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THESIS

DESCRIPTION OF GROWTH STAGES AND EFFECT OF MINERAL AND ORGANIC FERTILIZERS ON JATROPHA (*Jatropha curcas* L.)

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A Thesis Submitted in Partial Fulfillment of
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Nyi Nyi 2012: Description of Growth Stages and Effect of Mineral and Organic Fertilizers on Jatropha (*Jathopha curcas* L.). Doctor of Philosophy (Tropical Agriculture), Major Field: Tropical Agriculture, Faculty of Agriculture at Kamphaeng Saen. Thesis Advisor: Mr. Weeraphan Sridokchan, Ph.D. 62 pages.

Jatropha is a deciduous plant and grown in tropical regions for producing biodiesel.The plant is newly cultivated and not much about the crop is known. This research was done with the objectives of (1) to describe the growth stages of jatropha and (2) to evaluate the effect of mineral fertilizers and pig manure foliar fertilizer on jatropha plants established by seedling and stem cutting, and (3) to determine relationships between chlorophyll meter reading, nitrogen content, photosynthetic pigments and chlorophyll fluorescence in leaves.

Phenological growth stages of jatropha plant can be described using BBCH scale; 7 principal growth stages comprise of 21 secondary growth stages.

Three-years old jatropha plants of Korat variety grown by seedings and cuttings were applied by six fertilizer treatments viz. urea (N), N+triple super phosphate (N+TSP), N+muriate of potash (N+K), N+TSP+K, organic fertilizer of pig manure foliar fertilizer(PMF), and control (no fertilisation). Plants grown from seedlings were higher in height, canopy diameter, total number of branch (Tbr) and branch diameter (Dia) than grown from cuttings, except leaf greenness by SPAD reading. Plants treated with mineral fertilizers were higher in SPAD reading, height, canopy diameter, Tbr, and Dia than pig manure foliar fertilizer. Plants from cutting were higher in inflorescence bearing branches (Bbr), total number of fruits (Tfr), total number of seeds (Tsd), and seed weight (Swt) than seedings. N:P ratio in mineral fertilizer treatments were higher than that of pig manure foliar fertilizer treatment . N treatment showed the highest yield of 109.68 g. There was no difference in kernel oil percentage, and seed cake N content.

There were good relationships between SPAD reading with total N concentration (R^2=0.99), as well as with content of chlorophyll *a* (R^2=0.97), chlorophyll *b* (R^2=0.96) and carotenoid (R^2=0.75). Maximum fluorescence variable (F_m) and maximum quantum efficiency (F_v/F_m) were linearly increased with SPAD value and became stable when the SPAD value was higher than 20 until 60. The sharp decrease in F_v/F_m when SPAD value was less than 20 refers to the critical point of photosynthesis efficiency in jatropha leaf.

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Student's signature Thesis Advisor's signature

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LIST OF ABBREVIATIONS

ADP	=	Adenosine diphosphate
Al	=	Aluminium
ATP	=	Adenosine triphosphate
BBCH	=	Biologische Bundesanstalt, Bundessortenamt, CHemische Industrie
Bbr	=	Bearing branch
ºC	=	Degree celsius
C.V	=	Coefficient of variation
Ca	=	Calcium
Car	=	Carotenoid
Chl	=	Chlorphyll
cm	=	centimeter
df	=	Degrees of freedom
Dia	=	branch diameter
DMSO		Dimethylsulphoxide
EC	=	Electrical conductivity
Fe	=	Iron
F_m	=	Maximum fluorescence (dark adapted)
Fsp	=	Filled seed percentage
F_v/F_m	=	Variable fluorescence (dark adapted)
g	=	gram
H_2O_2	=	Hydrogen peroxide
ha	=	hectare
HCl	=	Hydrochloric acid
HNO_3	=	Nitric acid
hr	=	hour
Hsw	=	Hundred seed weight
ICP-OES	=	Inductively coupled plasma - optical emission spectrometry

LIST OF ABBREVIATIONS (Continued)

K	=	Potassium
Kg	=	Kilogram
L	=	Liter
LHCII	=	Light garvesting complex II
LSD	=	Lease significant difference
m	=	meter
mm	=	milimeter
Mg	=	Magnesium
mg	=	Milligram
min	=	minute
Mn	=	Manganese
MS	=	Mean Square
n	=	Number of observation
N	=	Nitrogen
NADPH		Nicotinamide adenine dinucleotide phosphate
NH_4N	=	Ammonia nitrogen
nm	=	nano meter
NO_3N	=	Nitrate nitrogen
NPQ	=	nonphotochemical quenching
ns	=	not significant
OM	=	Organic matter
P	=	Probability
P	=	Phosphorus
pH	=	Logarithm of hydrogen ion concentration
PMF	=	Pig manure foliar fertilizer
ppm	=	Parts per million
PSII		Photosystem II
r		Pearson's correlation coefficient
RCBD	=	Randomized complete block design

LIST OF ABBREVIATIONS (Continued)

RGR	=	Relative growth rate
S	=	sulphur
SAR	=	Specific absorption rate
SD	=	Standard deviation
SLA	=	Specific leaf area
SOV	=	Source of variation
SPAD	=	Soil and plant analysis development
Swt	=	Seed weight per plant
Tbr	=	Total number of branches per plant
Tfr	=	Total number of fruits per plant
Tsd	=	Total number of seeds per plant
TSP	=	Triple super phosphate
$\mu mol/m^2$	=	micromole per square meter
v/v	=	Volume/volume
Yield	=	Effective quantum yield
Zn	=	Zinc

DESCRIPTION OF GROWTH STAGES AND EFFECT OF MINERAL AND ORGANIC FERTILIZERS ON JATROPHA (*Jatropha curcas* L.)

INTRODUCTION

Jatropha is a deciduous small plant belongs to family Euphorbiaceae. It was believed native to Central America and nowadays found in all tropical regions. It is not grazed by animals, can be grown in poor or stony soil, drought and disease resistant, multipurpose and yield oil which can be made into high quality biodiesel. With aspects of these characters, jatropha proved to be superior over other non edible oil plants and plant might be grown as commercial plantation crop in very near future.

Knowledge of the phenology of crop is essential for the correct timing of management practices such as fertilizer application, disease, insect pest and weed control. Although plant growth and yield is influenced by application of fertilizers, little information on jatropha plant was found. Jatropha has been mainly propagated by seedlings and stem cuttings. Leaf chlorophyll (Chl) content is an important indicator of the physiology status of the plant. There are reports that showed significant relationship between chlorophyll meter value and leaf Nitrogen (N) content in many crop species. The Chl meter, Soil Plant Analysis Development (SPAD) meter is a simple and portable diagnostic tool that measures the greenness on Chl content of plants. Even though jatropha has gained the interests for large plantation, there is no research on using SPAD portable Chl meter. The information can be useful for fertilizer management and physiological studies in future research works.

OBJECTIVES

1. To describe the growth stages of jatropha by using the uniform decimal code BBCH scale.

2. To evaluate the effect of mineral fertilizers and pig manure foliar fertilizer on jatropha plants established by different types of propagation method (seedling and stem cutting).

3. To establish relationships of chlorophyll meter reading, nitrogen content, photosynthetic pigments and chlorophyll fluorescence.

LITERATURE REVIEW

Jatropha

Jatropha is a deciduous plant which can reach a height of up to 5 m. The plant shows articulated growth with a discontinuity at each increment. Plant dormancy is induced by fluctuation in rainfall, temperature and light (Heller, 1996).

In 2008, jatropha was planted 900,000 ha globally and 760,000 ha (85 percent) in Asia. It was estimated that 12.8 million ha would be grown by the year of 2015 all over the world, and the largest producer in Asia would be Indonesia. Growth of biofuel industry is driven by government policy due to three main reasons, i.e. climate change, energy security and strategy to support rural developments. As it is a wild plant, its seed yield is highly variable. Information on the agronomy of jatropha and cultural practices contributing to yield is less known (Brittaine and Lutaladio, 2010).

There are some projects to develop new industries based on jatropha oil, China National Offshore Oil Corporation, one of the largest state-owned oil companies in China, has begun to build a 60,000 ton biodiesel factory in Hainan province based on the use of jatropha oil (Aizhu, 2010). Recently, Air New Zealand has completed a two-hour test flight powered by a 1:1 blend of biodiesel made of jatropha oil and conventional jet fuel (Wassener, 2008). Currently, Sinopec Group is the only state-owned energy firm producing biodiesel in China. It has a pilot biodiesel plant in the northern Hebei province, which produces 2,000 tons a year (Zhang, 2010).

Although plant growth and yield is influenced by application of fertilizers, little information on jatropha plant was found. However, jatropha plant might be grown as a commercial plantation crop in very near future. Jatropha has been mainly propagated by both seedlings and stem cuttings. Depending on type of seedling the rooting pattern is different. Seedlings develop thick primary tap root with four lateral roots and with abundant and straight lateral roots, whereas plants propagated by cutting only develop secondary roots (Heller, 1996). Therefore, this study was done to

evaluate the effect of mineral fertilizers and pig manure foliar fertilizer (PMF) on jatropha plants established by seedling and stem cutting.

Growth Stages and BBCH Scale

Phenology is defined as the study of seasonal timing of life cycle events (Rathcke and Lacey, 1985). Knowledge of crop phenology is fundamental in plant-related sciences. It comprises (a) a useful tool for scheduling management applications (e.g. irrigation, fertilization, pesticide application) (Arcila-Pulgarin *et al.*, 2002; Proctor *et al.*, 2003), (b) the basis for the construction of crop growth simulation models and (c) a reference for assessing crop performance under variable inputs and conditions (Barłóg and Grzebisz, 2004).

Based upon Zadoks descriptions of cereals (Zadoks *et al.*, 1974), a general, uniform decimal code, known as the Biologische Bundesanstalt, Bundessortenamt, CHemische Industrie (BBCH) scale, was proposed by Blei-holder et al. (1991) and Lancashire et al. (1991). A more advanced scale, the extended BBCH scale, was proposed by Hack et al. (1992) and Hess et al. (1997). Later, the 'BBCH–Monograph' representing a group of 27 crops and weeds was published (Meier, 2001). Recently the Global Phenological Monitoring Network introduced and accepted the BBCH scale and its coding system to be used as the standard system to describe phenological stages of plants (van Vliet *et al.*, 2003).

The extended BBCH-scale is a system for a uniform coding of phenologically similar growth stages of all mono- and di-cotyledonous plant species. The entire development of the plants is subdivided into ten clearly recognizable and distinguishable longer-lasting developmental phases. These principal growth stages are described using numbers from 0 to 9 in ascending order. The principal growth stages are described in Table 1.

Table 1 Principal growth stages of the extended BBCH scale

Stage	Description
0	Germination/ sprouting/ bud development
1	Leaf development from main stem
2	Formation of branches (omitted in tree)
3	Stem elongation (main shoot, skipped in jatropha)
4	Development of harvestable vegetative plant parts (this stage is not concerned with jatropha and omitted)
5	Inflorescence emergence (main shoot)/ heading (date and percentage on total inflorescence)
6	Flowering (main shoot) (date and percentage on total flowering)
7	Development of fruit
8	Ripening or maturity of fruit and seed
9	Senescence, beginning of dormancy

Source: Adapted from Hess *et al*. (1997)

Secondary stages are used if points of time or steps in the plant development must be indicated precisely. In contrast to principal growth stages they are defined as short developmental steps characteristic of the respective plant species, which are passed successively during the respective principal growth stage. They are also coded by using the figures 0 to 9. The combination of figures for the principal and the secondary stages results in the two-digit code. An arithmetically greater code indicates a plant at a later growth stage. The principle growth stages need not to proceed in strict sequence, but can occasionally proceed in parallel. If two or more principal growth stages proceed in parallel, both can be indicated by using a diagonal stroke (example 51/69). If only one stage is to be indicated, either the more advance growth stage must be chosen or the principal growth stage of particular interest.

The time span of certain developmental phases of a plant can be exactly defined and coded by indicating two stages. For this purpose tow codes are connected with a hyphen. For instance, the code (51-69) describes the developmental phase from the appearance of the first inflorescence or flower buds until the end of flowering.

It is necessary to use primarily phenological criteria rather than homologous or analogous stages. Thus, germination of plants from true seed and sprouting from buds are classified in one principal growth stage, the principal growth stage 0, even though they are completely different biological process. In case of the BBCH-scales the descriptions are based on the actual characteristic features of the individual plant. If the scales are used for the definition of the development stage of a plant stand, the description should apply to at least 50% of the plants (Hack *et al.*, 1992). According to this universal scale, using phenological criteria and a consistent set of numeric codes, it is possible to establish a uniform coding to describe the growth stages of a large number of plant species (Arcila-Pulgarin *et al.*, 2002).

Jatropha Growth

Jatropha is unusual among tree crop, its most unusual feature is its modular construction. The plant's branching pattern and position of inflorescence conform to Leeuwenberg's model in the classification of architectural types introduced by Halle (1986). Each branch or axis terminates with an inflorescence, and 1-3 new orthotropic branches may begin to grow out from the axils of the last leaves formed by the apical meristem, at the same time the inflorescence is initiated. Other branches usually grow out from nodes near the base of the main stem and reiterate the architectural model. Each branch, along with its terminal inflorescence, constitutes a module and growth occurs by means of the more-or-less continuous accretion of modules. Plant growth and reproduction are thus tightly related in jatropha (Aker, 1997).

Leaves are 3-5 lobed, cordiform, stipules and deciduous. Inflorescence is complex and monoecious with protandry. First branching is racemose and subsequent is cyme. The inflorescence is axillary paniculate polychasial cymes formed terminally

on branches, possessing main and co-florescences with paracladia. Flowers are unisexual, monoeceious, greenish yellow colored in terminal long, peduncled paniculate cymes. (Divakara *et al.*, 2010). There are fewer female than male flowers and these are carried on the apex of the inflorescence, with the more numerous males borne lower down. The ratio of male to female flowers averages 29:1 but this is highly variable and may range from 25-93 male flowers to 1-5 female flowers produced on each inflorescence (Raju and Ezradanam, 2002). Normally the male to female ratio varies from 16/27: 1 to 108: 1 (Surwenshi *et al.*, 2011). The ratio varies from plant to plant, population to population and it also changes with time, climate and nutrition (Chang-wei *et al.*, 2007). The fruits are ellipsoidal, green and fleshy, turning yellow and then brown as they age. Fruits are mature and ready to harvest around 90 days after flowering. Jatropha is not sensitive to day length (flowering is independent of latitude) and may flower at any time of the year (Heller, 1996). Rainfall induces flowering and, in areas of unimodal rainfall, flowering is continuous throughout most of the year. Flowering and fruiting are continuous, consequently mature and immature fruits are borne together. Each fruit contains two or three black seeds, around 2 cm x 1 cm in size. On average, the seeds contain 35 percent of non-edible oil (Brittaine and Lutaladio, 2010).

Plant Nutrition

Deficiencies of essential macronutrients and micronutrients as well as nutrient imbalance cause lower rate of photosynthesis. However, the concentration of each element can vary over a fairly wide range in leaves without significantly altering the rate of photosynthesis. Although chlorosis and necrosis of leaf tissues may accompany the decreased photosynthetic capacity of mineral-deficient leaves, photosynthesis is commonly reduced even when such visible symptoms are not evident. The effects of mineral nutrients on photosynthesis are complex and may be both direct and indirect. In mineral-deficient leaves the rate of net photosynthesis may be reduced by depressed chlorophyll synthesis, decreased capacity for photosynthetic electron transport, lowered activity of carboxylating and other enzymes, decreased

stomatal conductance and increased respiration. In a long term, total photosynthesis of mineral-deficient plants is greatly reduced by a decrease in leaf area.

Increased rates of photosynthesis often, but not always follow additions of fertilizer to woody plants. The response to fertilizer varies with amount, timing, and composition of the fertilizer, vigor; species, and age of the tree, stand density, soil moisture content, soil fertility, temperature, and light conditions (Pallardy and Kozlowski, 2008).

Although plant growth and yield is influenced by application of fertilizers, little information on jatropha plant was available. Although, jatropha plant might be grown as commercial plantation crop in very near future. Jatropha has been mainly propagated by seedlings and stem cuttings. Depending on type of seedling the rooting pattern is different, seedlings develop thick primary tap root and four lateral roots and with abundant and straight lateral roots, whereas plants propagated by cutting only develop secondary roots (Heller, 1996).

The essential role of N as a constituent of amino acids, the building blocks of proteins, is well known. It occurs in a variety of other compounds such as purines and alkaloids, enzymes, vitamins, hormones, nucleic acids, and nucleotides. Both leaf area development and photosynthesis depend greatly on N supply. Nitrogen deficiency is accompanied by failure to synthesize normal amounts of chlorophyll, resulting in chlorosis of older leaves and, when deficiency is severe, of young leaves also. In fruit and nut trees, N deficiency may be associated with leaf abscission, decreased fruit set, poorly developed fruit buds, and small and early maturing fruits (Pallardy and Kozlowski, 2008).

Nitrogen (N) used for tree growth can come from several sources. In addition to fertilizers and the mineralization of soil organic matter, a significant input can come from atmospheric deposition (Pearson and Stewart, 1993). A major contribution can also come from N stored within the tree itself, through internal cycling. In deciduous trees such internal cycling comprises the storage of N during winter and

remobilisation of N from store in the spring for new growth. During summer, the leaves become dominant sink for N and the annual cycle is completed when a proportion of leaf N is withdrawn during canopy senescence, for storage again during winter (Millard, 1995). Many deciduous fruit orchards show little response to the application of N fertilizers. Mineralisation of soil organic matter and atmospheric deposition may provide sufficient N for uptake by the trees (Tagliavini *et al.*, 1980). Although the N fertilization can promote fruiting (Titus and Kang, 1982), generous N supply can reduce the quality of fruit (Tagliavini *et al.*, 1980).

Phosphorus plays an indispensable role as a universal fuel for all biochemical activity in living cells. High-energy adenosine triphosphate (ATP) bonds release energy for work, when converted to adenosine diphosphate (ADP). Beyond their role in energy-transferring processes, phosphate bonds serve as important linkage groups. Phosphate is a structural component of phospholipids, nucleic acids, nucleotides, coenzymes, and phosphoproteins. Phospholipids are important in membrane structure. Nucleic acids of genes and chromosomes carry genetic material from cell to cell. As a monoester, phosphorus provides an essential ligand in enzymatic catalysis. Phytic acid, the hexaphosphate ester of myo-inositol phosphate, is the most common phosphorus reserve in seeds. Inorganic and organic phosphates in plants also serve as buffers in the maintenance of cellular pH (Sanchez, 2007).

Phosphorus is mobile in plants and, when a deficiency of phosphorus occurs, phosphorus is moved from the older leaves to the younger leaves at the top of the plant thus deficiency symptoms first appear on the older, or bottommost leaves. The deficiency symptom is commonly a purplish color as a result of increased anthocyanin development in phosphorus deficient tissue. Some of the phosphorus deficiency symptoms of plants are not specific. The growth of both shoots and roots is greatly reduced. The major problem in phosphorus uptake from soils by roots is the very low solubility of most phosphorus compounds, resulting in a low concentration of phosphate ions in the soil solution at any one time.

Availability the phosphorous ions in the soil solution are a function of pH. Above pH 7.2, phosphate ion tends to precipitate as insoluble calcium phosphate compounds that are slowly converted to apatite. In acid soils, there is much less calcium (Ca^{2+}) and much more alumunium (Al^{3+}) and iron (Fe^{3+}) in solution than in calcareous soils. This results in precipitation of phosphate ions as insoluble aluminum and iron phosphates. The formation of these insoluble calcium, aluminum, and iron compounds is called phosphorus fixation. The optimum pH for phosphorus availability is near 6.5, where there is the least potential for phosphorus fixation. Commonly, only 10 to 20 percent of phosphorus in fertilizer is used by plants during the year of fertilizer application. Therefore, repeated use of phosphorus fertilizers results in an increase in soil phosphorus content, and in many instances, soils have become sufficiently high in phosphorus that further additions of phosphorus fertilizers do not increase plant growth (Foth, 1990).

Potassium (K) is the primary osmolyte and ion involved in plant cell membrane dynamics, including the regulation of stomata and the maintenance of turgor and osmotic equilibrium. It also plays important roles in activation and regulation of enzyme activity. Potassium is a soil exchangeable cation and is actively absorbed by plant roots. It is a major component of many soils and is ultimately derived from the weathering of soil parent materials such as potassium-aluminum-silicates in the soil. Potassium, though a part of the cation exchange complex, is only weakly held to the soil particles and is highly leachable. Potassium deficient plants generally form necrotic lesions or more generalized leaf necrosis after a relatively short period of chlorosis. In severely limiting conditions, there can be general bud death. As with nitrogen deficiency, symptoms of potassium deficiency first tend to appear in more mature leaves, as the plant will move potassium to actively growing, younger tissues. Most plants require potassium in fairly high concentration, and as a result, potassium is a common major constituent of commercial fertilizers, particularly in agricultural systems where the removal of plant parts (e.g., fruits) from the site strip potassium from the local cycling system. Sodium, another monovalent cation, can sometimes substitute for potassium in certain plants (Wiedenhoeft, 2006).

In addition to nutrients being added to the soil as fertilizers, some mineral nutrients can be applied to the leaves as sprays, in a process known as foliar application, and the leaves can absorb the applied nutrients. In some cases, this method can have agronomic advantages over the application of nutrients to the soil. Foliar application can reduce the lag time between application and uptake by the plant, which could be important during a phase of rapid growth. It can also circumvent the problem of restricted uptake of nutrients from the soil. For example, foliar application of mineral nutrients such as iron, manganese, and copper may be more efficient than application through the soil, where they are adsorbed on soil particles and hence are less available to the root system. Nutrient uptake by plant leaves is most effective when the nutrient solution remains on the leaf as a thin film (Mengel and Kirkby 1987). Production of a thin film often requires that the nutrient solutions be supplemented with surfactant chemicals, such as the detergent, that reduce surface tension. Nutrient movement into the plant seems to involve diffusion through the cuticle and uptake by leaf cells. For foliar nutrient application to be successful, damage to the leaves must be minimized. If foliar sprays are applied on a hot day, when evaporation is high, salts may accumulate on the leaf surface and cause burning or scorching. Spraying on cool days or in the evening helps to alleviate this problem. Addition of lime to the spray diminishes the solubility of many nutrients and limits toxicity. Foliar application has proved economically successful mainly with tree crops and vines such as grapes, but it is also used with cereals. Nutrients applied to the leaves could save an orchard or vineyard when soil-applied nutrients would be too slow to correct a deficiency. In wheat, nitrogen applied to the leaves during the later stages of growth enhances the protein content of seeds (Taiz and zeiger, 2002).

Recycling of the waste from a farm is the heart of sustainable farming system. Annually, Thailand produce 15 million head of pigs and most of the pig farms have waste treatment system. Recently, utilization of pig farm waste as fertilizer for plant production has increased. Pig farm waste including pig manure, pig manure extract, pig farm waste water and slurry were used as soil and foliar fertilizer for rice, cassava, sugarcane, vegetable, orchards and ornamental plants since 2003. The results on these crops showed the pig farm waste is effective organic fertilizer for improving yield and

quality of the crop, if it was applied in appropriate methods and quantity (Kanto, 2011).

Photosynthetic Pigments

Leaf chlorophyll (Chl) content is an important indicator of the physiology status of the plant. Chl *a* and Chl *b* are essential pigments in converting light energy to chemical energy (Steele *et al.*, 2008) thus the Chl contents are directly linked to primary production of plants (Curran *et al.*, 1990). Chl content is positively related with leaf N concentration, and the SPAD estimation can be linked to foliar N content of plant species including hardwood plants (Chang and Robison, 2003). There are reports that showed significant relationship between cholorphyll meter value and leaf Nitrogen (N) content in many crop species.

The Chl meter, Soil Plant Analysis Development (SPAD) meter is a simple and portable diagnostic tool that measures the greenness on Chl content of plants. The SPAD meter estimates the Chl content in leaf by emitting two wavelengths of 650 nm (red) and 940 nm (far red). Which are transmitted alternately through the leaf. The SPAD value is calculated instantly from the optical density differential between the red and infrared wavelength detected by the photodiode (MINOLTA, 1989). SPAD meter directly detect the Chl *a* and *b*, it can provide Carotenoid (*Car*) content in leaf indirectly. Two main functions of *Car* are photoprotection and light collection. Xanthophyll cycle is a group of *Car* and play important role of photo-protection in photosynthesis (Demmig-Adams *et al.*, 1996).

The method of chlorophyll fluorescence can be employed to investigate the photosynthetic performance of the plant. Light energy trapped by the photosynthetic pigments cause primary photochemical reactions. When there is no water splitting (charge separation) occur, excited molecules release energy as heat and/or chlorophyll fluorescence (Krause and Weis, 1991). Fluorescence can be measured easily and different components of fluorescence (photochemcial and non photochemical) variables revealed the aspects of photosynthesis process (Roháček, 2002). Chl *a*

fluorescence is a small fraction (1-3%) of dissipated energy from photosystem II (PSII) and it can serve as the probe for the various types of stresses in the plant photosynthesis system (Schreiber *et al.*, 1988). There are information on association between fluorescence variables and SPAD meter reading in some crops such as papaya, coffee and some trees (Netto *et al.*, 2005; Netto *et al.*, 2002; Percival *et al.*, 2008) but not in jatropha. Association between SPAD readings and fluorescence measurement can provide important information for interpretation of data from chlorophyll meter.

MATERIALS AND METHODS

1. Description of jatropha growth stages

1.1 Plant materials

Seeds of seventeen jatropha nut cultivars including 9 Thailand cultivars, 6 Myanmar cultivars, 1 India cultivar and 1 Vietnam cultivar, were collected from its host countries (Appendix Table 1). All the seeds were checked for the physical damage and kept under room temperature.

1.2 Culture condition

Plastic bags (12 cm x 18 cm) were filled with loamy soil for seedling. The jatropha seeds were laid under the soil about 2 cm deep and watered twice a week. The nursery was set under the 50% shading net. Four month old seedlings were transplanted to the field at the spacing of 2m x 2m. Fertilizer and irrigation were applied as necessary.

1.3 Observed variables

For the germination stage, number of days to germination and germination percentage were recorded. During the vegetative phase, plant height, number of leaves (including abscised leaves), stem diameter at the hypocotyls node, length of internodes, number of branches and length of branches were measured. For the reproductive phase, number of inflorescence, length of inflorescence, date of flowering, number of pistilate/staminate flowers, number of fruits set in each inflorescence, diameter and length, and number of fruits were observed twice a week. Meteorological data was obtained from Kamphaeng Saen agro-meterological station (Appendix Figure 1).

1.4 Statistical analysis

A completely randomized design (CRD) was used where 10 plants for each variety were observed as a sample. Descriptive statistics, percentile values and box plot were used to reveal differences among jatropha accessions.

2. Effect of mineral and organic fertilizers on seedling and cutting

2.1. Plant materials

Three years old jatropha standing plants (Korat accession) established by seedlings and cuttings with 2 m x 2 m spacing were used as plant material. Plants were heavily pruned twice, first in February 2009 (summer) and second in July 2009 (on set of rainy season) while the plants were flowering so as to suppress the nutrient cycling of the plant. The soil moisture was maintained not more than 60 centibar at 30 cm depth by using six tensiometers and drip irrigation system.

2.2 Experiment

The experiment was conducted in a field at Kamphaeng Saen campus, Kasetsart University, Thailand (14º 01´N, 94º 59´ E and 7.5 m above sea level) during February 2009 to February 2010. Agro-meteorological data were presented in appendix figure 2 and 3. The soil was silty loam, available phosphorus (P) was high amount and low in potassium (K) (Table 2). Pig manure foliar fertilizer (PMF) had large amount of Ca, Mg and Fe in addition to N, P and K.

The experiment was set up as split-plot in Randomized Complete Block Design (RCBD) with three replications. Type of seedlings (cutting and seedlings) and fertilizer treatments were assigned as whole plot factor (Factor A) and subplot factor (Factor B), respectively. Applications of fertilizer were started one month after the 2nd heavy pruning. There were six treatments viz. T1=urea (N), T2=N+triple super phosphate (N+TSP), T3= N+muriate of potash (N+K), T4=N+TSP+K, T5=organic

fertilizer of pig manure foliar fertilizer (PMF), and T6= control (no additional fertilizer). Mineral fertilizers were applied at the rate of 60 g N/plant, 40 g P/plant and 20 g K/plant. Nitrogen (N) fertilizer was split into three times with one month interval while P and K were used as basal application one month after second pruning. Pig manure foliar fertilizer (PMF) was prepared (1:10 v/v) with tap water and few drops of surfactant. Application rate was 350 ml/plant fortnightly for six times during vegetative phase. Each subplot had four plants and encircled with border plants, altogether there were 144 plants were observed in this experiment.

2.3 Observed variables

Plant height, canopy size were measured two-week interval. Mean value of two diagonal measurements were used to estimate the diameter of canopy assuming that was circle. Total number of branch (Tbr), mean value of five branches diameter (Dia) were measured at 1.0 m above soil level for each plants and inflorescences bearing branch (Bbr) were recorded. Mature and healthy leaf was collected between 7^{th} and 9^{th} leaf under the inflorescence (Laviola and Dias, 2008), while 50% of fruit set is visible (5mm) or the plant growth stage 71 of BBCH scale (Hess *et al.*, 1997).

2.4 Sample preparation

Leaf samples were washed with tap water, 0.01 M HCl and finally rinsed in de-ionized water., Leaf greenness (SPAD readings), leaf area, leaf fresh and dry weight were recorded. After air-dried for 72 hr at 45^oC, leaf samples were finely ground and sealed in the zip plastic bags and kept in a dessicator. Ground leaf sample of 0.5 gm was digested with 7.5 ml of concentrated HNO_3 at 150^oC for 30 min. Two drops of H_2O_2 was added while it started to boil. After cooling the test tube, the volume was made up to 25 ml by using volumetric flasks and kept at 4^oC until chemical analysis. Eight mineral elements namely phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S), iron (Fe), manganese (Mn) and zinc (Zn) concentration in samples were determined by inductively coupled plasma - optical

emission spectrometry (ICP-OES). Total N was determined by micro Kjeldahl method.

Fruits were harvested during November 2009 until end of February 2010, until the plant became dormant. Fruits were sun dried, and de-husked manually, filled seed percentage (Fsp) and hundred seed weight (Hsw) was recorded. Kernel oil content was accessed by soxhlet extractor using petroleum ether as the solvent and kernel cake N content was determined.

3. Investigation on indicator of leaf nitrogen status, chlorophyll and carotenoid

3.3 Sample Collection

Leaf samples were collected from three years old jatropha (Korat variety) grown in a experiment field at Kasetsart University, Kamphaeng Saen, Thailand (14º 01´N, 94º 59´ E and 7.5 m above sea level) on 24th February 2010. Healthy mature leaves free from shading were collected. The leaves were kept in an ice box, washed with tap water and rinsed by deionized water. Leaf discs, each at the size of 117.7 mm^2 were cut avoiding the leaf vein. The mean value of three readings from portable Chl meter (SPAD-502) was recorded on each leaf disc.

3.4 Total N determination

The leaf discs were divided into 12 groups according to the SPAD reading values (0-5, 6-10, 11-15, …, 56-60), ranging from yellow to dark green colors. Minimum number of leaf discs in each group was 200. The leaf discs were dried in hot air oven at 50^{o}C for 72 h, then ground to pass 20 mesh net and stored in a desiccators until use. Ground leaf sample of 0.2 gm was digested with 4 ml of concentrated sulphuric acid in a digestion block at 350º C for 3 h. The volume was adjusted to 50 ml by deionized water, then 10 ml of the solution was used for determination of total N by micro-Kjeldahl method.

3.5 Chlorophyll extraction and analysis

Each leaf disc of SPAD value from 0 to 58 was cut into a smaller circular disc of the size 50.28 mm^2 and dipped into a test tube containing 4 ml of dimethylsulphoxide (DMSO). The test tube was wrapped with aluminum foil to protect from light, then incubated at 70° C in a hot water bath for 30 min (Hiscox and Israelstam, 1979). After cooling to room temperature, the extracted aliquot was analyzed spectrophotometrically by Genesys UV-10 at wavelengths of 480, 649 and 665 nm. The concentration of Chl *a*, Chl *b* and carotenoid (*Car*) were determined according to the equation suggested by Wellburn (1994).

3.6 Fluorescence measurement

The leaf discs of size 117.7 mm^2 and SPAD reading values ranging from 0-58 were saturated with deionized water by placing abaxial surface down on moist germinating paper sealed with polythene film which is permeable to air but not water (Percival and Sheriffs, 2002). Two sets of leaf disc samples were kept for 18 hr at ambient temperature and light.

Prior to dark adapted measurement, one set of leaf sample was kept in a dark cabinet for 30 min. Weak green light (5 W) was lit during working in the dark room where minimum fluorescence $F_{0,}$ maximum fluorescence F_m, and maximum quantum efficiency of PSII photochemistry (F_v/F_m) were recorded. F_v is the maximum variable fluorescence (F_m - F_0) when all nonphotochemical process are at the minimum, i.e. under the dark adapted situation. All the symbols and nomenclatures were used according to Kooten and Snel (1990). Light adapted measurement was performed using leaf clip holder (2030-B) and external halogen lamp (2150-H). Both light and dark adapted conditions were recorded by PAM-2100 fluometer at a standard setting. The intensity and duration of saturation pulse were preset following the hand book at 10 and 0.8 sec, respectively. Nonphotochemical quenching (NPQ) was calculated by the equation of Bilger and Bjorkman (1990) as $NPQ = \left(F_m/F_m^{'}\right) - 1$.

RESULTS AND DISSCUSSION

1. Description of jatropha growth stages

1.1 Phenological growth stages of jatropha

For fruit trees, the BBCH scale was applicable to eight out of the ten principal stages, beginning from germination (stage 0) and ending with initiation of dormancy (stage 9) (Finn et al, 2007). Three principal growth stages were assigned to vegetative growth, which describe germination/bud development (stage 0), leaf development (stage 1) and stem elongation (stage 3). The latter being shared with flower initiation (stage 5), flowering (stage 6), fruit growth (stage 7), and maturity of fruit (stage 8) and dormant or senescence (stage 9) to complete the code. For the vegetative development in jatropha plant, stem elongation (stage 3) can be skipped so as the leaf development and stem elongation running in parallel. Totally seven principal growth stages could be applicable in jatropha plant.

1.2 Principal growth stage 0; germination

The principal growth stage 0 of jatropha takes place in the soil. Stage 00 is given when the dry seed was deposited in the soil, and stage (05) is assigned whenever the radical penetrates from the seed. Formation of lateral roots is coded as stage (06). Hypocotyl with cotyledons growing toward soil surface (hook stage) is coded as stage (08) and emergence of cotyledons breaking through the soil surface is coded as stage (09) to complete the germination process (fig 1).

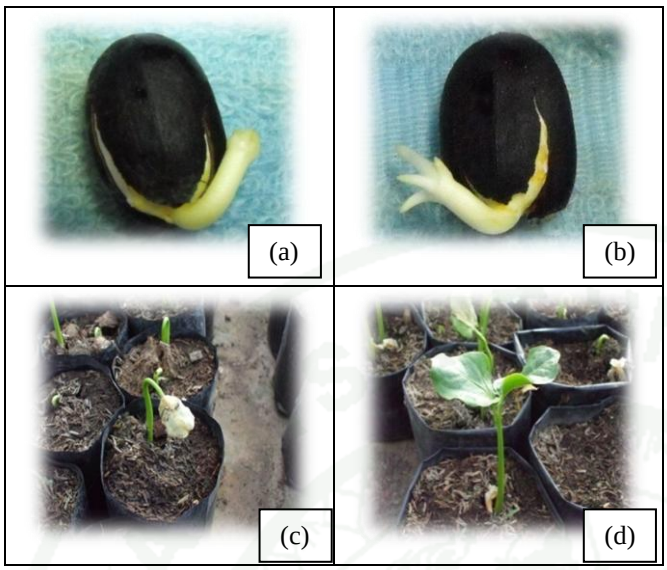

Figure 1 Principal growth stage 0; Germination
(a)Stage 05, radical penetrates from the seed coat
(b) Stage 06 formation of lateral roots
(c)Stage 08 hypocotyl emerges from soil surface with cotyledon (hook stage)
(d) Stage 09 cotyledon emergence

1.3 Principal growth stage 1; leaf development on the main stem

Leaf development stage describes aerial development of the young plant and coded as principal growth stage 1. It begins with the stage (10) when the cotyledons are completely unfolding (Fig 2). In jatropha, the subsequent development is characterized by the formation of one juvenile leaf (leaf margin is serrate and leaf blade is cordate). Followed by normal leaves (lobed leaf) which develop alternately on the main stem. The number of true leaves determine the coding of growth stages. A true leaf is regarded as the fully unfold green leaf. Abscised leaves were also included in the assessment. The abscised leaves can be recognized by the scars on stem. Leaf scars of cotyledonary leaves situate oppositely on main stem and serve as the markers for counting the leaf number. The leaf developmental stage runs until flower initiation. In this study, maximum number of leaves on main stem until flower initiation was 63 and thus it would be convenient to present as percentile value. For the leaf development on main stem, code (11) indicates 10% of leaves have developed

on main stem and continue until code (19) when 90% of leaves were developed on main stem.

1.4 Principal growth stage 2; formation of side shoots

Formation of side shoots occurred just before flower initiation. At that time, many leaves are emerged even form the abscised leaf scars. As for the tree, formation of side shoot development stage can be omitted as the BBCH scale is interested only on characteristic on the main stem.

1.5 Principal growth stage 3; main stem elongation

Elongation of main stem occurs parallel to the development of the leaves. For this reason, description and coding in principal growth stage 3 can be omitted. It is not a phenological phenomenon, rather dependent of growing conditions.

1.6 Principal growth stage 4; development of harvestable vegetative plant parts

As the jatropha is a tree crop and this growth stage code is not applicable and it could be omitted.

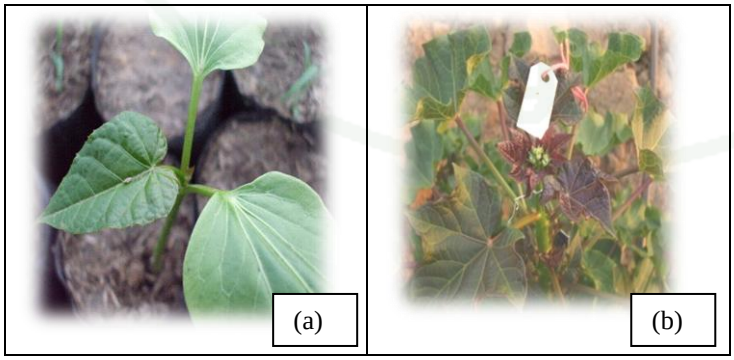

Figure 2 Principal growth stage 1; leaf development on the main stem
(a) Stage 11, two cotyledonary leaves situated oppositely and the juvenile leaf was unfold
(b) Stage 19 end of leaf development stage when inflorescence is visible (less than 5mm)

Figure 3 Principal growth stage 5; Inflorescence emergence
(a) Stage 51 inflorescence is visible (5 mm in size)
(b) Stage 55 individual flower buds are visible
(c) Stage 59 one or few flowers open and petals are visible

1.7 Principal growth stage 5; inflorescence emergence

This stage begins when the first inflorescence or flower bud is visible, code (51) and ends when the first flower petals are visible, code (59). During the inflorescence elongation stage, when individual flowers are visible it is defined the stage (55).

1.8 Principal growth stage 6; Flowering

Flowering stage is one of the principal stage and the code is (60). Secondary stage of flowering stage is described in percentile value. Thus code (61) stand for 10% of total flowers are open and (65) stands for 50 % of total flowers are open. The code (69) is given for the end of flowering. In jatropha male flowers are prominent and easily recognized. When the first male flower open it was code (60) and all the male flowers should be counted at this stage.

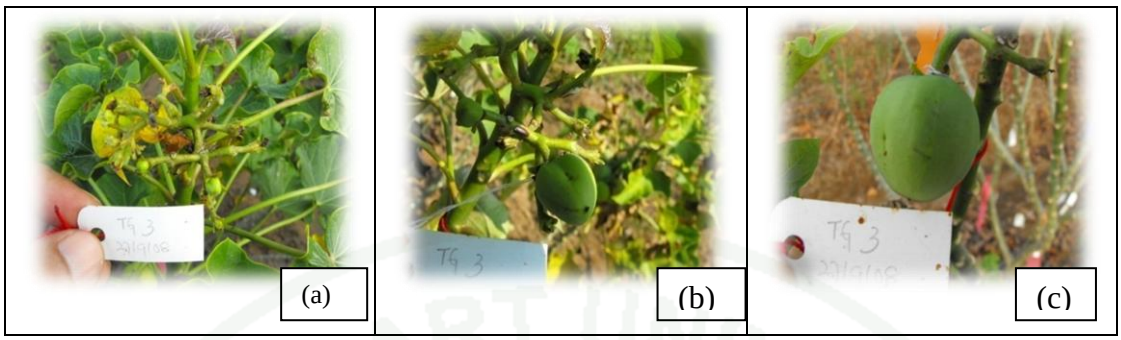

Figure 4 Principal growth stage 7; Fruit development
(a) Stage 71, fruit is visible (approximately 5 mm in diameter)
(b) Stage 75, fruit size has developed to 50% of final fruit size
(c) Stage 79 fruit developed to maximum size, the color of fruit is pale green and petal begin to dry

1.9 Principal growth 7; Fruit development

This stage runs from fertilization to the point where the fruits have achieved the final size. In the general BBCH scale, the phenological development of fruits is described in principal growth stage 7.

For the jatropha just after being fertilized, when fruit size is less than 5 mm, it is coded (70) and when the fruit is visible (5 mm) the fruit development stage is (71) and half of the final fruit size is stage (75). At the fruit development stage (79) the fruit get its maximum size and color is pale green and petal starts to dry.

1.10 Principal growth stage 8; Ripening

Due to extended flowering period, fruits do not ripe simultaneously. The definition of ripening in BBCH scale cannot be directly applied to jatropha. In the BBCH scale the number of ripe fruits is expressed as proportion of the total number of fruits. Individual fruit in an inflorescence should be described. Fruit ripening occurs as the fruit changes the colour from pale green to yellow code (81) and when the fruit is yellowish code (89). It is fully ripe and beginning of fruit abcission.

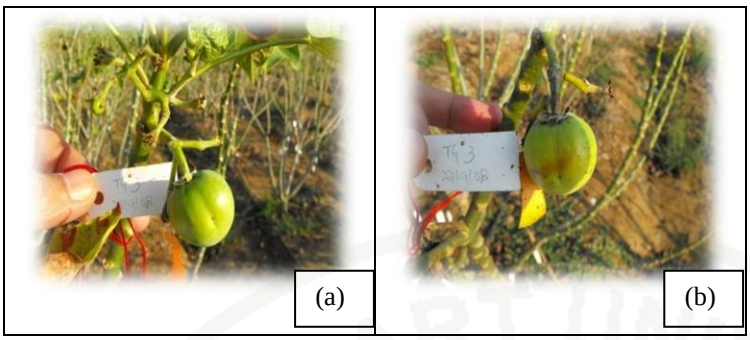

Figure 5 Principal growth stage 8; Ripening of fruit
(a) Stage 81, fruit color is pale green
(b) Stage 89, fruit color is yellowish green

Figure 6 Principal growth stage 9; Dormant period

1.11 Principal growth stage 9; Dormant period

As the jatropha is deciduous plant, during winter when the temperature fall down and water is limited, the plant dropped the leaves and stay in dormant. All the leaves are fall down and only the bare branch can be seen when the temperature dropped in November (Fig. 6).

1.12 Discussion

In this experiment there are 17 cultivars/accession from different locations, to ensure that the scale is appropriate for diverse cultivars. Phenological

stages are specific for each cultivars or accession and allowing us to distinguish and describe different cultivars (Sanz-Cortes *et al.*, 2002).

The variation in leaf number on main stem at flower initiation for each cultivar was shown in Figure 1. Most of the cultivars started flower initiation around leaves number of 50. The smaller leaf number shows early flowering (ywa ngan variety got flower initiation at leave number 32 and finished at54). It was generally accepted plants from higer latitude flower earlier than do plants from lower latitude, when these are grown in lower latitude (Ratche, 1985). In Figure 8, most of the different accessions flower initiate at 90 days after transplanting and it showed environment had much influence on flower initiation as there was rain on 27th February, 2008 (20.9 mm) (Appendix figure 1).

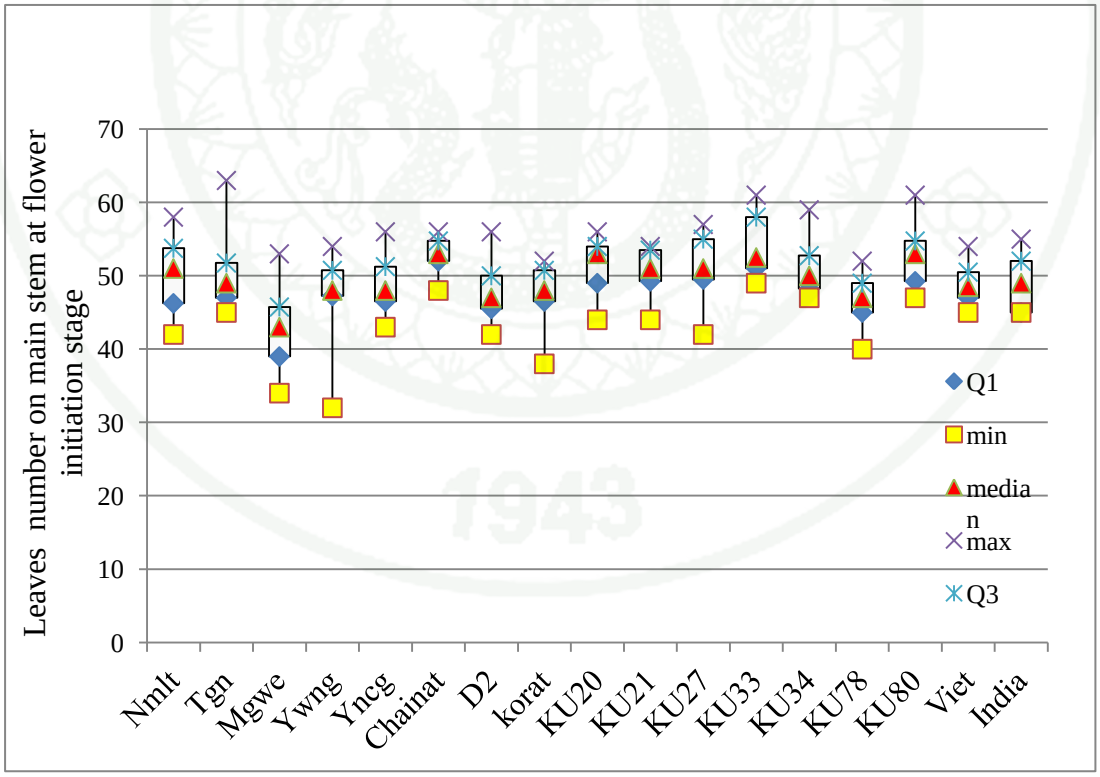

Figure 7 Number of leaves on main stem at flower initiation

Earliest flower initiation was Ywangan (at number of leaves = 32) and the latest flower initiation was Taungoo (number of leaves = 63). The box plots (Fig. 9) reveal uniformity of flower initiation. The longer the box plot the more variation in flower initiation.For example if we have to choose for synchronous flower initiation, we can choose Chainat variety as it has shorter box plot. India and Vietnam varieties also showed uniform flower initiation.

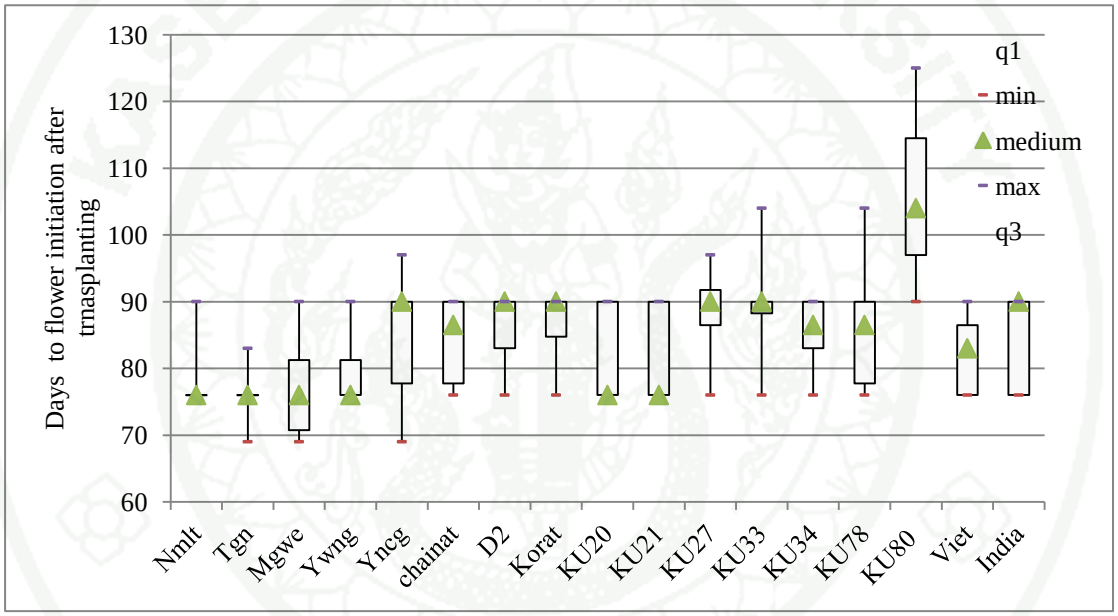

Figure 8 Days to flower initiation after transplanting

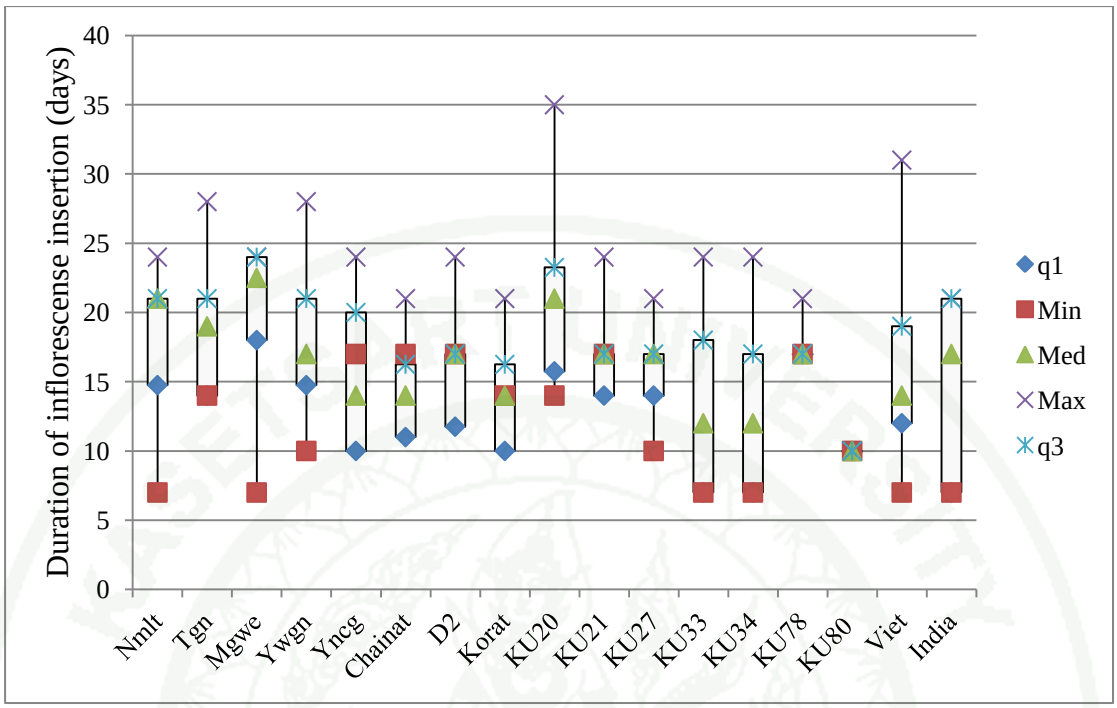

Figure 9 Duration of inflorescence initiation (days)

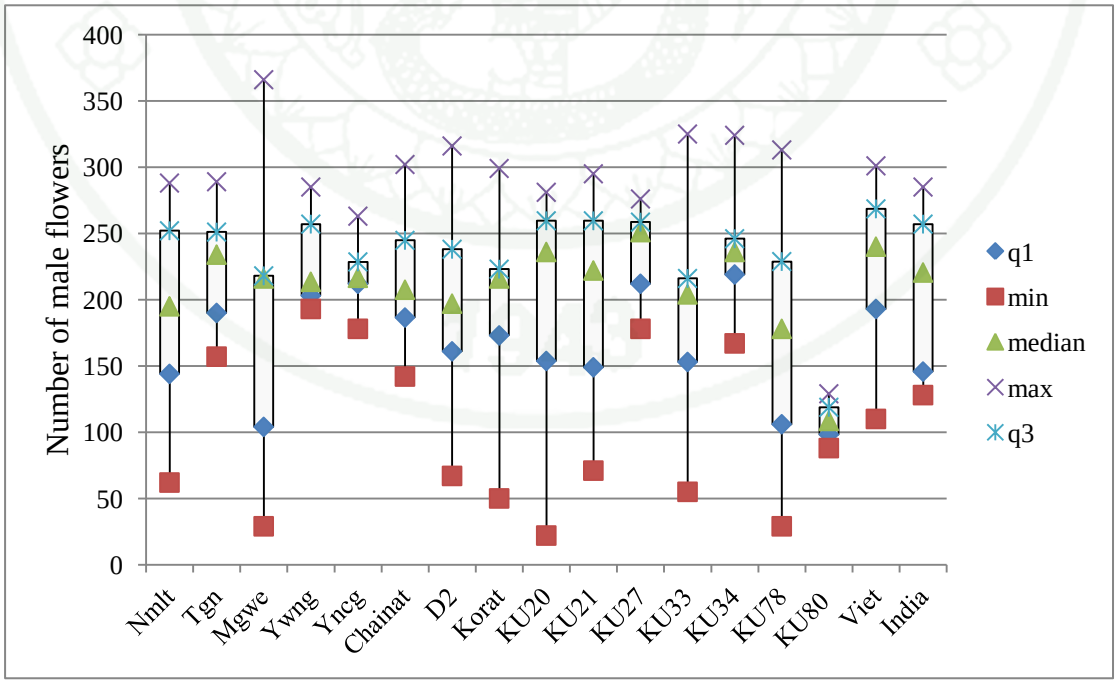

Figure 10 Number of male flowers in each inflorescence

Number of male flowers for each variety, was about 200 male flowers. While Magwe variety showed highest variation in bearing male flowers, Yenanchaung variety showed less variation among the observed cultivars (Fig. 10). As the male flower is prominent and easy to observe. Higher male flower bearing inflorescences believe to also set higher female flowers. To obtain stable yield we should choose the high stable number of male flowers, for example Yenanchaung variety.

The inflorescence length and fruit diameter are shown in Figure 11 (48 sample plants from 17 varieties). The length of inflorescence was highly variable and ranged between 15- 60 mm, while the median value was 35 mm. The fruit size in diameter ranged 16.19 to 28.33 mm, while the median value is 24.18 mm. Since the sample dropped off due to frequent disturbance (inflorescence length and fruit size were measured twice a week) and thus could not get enough data to describe difference between the accessions. These traits should be measured only once while there was opening of first flower for the inflorescence length and when the fruit turns yellowish (final size).

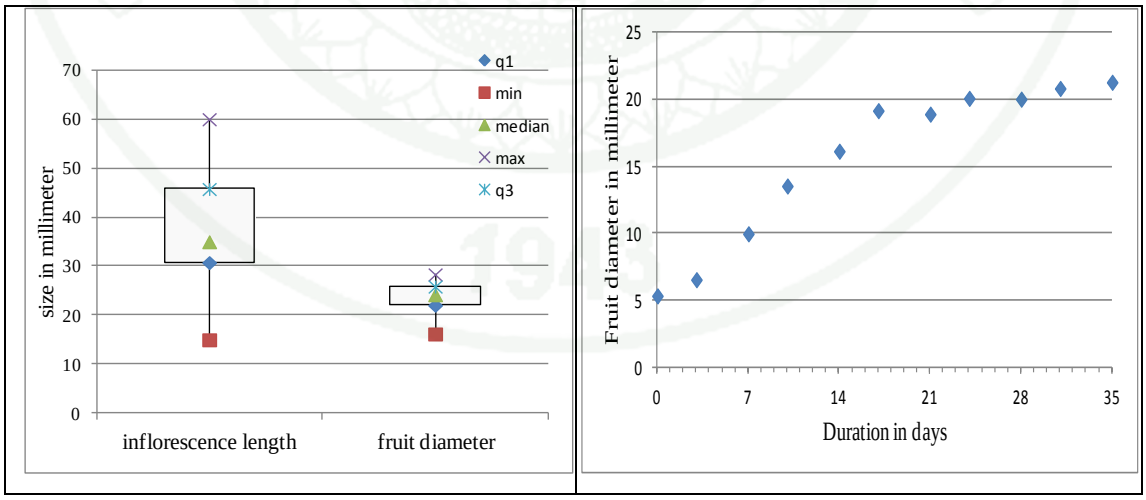

Figure 11 Size of inflorescence and fruit diameter

2. Effect of mineral and organic fertilizers on seedling and cutting

2.1 Vegetative characteristics

The significant effect of propagation method was observed in plant height; cutting (130.5 cm) and seedling (140.42 cm) at the time of first fruit set was visible (Table 3). Among fertilizer treatments, T3 and T4 resulted in maximum plant height, which were significantly higher than those of T5 and T6 (control). The lowest mean value of plant height (107.92 cm) was resulted from T5 (PMF). The stunted plant height of T5 might be due to the adverse effect of pig manure foliar fertilizer in this study.

Larger canopy size was observed in plant grown from seedling (17765 cm^2) as compare to those grown by cuttings (13349 cm^2). There was no significant effect of mineral fertilizers on canopy size, since the mean values of canopy size were not significantly different from control. Noticeably, pig manure foliar fertilizer (T5) showed the smallest canopy size (1141 cm^2) as compared to other treatments.

Total number of branches responded to both seedling type and fertilizers. The plants grown from seedlings were significantly higher in number of branches (32) than those from cutting (23). The maximum number of branches (30) was observed in T1, however the mean values of number of branches were not significantly different among the mineral fertilizer treatments (T1, T2, T3, and T4). Surihan *et al*. (2011) found that different levels of pruning did not have significant effect on branch number and length but the application of fertilizer increased the branch number and length. The result of present study indicated that number of branches become low when N was accompanied with either P or K. It implied that there was sufficient amount of P and K in the experimental soil to sustain vegetative growth. Here again, T5 (pig manure foliar fertilizer) showed the minimum number of branches.

The branch diameter (Dia) was significantly different between jatropha plants developed from cuttings (1.29 cm) and seedlings (1.47 cm). Among the mineral

fertilizer treatments (T1, T2, T3 and T4), mean values of Dia were not significantly different, but all except T2 were significantly higher than that of control. The pig manure foliar fertilizer (T5) treatment resulted smaller mean value of Dia (1.21 cm) as compare to the mineral fertilizer treatment, although the value was not significantly different from control T6.

Achten *et al*. (2010) reported that diameter of branch reflected the growth of biomass and total leaf area. In the present study, mineral fertilizer treatments provided an increase in Dia, thereafter it might support to the growth of biomass and leaf area (Table 3).

SPAD meter detected the leaf greenness and represented the leaf N concentration indirectly (Percival *et al.*, 2008). SPAD readings were varied, significance differences were not observed within mineral fertilizer treatments (T1, T2, T3, and T4). The SPAD readings of mineral fertilizer treatments were significantly higher than those of pig manure foliar fertilizer (T5) and control (T6). SPAD reading had good relationship with Dia, height and canopy of the jatropha plant (Table 8).

2.2 Leaf nutrient concentration

Leaf N concentration was not effected by seedling type, but the differences were observed among different fertilizer applications (Table 4). T4 resulted the maximum concentration (3.19 %) which was higher than T2 and T3, but significantly higher than those of T1, T5 and T6 (control). The mineral fertilizer treatments were significantly higher in leaf N than that of T5 (PMF), which was not different from T6 (control) and relatively low value.

N : P ratio in leaf sample reflects the N and P availability. Koerselman and Meuleman (1996) suggested that the ratio <14 indicates limitation of N and that of >16 indicates limitation by P. In our results, low N : P ratio in T5 and T6 could be the limited availability of N because its values were 9.71 and 10.99 (Table 4). Garrish

et al. (2010) found that P concentration varied as a function of transpiration at constant P solution, and transpiration rate is influenced by N concentration in the solution. Water use efficiency is the function of N availability and not because of P. Generally, plants with high N:P ratio allocate less biomass in root (Güsewell, 2004). Our results tended to be in line with this statement as the aerial biomass, viz. plant height, Tbr and Dia, of mineral fertilizer treatment (T1, T2, T3 and T4) was higher than those of pig manure foliar fertilizer (T5) and control (T6). It could be assumed that T5 and T6 seem to be higher investment of assimilate in root system.

Leaf P concentration was not different among mineral fertilizer treatments (T1, T2, T3 and T4). However, the concentration was significantly lower than those of T5 and T6. Accumulation of higher P in T5 and T6 would be due to the less sink of low aerial biomass and yield (Table 3 and 7). There was no different of K concentration in leaves among mineral fertilizer treatments and control (T6). Significantly lower K value obtained in pig manure foliar fertilizer (T5) might be the reason of high concentration of Ca and Mg in pig manure foliar fertilizer (Table 2).

Significantly higher concentration of leaf Ca was found in T5 and T6 comparing to mineral fertilizer treatment except T3. Similarly, Mg concentration in T5 and T6 were higher than other treatments.

Pig manure foliar fertilizer (T5) resulted in significantly lower concentration of leaf S (1289.17 mg/kg) and Zn (8.67 mg/kg) (Table 3). Accumulation of high amount of P (2617.5 ppm) might interfere the uptake of Zn (8.67 ppm) in T5 (Table 3) similar result was found in wheat (Zhu *et al.*, 2001). For most crops, typical leaf concentration of Zn ranged between 15 to 20 mg/ kg (Broadley *et al.*, 2007). Chave et al. (2009) found that Zn affect the Cu content in leaf of jatropha. Since Zn is related to enzyme activities, malfunction of plant growth and yield might be the reason of deficiency in this microelement.

The result of significant difference in specific leaf area (SLA) indicated difference in leaf thickness. An interaction was observed between type of seedlings and fertilizer applications (data not shown). Although no significant effect was observed in fertilizer application, the significant effect was observed in type of seedlings, thus it required to analyse simple effect of fertilizer on each seedling and cuttings of jatropha plants.

It was found that fertilizer treatments had significant effect on specific leaf area (SLA) of plants grown from cutting (162.11 $cm^2.g^{-1}$) as compare to those grown from seedlings (147.91 $cm^2.g^{-1}$) (Table 5). Maximum SLA was found in T4 (170.16 $cm^2.g^{-1}$). The minimum SLA from T5 (151.21 $cm^2.g^{-1}$) was significantly lower than those of mineral fertilizer treatments, except T3 (Table 6). Garnier *et al*. (1997) reported that SLA and N in perennial plants have positive relationship. Similar results was found in our experiment as the T4 had highest leaf N concentration (3.19%) and highest in SLA (170.16 $cm^2.g^{-1}$) of cutting plants (Table 4 and 5).

Specific leaf area (SLA) reflects leaf thickness and closely relates with relative growth rate (RGR), Osone *et al*. (2008) reported that SLA is associated with Specific Absorption Rate (SAR) of the root, positively affects leaf/root ratio, leaf N, and maximum photosynthetic rate. In our experiment, difference in SLA between plants from cuttings and seedlings might be because of the difference in root systems. The cutting plants have adventitious root system, which is believed to have higher root surface and absorption comparing to seedling plants.

2.3 Reproductive characteristics

Cuttings and seedlings showed significant difference in yield related variables of Bbr, Tfr, Tsd, and Swt. Higher mean values of those variables were observed in cutting plants (Table 7).

Mineral fertilizer treatments (T1, T2, T3 and T4) gave significantly higher mean value of yield related variables than those of pig manure foliar fertilizer (T5) and control (T6). There was no significance between T5 and T6.

Among the mineral fertilizer treatments, each plant of T1 gave the highest Swt 109.68 g, while T2, T3 and T4 had 69.67 g, 91.50 g and 73.95 g, respectively. This result indicated that the plants did not respond to the treatments of T2, T3 and T4. Since the experimental site had sufficient P and K (Table 2), it could be interpreted that there was no additional requirement of P or K or both. The results of T1 (N only) treatment were agreed with the Yong *et al*. (2010) who suggested to use N nutrition to improve the yield of jatropha. T5 and T6 showed the same result of yield related variables, suggesting that spraying of pig manure forliar fertilizer (PMF) without using basal mineral fertilizer was not effective. Since the pig manure fertilizer was rich in microelements, it might be effective when using together with some mineral fertilizers in jatropha.

It was found that there was no difference of Fsp and Hsw for both factors (type of seedling and fertilizer treatments). Fsp and Hsw might be much more influenced by other factors rather than fertilizer treatments and type of propagation methods.

2.4 Relationship between vegetative and Reprodutive characteristics

The branch diameter (Dia) had highly significant relationship with other vegetative variables of plant height (r = 0.71), canopy size (r = 0.78) and total number of branches (r = 0.55) but there was no significant relationship between Dia and reproductive variables (Table 8). Dia was a reliable trait to estimate the aerial biomass of the jatropha plants. Regarding reproductive variables, Bbr was positive and highly significant relationship with Tfl (r = 0.98), Tfr (r = 0.87), Tsd (r = 0.85) and Swt (r = 0.85). Negative relationship (r = -0.40) was found between Fsp and Bbr. Among the yield related variables, Bbr was the most important character. The vegetative and reproductive variables of jatropha plant were significantly associated with not only

SPAD meter readings but also SLA. SPAD reading had significant positive relationship with Dia (r = 0.50) and Bbr (r = 0.65). Significant and negative association between SLA and Dia (r = -0.33), while there was significant positive correlation with Bbr (r = 0.40).

2.5 Seed quality

There is no difference in kernel oil percentage and kernel cake N content for types of seedlings and fertilizer treatments (Table 9). Seed oil and protein synthesis is mainly genetically controlled. Since jatropha is a wild plant, application of fertilizers did not affect quality of seed yield in this experiment.

Table 2 Physico-chemical properties of the experimental soil and analysis of pig manure foliar fertilizer

Trait	Soil		Pig manure($mg.L^{-1}$)
	mean±SD	range	range
OM (%)	1.14±0.24	0.81-1.46	-
N ($mg.kg^{-1}$)	no data	no data	0.09-0.10
P ($mg.kg^{-1}$)	92.4±25.56	56.29-122.65	0.02-0.03
K($mg.kg^{-1}$)	154.22±59.83	95.04-266.95	0.13-0.16
Ca ($mg.kg^{-1}$)	3667.78±1679.84	2507.41-6967.54	45.00-95.00
Mg ($mg.kg^{-1}$)	229.29±43.83	163.74-297.99	197.00-229.00
S ($mg.kg^{-1}$)	447.95±268.97	171.43-916.94	-
Fe ($mg.kg^{-1}$)	32.92±9.71	23.41-49.31	8.00-19.00
Mn ($mg.kg^{-1}$)	44.37±3.82	38.88-49.86	1.00-8.00
Zn ($mg.kg^{-1}$)	1.49±0.40	0.92-2.06	6.00-8.00
pH	7.11 ± 0.19	-	-
EC	0.85 ± 0.36	-	-
Sand (%)	27.48±3.78	23.42-33.09	-
Silt (%)	48.73±2.97	44.73-52.03	-
Clay (%)	23.80±1.80	22.18-27.09	-

Table 3 Mean comparison on vegetative characters in different types of seedlings and fertilizer treatments

Factor	Height (cm)	Canopy (cm^2)	Tbr	Dia (cm)	SPAD
Type of seedling (A)					
Cutting	130.50b	13349.5b	23.36b	1.29b	34.26
Seedling	140.42a	17765.8a	32.22a	1.47a	33.92
LSD	5.86	1375.6	2.54	0.064	1.35
Fertilizer (B)					
T1	137.75bc	16007a	30.00a	1.48a	35.45a
T2	137.08bc	16575a	26.33ab	1.39ab	35.81a
T3	148.75a	17521a	28.75a	1.47a	36.35a
T4	146.25ab	16247a	29.58a	1.45a	35.60a
T5	107.92d	11410b	22.92b	1.21c	29.44c
T6	135.00c	15585a	29.17a	1.30bc	31.90b
LSD	10.14	2382.7	4.40	0.11	2.35
P-value (A)	0.0013	<0.0001	0.0091	<0.0001	0.5301
(B)	<0.0001	<0.0001	0.0168	<0.0001	0.0001
(A x B)	0.3247	0.0685	0.0523	0.7214	0.1460
C.V. (A)	10.56	23.33	12.98	17.90	5.51
(B)	9.16	18.73	19.37	9.81	8.44

Mean in each trait followed by the same letter are not significantly different as compared by Fisher's least significant difference (lsd) at 0.05 probability level

Table 4 Mean comparison of mineral element concentration in leaves of jatropha among fertilizer treatments

Factor	N/P	N (%)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	S (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)
Seedling type Factor (A)										
Cutting	13.3	2.8	2259.7	12887.2	10038.9	6170.8	1618.1	45.5	55.5	12.5
Seedling	13.0	2.7	2204.7	12294.4	10733.6	5981.1	1540.2	46.8	59.9	11.2
LSD	1.6	0.1	202.2	751.9	889.4	460.4	81.7	6.1	4.6	1.0
Fertilizer Factor (B)										
T1	14.1^{a}	2.8^{b}	2052.5^{b}	12567.5^{a}	9961.7^{b}	5560.8^{b}	1615.0^{a}	42.4	51.9^{c}	11.7^{b}
T2	13.9^{a}	2.9^{ab}	2152.5^{b}	12349.2^{ab}	9171.7^{b}	5405.8^{b}	1592.5^{a}	47.9	52.0^{c}	13.0^{ab}
T3	14.2^{a}	2.9^{ab}	2052.5^{b}	12440.0^{ab}	10376.7^{ab}	5542.5^{b}	1605.0^{a}	43.1	57.3^{bc}	11.3^{b}
T4	15.9^{a}	3.1^{a}	2010.0^{b}	13413.3^{a}	9519.2^{b}	5559.2^{b}	1732.5^{a}	48.0	54.1^{bc}	12.2^{b}
T5	9.7^{b}	2.5^{c}	2617.5^{a}	11260.8^{b}	11700.0^{a}	6915.0^{a}	1289.1^{b}	44.5	60.7^{b}	8.6^{c}
T6	10.9^{b}	2.6^{bc}	$2508.^{a}$	13514.2^{a}	11588.3^{a}	7472.5^{a}	1640.8^{a}	51.0	70.3^{a}	14.1^{a}
LSD	2.8	0.3	350.2	1302.5	1540.5	797.4	141.6	10.5	8.0	1.8
P-value (A)	0.7401	0.3272	0.8060	0.1157	0.5051	0.2304	0.1998	0.7738	0.6260	0.4293
(B)	0.0014	0.0023	0.0044	0.0203	0.0101	<0.0001	<0.0001	0.5144	0.0008	0.0001
(A x B)	0.9347	0.5398	0.7524	0.8853	0.5536	0.5948	0.5839	0.2775	0.2994	0.7726
C.V. (A)	18.4	6.9	26.4	0.8	24.9	5.4	7.8	25.2	41.2	33.5
(B)	17.6	8.7	13.0	8.5	12.3	10.9	7.4	18.9	11.4	12.6

Means in each trait followed by the same letters are not significantly different as compared by Fisher's least significant difference (lsd) at 0.05 probability level

Table 5 Mean square of specific leaf area (SLA) as affected by cutting and seedling

SOV	*df*	Cutting			Seedling		
		MS	F	*P*-value	*MS*	F	*P*-value
Blk	2	12.65	0.15	0.86	184.36	1.29	0.29
Fertilizer	5	296.33	3.58	0.01	175.73	1.23	0.32
Error	28	82.87			142.56		
C.V (%)		5.62			8.07		
Mean		162.11			147.91		

Table 6 Mean comparison of specific leaf area (SLA) of jatropha among fertilizer treatments

Fertilizer Treatment	SLA ($cm^2.g^{-1}$)	
	Seedling	Cutting
T1	153.74	163.27^{ab}
T2	140.57	163.27^{ab}
T3	141.74	159.00^{bc}
T4	149.95	170.16^{a}
T5	151.07	151.21^{c}
T6	150.43	159.97^{abc}
P-value	0.3203	0.0126
LSD	14.12	10.77

Means followed by the same letters are not significantly different by Fisher's least significant difference (lsd) at 0.05 probability level

Table 7 Mean comparison of reproductive characteristics among fertilizer treatments

Factor	Bbr	Tfl	Tfr	Tsd	Swt	Fsp	Hsw
Seedling type (A)							
Cutting	9.67a	11.17	57.87a	141.86a	85.54a	82.95	58.33
Seedling	6.75b	7.57	26.77b	69.20b	41.46b	86.77	61.81
Fertilizer (B)							
T1	12.26a	15.01a	69.63a	179.83a	109.68a	85.42	62.10
T2	10.15ab	11.50b	48.29b	114.37b	69.67b	80.02	61.74
T3	9.42b	11.08b	60.17ab	148.58ab	91.50ab	81.67	58.41
T4	10.00ab	11.17b	49.92b	124.00b	73.95b	84.07	58.91
T5	2.90c	2.93c	13.51c	34.66c	18.08c	91.06	55.78
T6	4.54c	4.52c	12.43c	31.73c	18.13c	86.94	63.49
Mean	8.76	10.05	46.08	114.35	68.78	84.07	59.55
P-value (A)	0.0444	0.0680	0.0026	0.0048	0.0118	0.1975	0.1398
(B)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.1061	0.7856
(A x B)	0.7558	0.4195	0.0340	0.4030	0.0158	0.8093	0.0788
C.V. (A)	42.50	50.01	35.92	38.26	44.96	9.77	10.59
(B)	33.96	34.64	41.45	42.75	43.30	9.57	20.06

Means in each trait followed by the same letters are not significantly different as compared by least square method at 0.05 probability level, Bbr = inflorescense bearing branch, Tfl= total number of inflorescence, Tsd = Total seed number, Swt = seed weight, Fsp = Filled seed percentage, Hsw = 100 seed weight

Table 8 Relationships between vegetative and reproductive characteristics

	SPAD	SLA	Dia	Height	Canopy	Tbr	Bbr	Tfl	Tfr	Tsd	Swt	Fsp
SLA	-0.11^{ns}											
Dia	0.50**	-0.33*										
Height	0.66***	-0.15^{ns}	0.71***									
Canopy	0.39*	-0.47**	0.78***	0.78***								
Tbr	0.03^{ns}	-0.40*	0.55***	0.54***	0.75***							
Bbr	0.65***	0.40*	0.26^{ns}	0.39*	0.10^{ns}	-0.13^{ns}						
Tfl	0.63***	0.37*	0.30^{ns}	0.34^{ns}	0.10^{ns}	-0.16^{ns}	0.98***					
Tfr	0.44**	0.51**	0.17^{ns}	0.18^{ns}	-0.06^{ns}	-0.30^{ns}	0.87***	0.91***				
Tsd	0.44**	0.49**	0.19^{ns}	0.17^{ns}	-0.05^{ns}	-0.27^{ns}	0.85***	0.91***	0.99***			
Swt	0.47**	0.49**	0.20^{ns}	0.19^{ns}	-0.06^{ns}	-0.28^{ns}	0.85***	0.90***	0.99***	0.99***		
Fsp	-0.19^{ns}	-0.09^{ns}	-0.01^{ns}	-0.37*	-0.09^{ns}	0.07^{ns}	-0.40*	-0.35*	-0.30^{ns}	-0.25^{ns}	-0.25^{ns}	
Hsw	0.31^{ns}	0.00^{ns}	0.20^{ns}	0.16^{ns}	0.03^{ns}	-0.01^{ns}	0.05^{ns}	0.04^{ns}	0.05^{ns}	0.04^{ns}	0.14^{ns}	0.13^{ns}

*, ** and *** significantly different at 0.05, 0.01, and 0.001 probability levels, SPAD = SPAD reading, SLA = specific leaf area, Dia = branch diameter, Height = plant height, Canopy = plant canopy size, Tbr = total branch number , Bbr = inflorescence bearing branch, Tfl = Total inflorescence number, Tfr = Total fruit number, Tsd = Total seed number, Swt = seed weight, Fsp = Filled seed percentage, Hsw = 100 seed weight

Table 9 Mean comparison of oil percentage in kernel and N in kernel cake

Factor	Oil in kernel (%)	N (%) in cake
Seedling type (A)		
Cutting	41.12	7.63
Seedling	43.15	7.79
Fertilizer (B)		
T1	41.02	7.90
T2	45.11	7.97
T3	42.29	7.84
T4	42.06	7.71
T5	40.16	7.30
T6	42.16	7.51
Mean	42.22	7.69
P-value (A)	0.48	0.43
(B)	0.64	0.69
C.V. (A)	15.38	6.67
(B)	11.03	9.82

3. Investigation on indicator of leaf nitrogen status, chlorophyll and carotenoid

3.1 SPAD meter and leaf nitrogen

Total N concentration of the jatropha leaves increased linearly with the SPAD value (Fig. 12), showing a strong relationship with each other (R^2=0.986). The highest N concentration in jatropha leaf is nearly 4% in the group of 55-60 SPAD value. These results show higher relationship and N content when compared with the other plant species. The correlation coefficients between SPAD reading and leaf N concentration of sycamore, Englis oak and European beech are 0.86, 0.85 and 0.93, respectively with leaf N concentration all under 3.3% (Percival *et al.*, 2008). Peng et al., (1995) suggested finding relationship between chlorophyll meter reading and leaf area-based N concentration for all growth stages of rice leaves. Our work showed a near-perfect relationship as we used the leaf disc samples avoiding the leaf veins. This result is clear that SPAD meter can be used as N measurement technique for jatropha leaf blade as there was a strong linear correlation. In addition, the results also implied a good efficiency of N usage of jatropha plant as detected of high N concentration in its leaves and most of the N is used as the photosynthetic pigments in leaf blade.

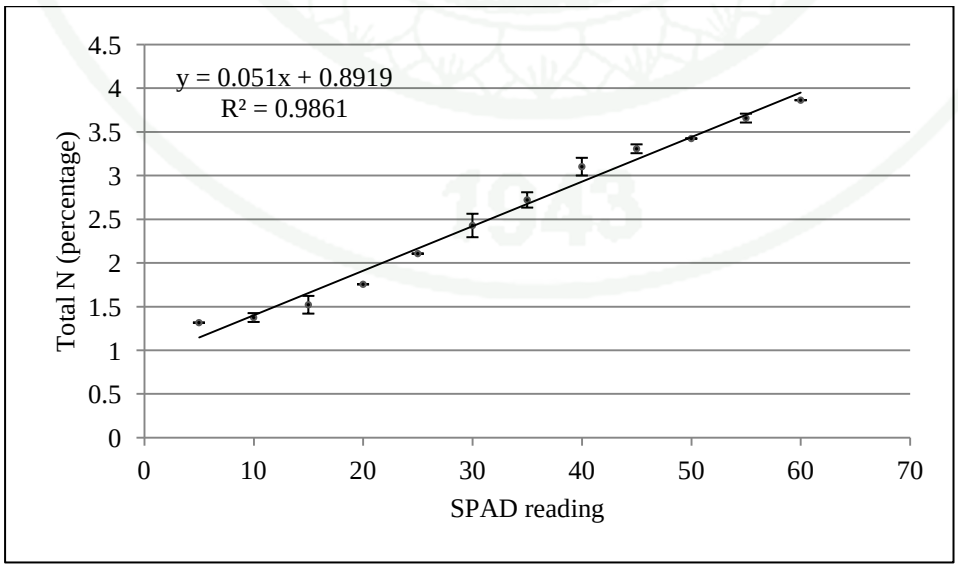

Figure 12 Relationship between SPAD value and total N in jatropha leaves

3.2 SPAD meter and photosynthetic pigments

The relationship between SPAD reading and photosynthetic pigment concentration was curvilinear as shown in Fig 13. The coefficient of determination (R^2) between SPAD reading and Chl *a*, Chl *b* and *Car* were 0.97, 0.96 and 0.75, respectively. When SPAD reading values were higher than 20, all kinds of photosynthetic pigments were sharply increased, especially Chl *a* which showed the strong linear response. Chl *a/b* ratio slightly decreased when the SPAD value decreased. When SPAD values were lower than 20, Chl *a/b* ratio became lower than 3.0. After this critical point, the Chl *a/b* ratio was sharply decreased when the SPAD reading values kept decreasing (Fig 14c). Decrease in Chl *a/b* ratio is considered a result from oxidative stress, causing Chl *a* to degrade earlier than Chl *b* (Rout *et al.*, 1997). It is generally accepted that healthy leaves should have the Chl *a/b* ratio above 3.0, Chl *a*/*b* ratio increases with decreasing N availability. Hikosaka (1996) explained that when N supply becomes limiting under high light, the proportional allocation to PSII increases on the expense of decreased N allocation to Rubisco, while N allocation to Light Harvesting Complex II (LHCII) is maintained at a similar level. Consequently, the ratio of PSII to LHCII (Chl *a*/*b* ratio) increases with decreasing N availability. Although SPAD meter uses the 650 nm wave length for detecting photosynthetic pigments, it is possible to estimate the *Car* concentration as there is a good curvilinear realationship (R^2=0.74) as shown in Fig 13d. There is no good relationship when SPAD values are under 20 and it might reflect a poor association between Chl *a* and/or Chl *b* with *Car* at that point of SPAD readings. Similar trend was found in coffee plant when the SPAD values were under 40 (Netto *et al.*, 2005). Carotenoid has two main functions, light collection and photo-protection. Particular group of carotenoids, such as those of xanthophyll cycles, play key role in photoprotection in photosynthesis under environmental stress (Demmig-Adams *et al.*, 1996).

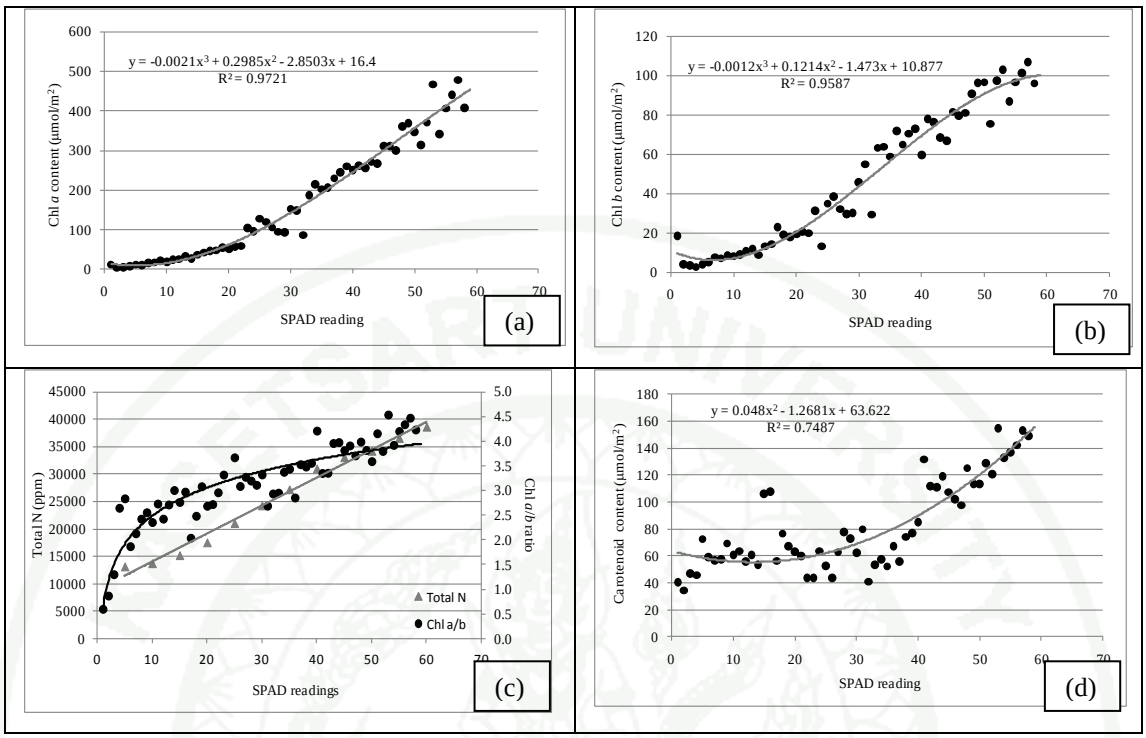

Figure 13 Relationship between SPAD readings and photosynthetic pigment content in jatropha leaves (a) Chl *a* content (µmol/m^2), (b) Chl *b* content (µmol/m^2), (c) Chl *a/b* ratio and total leaf N (ppm), (d) *Car* content (µmol/ m^2)

The maximum flourescence variable of F_m reflects the redox potential of plastoquinone pool (Maxwell and Johnson, 2000). It was found that the relationship between F_m value and SPAD value fitted well in a curvilinear manner (Fig 15a). There was a sudden reduction in F_m value after the SPAD reading of 20. Netto et. al., (2002; 2005) reported the relationships between F_m and SPAD readings in papaya and coffee leaves in which F_m values were sharply decreased when SPAD values were lower than 40. Lower value of F_m and nonphotochemical quenching (NPQ) for the same SPAD reading implied lower amount of photosynthetic pigments (Miranda *et al.*, 1981). In our experiment, it could be concluded that the decrease in F_m value with lower SPAD readings was the effect of lower amount of photosynthesis pigments in PSII reaction centers (Fig 14a, 14b and 15a). Baker (2008) pointed out an interprectation of non-photochemical quencing, which might accompany with photoinactivation and significant decrease of F_m or increase in mimimum

fluorescence at dark adapted (F_0). This implies that both optical and physiochemical properties of leaf should be investigated together when the leaf is under stress condition.

3.3 SPAD meter and fluorescense variables

Maximum quantum efficiency of PSII photochemistry (F_v/F_m) reflects the reduction of plastoquinone A and it was frequently used as a stress indicator (Baker, 2008). In a normal healthy leaf, F_v/F_m value varies between 0.79 to 0.84 (Björkman and Demmig, 1987). However, if F_v/F_m is below 0.6, the plants were affected in terms of survival, growth and necrosis of the leaf (Maki and Colombo, 2001). In our result, jatropha leaves showed the optimum F_v/F_m value in the range of 0.67 – 0.82 and abruptly decreased when the SPAD values were 20 and lower as shown in Fig 15b. The sharp decrease in F_v/F_m value might be due to photoinhibition and disintegration of LHCII and PSII. Seasonal variation of F_v/F_m value is not much different in most plant species of pine, evergreen broadleaf trees and grasses (Weng *et al.*, 2006). Netto et. al. (2002) reported in papaya leaves, that SPAD values of less than 40 showed a decrease in F_v/F_m values, causing reduction in photosynthesis quenching coefficient (q_p) and possibly dissipation in the form of heat. F_v/F_m value would be a suitable stress indicator for jatropha during a cropping season. Kitajima and Hogan (2003) found that leaf N limitation and high light intensity did not cause photoinhibition (i.e. no change in quantum yield or in dark acclimate, F_v/F_m) in four tropical woody species of the Bignoniaceae. It was revealed by SPAD values in that experiment, proper functioning of photosynthesis in jatropha leaf blade required 2% of N by its weight. The estimate of minimum leaf N requirement by using SPAD reading also showed a similar result in sycamore, English oak and European beech with respectively 1.5, 1.5 and 2.0% of N in their leaves (Percival *et al.*, 2008). Effective quantum yield or operating efficiency of PSII (Yield) is close to overall quantum yield of photosynthesis. It is a measure of the proportion of the light absorbed by PSII that is used in photochemistry. For the C_3 plant leaves, linear relationship is observed between Yield and assimilation of CO_2 if the photorespiration is suppressed and

create the situation when CO_2 assimilation is the only sink for ATP and NADPH (Baker, 2008).

In our experiment, a decrease in Yield value between the SPAD reading from 60 to 20 might be the result of photorespiration and reduction in total amount of photosynthetic pigments content in leaf samples (Fig 14a, 14b and 15c). At the SPAD value of 20, there is a sudden drop of Yiled value, implying no net photosynthesis and showing a damage in light harvesting complex. NPQ is the quenching of fluorescence caused by the non-photochemical dissipation of energy as heat (Bilger *et al.*, 1995). Yield and NPQ fluorescence variables in Fig. 14 and 15 suggested that the decrease in these variables with the SPAD reading may associate with the reduction in quantity of photosynthetic pigments and photoinhibition.

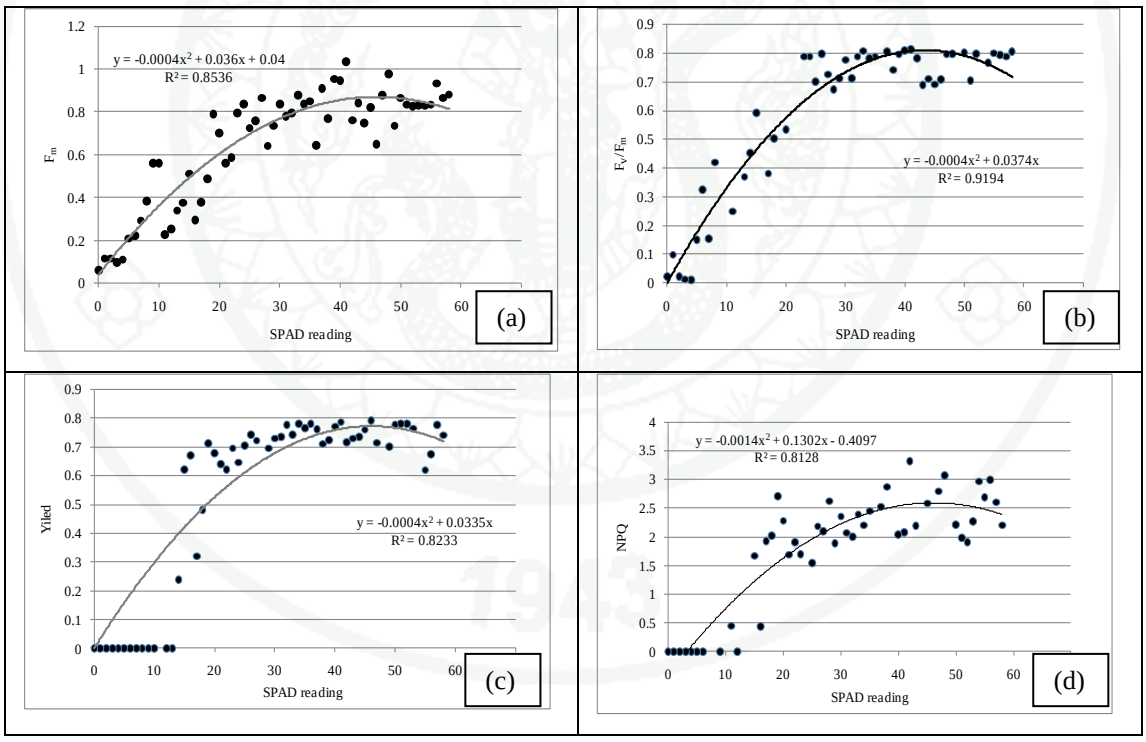

Figure 14 Relationship between SPAD readings and fluorescence variables in jatropha leaves, (a) maximum fluorescence in dark adapted, (b) maximum quantum efficiency of PSII photochemistry (F_v/F_m) or variable fluorescence in dark adapted, (c) effective quantum yield operating efficiency of PSII (Yield), (d) nonphotochemical quenching (NPQ)

CONCLUSION AND RECOMMENDATION

Conclusion

1. Jatropha plant growth stages could be described using BBCH scale. Among 10 principal stages of BBCH scale, 7 stages were relevant to describing the growth stage of jatropha. In addition, 21 secondary growth stages could be added to describe plant development stages. More detailed study of growth stages would facilitate identifying the specific accessions or cultivars.

2. Fertilizer treatments on jatropha plants grown from seedlings and cuttings showed different effects. Cutting gave higher seed yield while seedling had larger aerial biomass. The branch diameter (Dia) could be used to assess aerial biomass of the plant and inflorescence bearing branch (Bbr) from which both of them reflected seed yield. Chlorophyll meter (SPAD) was portable and reliable instrument to monitor the plant status.

3. SPAD meter is a portable, convenient, affordable and non-destructive tool to estimate photosynthetic pigment concentration and status in jatropha plant. The instrument can be used for N management in jatropha field as well as for advanced interpretation of photosynthesis process. The SPAD values under 20 indicate the critical point of impairment in photosystem of the jatropha leaf.

Recommendation

Uniform coding system should be used for sharing information among researchers as well as to specifying stages suitable for application of inputs. Nitrogen fertilizer can enhance both biomass and seed yield of the jatropha plant. Further study is required to obtan the optimum rate and time of application in conjunction with well described growth stages.SPAD meter is the portable, nondestructive and reliable instruments that can be employed to support the monitorin of response to nutrient inputs.

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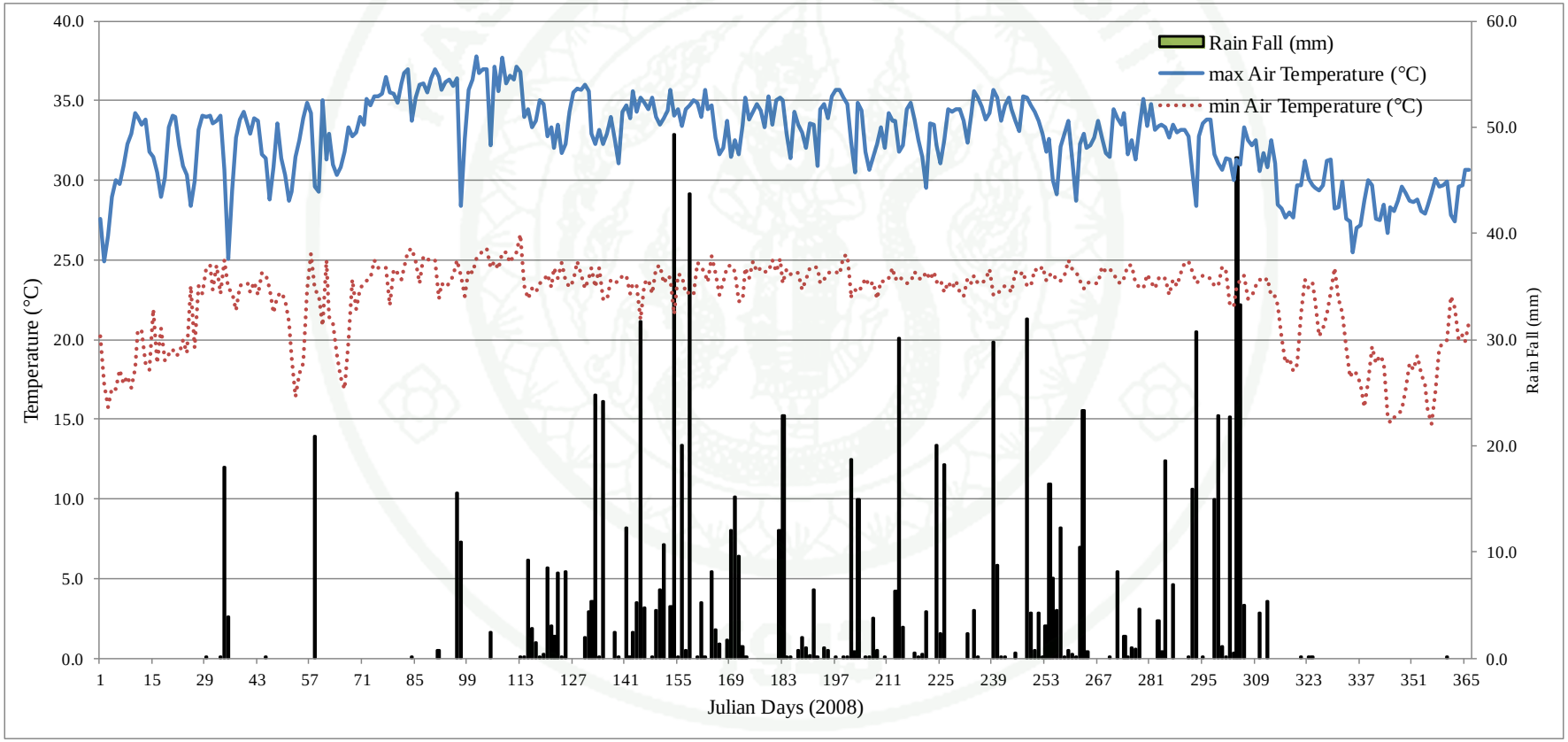
APPENDIX

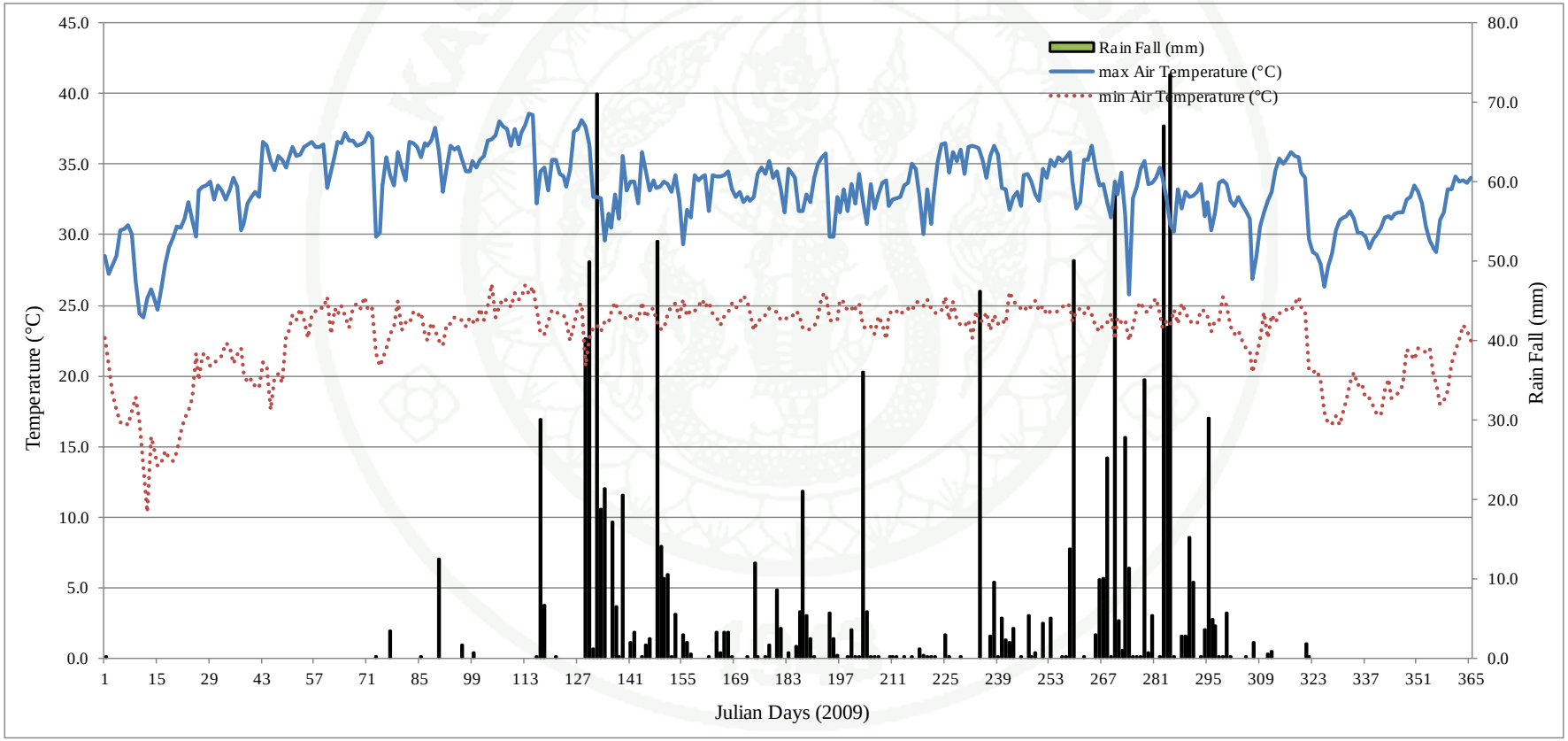
Appendix Table 1 Origin and name of jatorpha accession used in phenology growth stage study

Name of accession	Country	Origin of seed
Namlatt (Nmlt)	Myanmar	Shan
Taungngoo (Tgn)	Myanmar	Pegu
Magwe (Mgwe)	Myanmar	Magwe
Ywangan (Ywng)	Myanmar	Shan
Yenanchaung (Yncg)	Myanmar	Magwe
Chainat	Thailand	Chainat
D2	Thailand	Mandalay
korat	Thailand	Korat
KU20	Thailand	Mukdahan
KU21	Thailand	Mukdahan
KU27	Thailand	Ubon Ratchathani
KU33	Thailand	Surin
KU34	Thailand	Amnat Charoen
KU78	Thailand	Nakhon Si Thammarat
KU80	Thailand	Phattalung
Viet	Vietnam	Vietnam
India	India	India

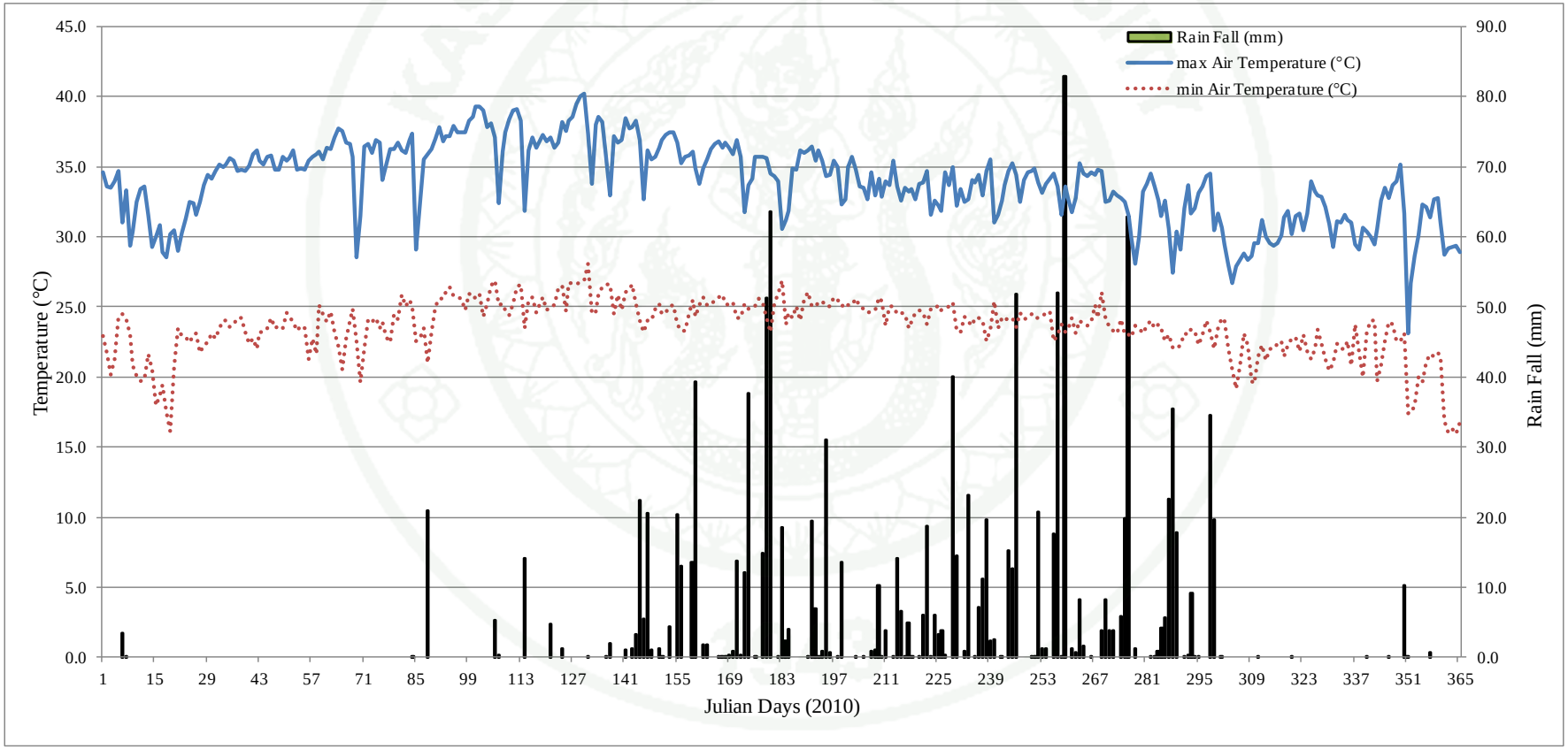
Appendix Figure 1 Rain fall (mm) and air temperature (minimum, maximum) in 2008



Appendix Figure 2 Rain fall (mm) and air temperature (minimum, maximum) in 2009



Appendix Figure 3 Rain fall (mm) and air temperature (minimum, maximum) in 2010



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