.000
con	5.353	7	.765	3.823	.001
rep	.322	4	.080	.402	.807
Error	62.406	312	.200		
Total	564.516	324			
Corrected Total	68.291	323			

a R Squared = .086 (Adjusted R Squared = .054)

Tests of Between-Subjects Effects
Dependent Variable: stem diameters at 47 weeks

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	206.46	31	6.66	55.60	0.000
Intercept	1059.34	1	1059.34	8844.45	0.000
con	8.95	7	1.28	10.68	0.000
rep	156.22	3	52.07	434.76	0.000
Error	1.81	21	0.09	0.72	0.818
Total	151.15	1262	0.12		
Corrected Total	1648.09	1294			
	357.61	1293			

a R Squared = .086 (Adjusted R Squared = .054)

Treatment	Means			
	14	24	39	47
water	0.53^{b}	0.73^{b}	1.40^{c}	1.05^{ab}
0%	0.39^{a}	0.59^{a}	0.99^{a}	0.83^{a}
0.05%	0.49^{b}	0.69^{b}	1.22^{bc}	0.97^{ab}
0.10%	0.55^{b}	0.75^{b}	1.34^{c}	1.04^{cd}
0.20%	0.53^{b}	0.73^{b}	1.23^{bc}	1.00^{bc}
0.40%	0.49^{b}	0.69^{b}	1.03^{ab}	0.92^{b}
0.80%	0.65^{c}	0.85^{c}	1.33^{c}	1.11^{de}
1.60%	0.69^{c}	0.89^{c}	1.40^{c}	1.14^{e}

Table F. 5.7: number of leaf of *J. curcas* after application at 14, 24, 39 and 47 weeks number of leaf (leaf)

Tests of Between-Subjects Effects
Dependent Variable: number of leaf at 39 weeks

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1224.723(a)	11	111.338	1.590	.101
Intercept	24286.337	1	24286.337	346.730	.000
con	1096.928	7	156.704	2.237	.031
rep	145.622	4	36.406	.520	.721
Error	21853.681	312	70.044		
Total	51771.000	324			
Corrected Total	23078.404	323			

a R Squared = .053 (Adjusted R Squared = .020)

Tests of Between-Subjects Effects
Dependent Variable: number of leaf at 47 weeks

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	31310.80	31	1010.03	30.26	0.000
Intercept	41480.80	1	41480.80	1242.89	0.000
con	447.93	7	63.99	1.92	0.063
rep	25159.22	3	8386.41	251.28	0.000
Error	987.81	21	47.04	1.41	0.103
Total	42118.43	1262	33.37		
Corrected Total	122740.00	1294			
	73429.22	1293			

a R Squared = .053 (Adjusted R Squared = .020)

Treatment	Means			
	14	24	39	47
water	2	1	13.08[b]	7.07
0%	2	1	6.60[a]	5.57
0.05%	2	1	8.09[a]	5.74
0.10%	2	1	10.93[ab]	6.65
0.20%	2	1	8.51[ab]	5.65
0.40%	2	1	8.73[ab]	6.2
0.80%	2	1	11.03[ab]	7.18
1.60%	2	1	11.11[ab]	6.33

Table F. 5.8: Number of branch of *J. curcas* after application at 14, 24, 39 and 47 week

Treatment	Means			
	14	24	39	47
water	0	0	0.04[b]	0.08
0%	0	0	0a	0.11
0.05%	0	0	0a	0.14
0.10%	0	0	0a	0.32
0.20%	0	0	0a	0.14
0.40%	0	0	0a	0.29
0.80%	0	0	0a	0.3
1.60%	0	0	0a	0.04

Curriculum vitae

ประวัติคณะผู้วิจัย

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1986	ปริญญาโท	วท.ม.	ปฐพีวิทยา	มหาวิทยาลัยเกษตรศาสตร์	ไทย
1994	ปริญญาเอก	Ph.D.	Plant Sciences	Adelaide University	Australia

6. สาขาวิชาการที่มีความชำนาญพิเศษ (แตกต่างจากวุฒิการศึกษา) ระบุสาขาวิชาการ

- Agronomy & physiology of field crops & field-testing of transgenic plants;
- Plant Ecophysiology & abiotic stresses (nutritional, anoxic & oxidative);
- Micronutrients physiology: plant responses, uptake, efficiency mechanism & varietal screening, rhizosphere acquisitions, interaction with root disease;
- Chemistry of submerged soils & behaviours of heavy metal; quantify O_2 status in rhizosphere (soil & solution);
- Integrated ecosystem assessment, participatory scenario for natural resource management (Working member of Millennium Ecosystem Assessments)
- National & international management & policies on sciences, agriculture & natural resource

7. ประสบการณ์ที่เกี่ยวข้องกับการบริหารงานวิจัยทั้งภายในและภายนอกประเทศ โดย ถานภาพในการทำการวิจัยว่าเป็น ผู้อำนวยการแผนงานวิจัย หัวหน้าโครงการวิจัย หรือผู้ร่วมวิจัยในแต่ละข้อเสนอการวิจัย เป็นต้น

7.1 ผู้อำนวยการแผนงานวิจัย : ชื่อแผนงานวิจัย

1. พค. 2547. ได้รับการแต่งตั้งจาก วว. ให้เป็นประธานคณะทำงานโครงการ "การประเมินผลโครงการถ่ายทอดเทคโนโลยีของสถาบันวิจัยวิทยาศาสตร์และเทคโนโลยีแห่งประเทศไทยในปีงบประมาณ 2547"
2. สค. 2546. ได้รับเชิญจาก Millennium Ecosystem Assessment Secretariat ให้เป็น Coordinating Lead Author เพื่อรับผิดชอบการวิเคราะห์และเรียบเรียงรายงาน Sub-global Assessment Report Chapter 10: Sub-global Scenarios. ร่วมกับนักวิจัยนานาชาติอีก 10 คน

7.2 หัวหน้าโครงการวิจัย : ชื่อโครงการวิจัย

1979-86: Water Loss Characteristics of *Oxic Paleustules* soil in North-eastern Thailand under Selected Surface Management Practices. (M.Sc. Thesis, Kasetsart U., Thailand)

1989-93: The Influence of Zn Nutritional Status in Cereals on the Severity of *Rhizoctonia* Root Rot Disease. (Ph.D. Thesis, Adelaide U., Australia. (GRDC & DEET Scholarships).

1994-96: a) Physiology of submergence and post-anoxic tolerance in relation to carbohydrate, iron toxicity and free radicals (Hohenheim University & Scottish Crop Research Institute, UK.); b) Develop technique for using Near Infrared Reflectance Spectrometer to measure N & non-structural carbohydrate in stem of floodprone rice plants at seedling stage (IRRI-ACIAR Project).

1996-98: Mechanisms rhizosphere acquisitions and Zn efficiency in rice and develop phenotypic technique for screening Zn efficient varieties. (Part of the IRRI Floodprone Research Programme (FP1): Genetics and mechanisms of traits for tolerance to soils with adverse conditions).

1998-2002: Physiological response of cotton to waterlogging. (Part of Programme TA1, CSIRO Cotton Research Unit, CSIRO Plant Industry, AUSTRALIA)

7.3. งานวิจัยที่ทำเสร็จแล้ว :

INVITED CHAPTER:-

1. Lebel L., **Thongbai P** and Kok K. 2005. Chapter 10: Scenarios *in* **A Report of the Millennium Ecosystem Assessment.** (Will be published by the Millennium Ecosystem Assessment in November, 2005.)
2. เอกสาร 'ระบบประเมินผลการดำเนินงานรัฐวิสาหกิจ: การประเมินผลโครงการถ่ายทอดเทคโนโลยีปีงบประมาณ 2547' โดย สถาบันวิจัยวิทยาศาสตร์และเทคโนโลยีแห่งประเทศไทย 2547. (สงวนลิขสิทธิ์)
3. Bange, M.P., S. P. Milroy and P. Thongbai. 2004. Waterlogging: its impact on cotton. Pp137-140 in WATER Pak: A guide for irrigation management of cotton. Australian Cotton Research Institute & CSIRO Plant Industry, AUATRALIA.
4. **Thongbai P**, 2001. Waterlogging in Cotton. Chapter 12, *in* 'NUTRIpak**: A Practical Guide to Cotton Nutrition'**, 2001. I. Rochester (ed.). Australian Cotton Cooperative Research Centre. Narrabri, Australia.

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1. Millennium Ecosystem Assessment. 2005. **Ecosystems and Human Well-being: Synthesis**. Island Press, Washington D.C. 137pp.
2. Bange M P, Milroy S P, and **Thongbai P**. 2004. Growth and yield of cotton in response to waterlogging. ***Field Crop Res. 88:129-142.***
3. **Thongbai P**, Kirk G J D and Huguenin, O. 2002. Effect of rhizosphere oxygen released on rhizosphere Zn acquisition and Zn uptake of rice lines differed in Zn efficiency. Proceeding of the 17th World Congress of Soil Sciences, 14-21 August, 2002. Bangkok, Thailand.
4. **Thongbai P**, Kirk G J D and Quijano C. 2001. Effect of Fe plaque on rhizosphere Zn acquisition and Zn uptake of rice lines differed in Zn efficiency. pp 574-575 *in* **Proceedings the XIV International Plant Nutrition Colloquium**. Hannover, Germany. Kulwer Academic Publishers, Dordrecht, the Netherlands.
5. **Thongbai P,** Milroy S, Bange M, Rapp G and Smith T. 2001. Agronomic Responses of Cotton to Low Soil Oxygen during Waterlogging. **Proceeding of the 10th Australian Agronomy Conference**. Hobart, Tasmania. www.regional.org.au/au/asa/2001/.
6. **Thongbai P** and Goodman B A. 2000. Oxidative free radicals generation and post-anoxic injury of rices (Oryza Sativa L.) in an iron-toxic soil. ***J. Plant Nutrition***. 23: 1887-1900.
7. Kilcoyne S H, Bentley P M, **Thongbai P**, Gordon D C and Goodman B A. 2000. The application of Fe-57 Mőssbauer Spectroscopy in the investigation of iron uptake and translocation in plants. ***Nuclear Instruments & Methods in Physics Research Section B-Beam Interaction with Material & Atom***. 160:157-166.
8. **Thongbai P**, Kirk G J D and Senadhira D. 1999. Rhizosphere acquisition traits for phenotyping Zn efficient rice (Oryza sativa L.). In: **Plant Nutrition-Molecular Biology and Genetics**. G. Gissel - Nielsen and A. Jensen (eds). Kluwer Academic Publisher, The Netherlands; 1999. pp 253-260
9. **Thongbai P**, Guerta C. Q, and G. Kirk. 1999. Screening technique for zinc efficiency. *In* **IRRI Program Report for 1998**. The International Rice Research Institute, Los Baños, Philippines. pp 74-77.
10. **Thongbai P**, Quijano C and Kirk G J D. 1998. Performance of different Zn efficient rice lines in a Zn deficient soil. **International Rice Research Notes** 23: 18-19
11. **Thongbai P**, Neuemann G, Roemheld V and Marchner H. 1997. Quantitative study of pH and root exudates of Zn efficient and inefficient rice lines. **Report Project A/95/24286** submitted to Deutscher Akademischer Austauschdienst (DAAD), Bonn, Germany.
12. **Thongbai P**, Kongchum M, Setter T L, Puckridge D W, Conocono E A and Batten G D. 1995. Use of Near Infrared reflectance Spectroscopy (NIRS) for estimation of total nonstructural carbohydrates in rice plants: a new tool in screening for submergence tolerance. *In* **Fragile lives in fragile ecosystems**. The International Rice Research Institute, Los Baños, Philippines. pp 843-854.
13. Puckridge D W, Kongchum M, **Thongbai P**, Sattarasart A and Sinoupakarn S. 1994. Nitrogen uptake and yield of deepwater rice in the Central Plain of Thailand. ***Field Crop Res.*** 37:193-204.
14. **Thongbai P**, Hannam R J, Graham R D and Webb M J. 1993. Interaction between zinc nutritional status of cereals and *Rhizoctonia* root rot severity. I. Field observations. ***Plant and Soil.*** 153: 207-214.
15. **Thongbai P**, Graham R D, Neate S M and Webb M J. 1993. Interaction between zinc nutritional status of cereals and *Rhizoctonia* root rot severity. II. Effect of Zn on disease severity of wheat under controlled conditions. ***Plant and Soil.*** 153: 215-222.
16. **Thongbai P**, Webb M J and Graham R D. 1993. Zinc Deficiency predisposes winter cereals to *Rhizoctonia* root rot. *In* **Plant Nutrition from Genetic Engineering to Field Practices**. M J Barrow (ed). Kulwer Academic Publishers, Dordrecht, The Netherlands. pp 681-684.

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18. Puckridge D W, Wiengweera A, **Thongbai P**, Sangtong P, Sattarasart A, Kongchum M and Runtan S. 1991 Nitrogen uptake by deepwater rice during the pre-flood and flooded phases, in relation to dry matter and grain production. ***Field Crop Res***. 27:315-336.

19. Puckridge D W, Catling H D, Vongsaroj P, Boonyawivatana S, Niyomwit L and **Thongbai P**. 1989. Factors affecting yield of deepwater rice in the Central Plain of Thailand. ***Field Crop Res.*** 19:263-283.

20. **Thongbai P**, Puckridge D W, Sangthong P and Sattarasart A. 1988. Residual effects of rock phosphate and triple superphosphate in acid sulphate soil. *In* **Proceedings of 1987 International Deepwater Rice Workshop**. IRRI, Los Baños, Philippines. pp 439-448.

21. Puckridge D W and **Thongbai P**. 1988. Fertilizer management in deepwater rice. In **Proceedings of 1987 International Deepwater Rice Workshop.** IRRI, Los Baños, Philippines pp 387-406.

22. Puckridge D W, Chankasem L, Vongsaroj P, **Thongbai P** and Chinawong S. 1988. Effect of tillage and sowing methods on control of wild rice (*Oryza rufipogon*) in deepwater rice. In **the Proceedings of 1987 International Deepwater Rice Workshop.** IRRI, Los Baños, Philippines pp 593-598.

23. Kupkanchanakul T, Puckridge D W, Petchrit K, Kupkanchanakul K and **Thongbai P**. 1986. Production from deepwater rice and early maturing rice intercrops with different row combinations. ***Field Crop Res***. 14: 53-62.

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1. **Thongbai P**, Panichagoon P, Potiruksanont T and Sattarasart A. 1986. Research on Rice and Rice-based cropping systems, Northeastern Thailand, 1976-1986: A Synopsis of Research Programs and Individual Projects. International Rice Research Institute, Bangkok, Thailand. 170p.

2. Puckridge D W, Panichagoon P and **Thongbai P**.1988. Analysis of floodwater patterns. In the **Proceedings of 1987 International Deepwater Rice Workshop.** IRRI, Los Baños, Philippines. pp 35-46.

3. Lek Tek Seng, Puckridge D W and **Thongbai P**. 1988. Cultivation of deepwater rice and floating rice in Kampuchea. In the **Proceedings of 1987 International Deepwater Rice Workshop**. IRRI, Los Baños, Philippines. pp 159-167.

4. Puckridge D W & **Thongbai P** (eds). 1995. Proceedings for Planning Meeting of the Thai – IRRI Collaborative Research and Training Program 1994/1995. International Rice Research Institute, Bangkok, Thailand.

7.4. งานวิจัยที่กำลังทำ :

1. กลุ่มวิจัยวิจัยและพัฒนาพลังงานชีวภาพของมหาวิทยาลัยแม่ฟ้าหลวง
2. **โครงการความร่วมมือระหว่างมหาวิทยาลัยแม่ฟ้าหลวงกับบริษัทโตโยไทย จำกัด (มหาชน)เพื่อการวิจัยและพัฒนาสบู่ดำเพื่อการผลิตเป็นการค้า ระยะที่ 1**

ประวัติผู้ร่วมงานวิจัย (1)

1 ชื่อ **ภาษาไทย** นางสาวสมฤดี อ้นโต

ภาษาอังกฤษ SOMRUDEE ONTO

2 ระบุรหัสประจำตัวนักวิจัยแห่งชาติ -

3. ตำแหน่งปัจจุบัน อาจารย์

4. สถานที่ทำงานสำนักวิชาวิทยาศาสตร์ มหาวิทยาลัยแม่ฟ้าหลวง

333 หมู่ 1 ตำบลท่าสุด อำเภอเมือง จังหวัดเชียงราย 57100

โทรศัพท์ 053-916778

5 ประวัติการศึกษา

ปีจบการศึกษา	ระดับปริญญา (ตรี โท เอก)	อักษรย่อปริญญา และชื่อเต็มวิชาเอก	สาขา	ชื่อสถาบัน	ประเทศ
2543	ปริญญาตรี	วท.บ	ชีววิทยาพันธุศาสตร์	ม. เกษตรศาสตร์	ไทย
2547	ปริญญาโท	วท.ม	พันธุศาสตร์	ม. เกษตรศาสตร์	ไทย

6 สาขาวิชาที่เชี่ยวชาญ

- Genetics
- Molecular genetics

7. ประสบการณ์ที่เกี่ยวข้องกับการทำงานวิจัยทั้งในและต่างประเทศ โดยระบุสถานภาพในการทำงานวิจัย ว่าเป็นผู้อำนวยการแผนงานวิจัย หัวหน้าโครงการวิจัย หรือผู้ร่วมวิจัยในแต่ละข้อเสนอการวิจัย

7.1 ผู้อำนวยการแผนงานวิจัย : ระบุชื่อแผนงานวิจัย

7.2 หัวหน้าโครงการวิจัย : ชื่อโครงการวิจัย

1) หัวหน้าโครงการวิจัยเรื่อง "การศึกษาความหลากหลายทางพันธุกรรมของแตงกวาที่ต้านทานต่อโรคราน้ำค้างโดยใช้เทคนิค AFLP"

2) ผู้ร่วมวิจัยโครงการวิจัยเรื่อง "การศึกษาความสัมพันธ์ทางพันธุกรรมของสับปะรดพันธุ์ภูแล นางแล และภูเก็ต โดยใช้วิธีอาร์เอพีดี (RAPD analysis)"