RESPON PERTUMBUHAN DAN FISIOLOGIS JATROPHA HIBRIDA INTERSPESIFIK PADA CEKAMAN GARAM

GROWTH AND PHYSIOLOGICAL RESPONSE OF JATROPHA INTERSPECIFIC HYBRID UNDER SALT STRESS

SKRIPSI

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Diajukan guna melengkapi tugas akhir dan memenuhi salah satu syarat untuk menyelesaikan Program Sarjana (S1) Pertanian pada Program Studi Agroteknologi Fakultas Pertanian Universitas Jember

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menyatakan sesungguhnya bahwa karya tulis ilmiah yang berjudul : Respon Pertumbuhan dan Fisiologis Jatropha Hibrida Interspesifik pada Cekaman Garam adalah benar hasil karya sendiri, kecuali disebutkan sumbernya dan belum pernah diajukan pada institusi manapun, serta bukan karya jiplakan. Saya bertanggung jawab atas keabsahan dan kebenaran isinya sesuai dengan sikap ilmiah yang harus dijunjung tinggi.

Demikian surat pernyataan ini saya buat dengan sebenarnya, tanpa adanya tekanan dan paksaan dari pihak manapun serta bersedia mendapat sanksi akademik jika ternyata di kemudian hari pernyataan ini tidak benar.

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Thailand, August 2014
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Respon Pertumbuhan dan Fisiologis Jatropha Hibrida Interspesifik pada Cekaman Garam

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RINGKASAN

Biofuel sebagai salah satu jenis energi terbarukan diprediksi akan memegang peranan penting di masa depan. Konsumsi biofuel secara global diperkirakan akan naik dari 1.3 mboe d^{-1} pada 2011 menjadi 4.1 mboe d^{-1} pada 2035 (IEA, 2013). Jatropha (*Jatropha curcas* L.) telah umum diketahui sebagai salah satu sumber bahan baku biodiesel dan diprediksi sebagai alternatif sumber utama biofuel di masa depan. Jatropha diketahui memiliki kemampuan untuk tumbuh di tanah salin dan digolongkan sebagai jenis tanaman yang memiliki ketahanan moderat terhadap cekaman garam. Jatropha dapat tumbuh dan berproduksi pada kondisi salin hingga 4 dS m^{-1} (Diaz-Lopez et al., 2012). Namun, Jatropha diketahui memiliki beberapa sifat agronomis yang kurang menguntungkan seperti hasil minyak yang lebih rendah utamanya dibandingkan dengan tanaman kelapa sawit dan waktu pembungaan yang tidak seragam.

Memiliki beberapa sifat agronomis unggul seperti hasil minyak yang lebih tinggi dan waktu pembungaan yang seragam, kultivar Jatropha hibrida interspesifik dari *J. curcas* x *J. integerrima* diproyeksikan menjadi kultivar unggul alternatif di masa depan. Namun, sebagai penemuan baru, penelitian terkait Jatropha hibrida interspesifik masih sangat terbatas utamanya pada aspek ketahanannya terhadap kondisi lingkungan yang kurang menguntungkan seperti pada kondisi cekaman garam. Menarik untuk mengetahui bagaimana Jatropha hibrida interspesifik merespon kondisi cekaman garam mengingat tanaman

induknya (*J. curcas* L.) dikenal sebagai tanaman yang moderat toleran terhadap cekaman garam. Kegiatan penelitian ini bertujuan untuk membandingkan respon pertumbuhan dan fisiologis varietas Jatropha hibrida interspesifik dengan Jatropha non-hibrida pada cekaman garam.

Enam varietas Jatropha terdiri dari 2 jenis tanaman Jatropha yakni *Jatropha curcas* L. (3 kultivar) dan hibrida interspesifik dari *J. curcas* x *J. integerrima* (3 kultivar) digunakan sebagai tanaman percobaan dalam penelitian ini. Bahan stek Jatropha diperoleh dari tanaman Jatropha berumur 1 tahun yang ditumbuhkan di Land Development Department at Nakhon Pathom, Kamphaeng Saen District, Nakhon Pathom Province, Thailand. Penelitian dilakukan dengan sistem budidaya hidroponik di greenhouse milik Department of Agronomy, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Nakhon Pathom, Thailand mulai 2 April sampai 21 Mei, 2014. Rancangan acak lengkap (RAL) faktorial dengan 4 replikasi digunakan dalam eksperimen ini. Faktor A merupakan jenis varietas Jatropha yakni varietas hibrida interspesifik (V1, V2, V4) dan varietas non-hibrida (India, KUBP 35, KUBP 40). Faktor B adalah tingkat cekaman garam pada larutan Hoagland yang ditambah dengan NaCl yang memiliki tingkat konduktivitas elektrik (EC) 2.0 (kontrol), 8.0, dan 16.0 dS m^{-1}. Parameter pertumbuhan yang diukur meliputi tinggi tanaman, jumlah daun, total luas spesifik daun (portable LI-3000A leaf area meter) dan SPAD index (SPAD-50 Minolta Inc. Japan). Parameter fisiologis yang diukur meliputi *specific leaf area* (SLA), *leaf water potential* (Ψt), *solute concentration* (Cs), *leaf osmotic potential* ($\Psi\pi$), dan *leaf turgor potential* (Ψp). Dua langkah faktorial ANOVA dan tes beda nyata terendah digunakan sebagai metode statistik pembanding rataan dengan menggunakan program R stat ver 2.13.1

Hasil penelitian menunjukkan bahwa jumlah daun tanaman Jatropha baik kultivar hibrida maupun non-hibrida menunjukkan perbedaan non-signifikan pada tingkat salinitas moderat (8 dS m^{-1}) dan menunjukkan tingkat penurunan yang signifikan pada tingkat salinitas tinggi (16 dS m^{-1}). Kultivar hibrida interspesifik (V2) menunjukkan tren peningkatan jumlah daun pada tingkat cekaman garam moderat (8 dS m^{-1}), namun menunjukkan tren penurunan pada tingkat kegaraman yang lebih tinggi (16 dS m^{-1}). Pada aspek total luas daun, kultivar hibrida interspesifik (V2) merupakan varietas yang paling tidak terpengaruh dengan cekaman garam utamanya pada tingkat cekaman moderat, meskipun luas area daunnya lebih rendah dari kultivar non-hibrida (KUBP 35 dan KUBP 40). Kultivar non-hibrida maupun hibrida interspesifik (KUBP 35 dan V2) menunjukkan tingkat SPAD index tertinggi dan perbedaan yang tidak signifikan pada berbagai tingkat cekaman. Berat kering kultivar hibrida interspesifik maupun non-hibrida menunjukkan peningkatan signifikan pada tingkat cekaman garam moderat (8 dS m^{-1}) dan menunjukkan penurunan pada tingkat cekaman garam yang lebih tinggi (16 dS m^{-1}). Pola yang sama juga ditunjukkan oleh kultivar non-hibrida, kecuali India. Pola tersebut menunjukkan bahwa kultivar Jatropha baik hibrida maupun non-hibrida (V2, KUBP 35, KUBP 40) memiliki respon pertumbuhan yang baik pada cekaman garam dan dapat diduga moderat toleran terhadap cekaman garam.

Hasil pengukuran terhadap respon pertumbuhan menunjukkan bahwa kedua jenis Jatropha yakni hibrida interspesifik dan non-hibrida dapat diduga sebagai varietas yang moderat toleran terhadap cekaman garam. Kultivar hibrida interspesifik diduga sebagai kultivar yang memiliki potensi tinggi sebagai jenis yang tahan cekaman garam. Hal tersebut diindikasikan oleh tren peningkatan jumlah daun, total luas daun, dan berat kering kultivar V2 pada tingkat cekaman garam moderat (8 dS m^{-1}). Pola peningkatan berat kering pada tingkat salinitas yang lebih tinggi dari kontrol biasanya ditunjukkan oleh jenis tanaman yang dianggap sebagai tanaman yang toleran terhadap cekaman garam seperti *Alhagi pseudoalhagi* (Kurban et al., 1999), *Halophyrum mucronatum* (Khan et al., 1999), dan *Salicornia rubria* (Khan et al., 2001). Jenis kultivar non-hibrida juga dapat diduga sebagai jenis yang moderat toleran terhadap cekaman garam karena menunjukkan pola yang serupa dengan kultivar hibrida interspesifik utamanya pada aspek berat kering tunas. Berdasarkan hasil tersebut maka kultivar Jatropha baik non-hibrida maupun hibrida interspesifik dapat diklasifikasikan sebagai moderat toleran terhadap cekaman garam dan dapat diduga memiliki kemampuan adaptasi tertentu terhadap cekaman garam untuk mempertahankan performa pertumbuhannya.

Hasil yang diperoleh pada penelitian berkaitan dengan respon fisiologis Jatropha hibrida interspesifik dan non-hibrida terhadap cekaman garam menunjukkan bahwa kultivar hibrida interspesifik dan non-hibrida (V2, KUBP 35 dan KUBP 40) menunjukkan mekanisme adaptasi fisiologis terhadap kondisi cekaman garam. Hal tersebut diindikasikan oleh tren peningkatan *solute concentration* yang signifikan pada kondisi cekaman garam. Tren peningkatan Cs, $\Psi\pi$, dan Ψp mengindikasikan bahwa varietas tersebut memiliki kemampuan untuk beradaptasi dengan cekaman garam melalui mekanisme akumulasi *compatible solute* yang bertujuan untuk menjaga potensial air daun. Tren peningkatan *leaf osmotic potential* pada cekaman garam biasanya dituunjukkan oleh jenis tanaman yang toleran garam seperti *R. mucronata* (Aziz and Khan, 2001) dan *Urochondra setulosa* (Trin.) (Gulzar et al., 2003). Beda non-signifikan pada data potensial air daun antara tanaman tanpa cekaman (kontrol) dan tanaman tercekam garam menunjukkan bahwa mekanisme akumulasi *compatible solute* telah berhasil mempertahankan potensial air daun pada tanaman tercekam sehingga besarnya sama dengan kondisi tanpa cekaman garam (kontrol). Berdasar data Cs, $\Psi\pi$, dan Ψp, kita dapat menyimpulkan bahwa terdapat respon adaptif yang ditunjukkan oleh kultivar Jatropha hibrida interspesifik maupun non-hibrida dibawah cekaman garam melalui mekanisme akumulasi *compatible solute*. Respon adaptif yang ditunjukkan pada pengukuran tersebut mengkonfirmasi hasil pengukuran respon pertumbuhan yang menunjukkan kultivar hibrida interspesifik dan non-hibrida sebagai kultivar moderat toleran terhadap cekaman garam.

Secara keseluruhan dapat disimpulkan bahwa pertumbuhan varietas hibrida interspesifik dibawah cekaman garam lebih rendah dibandingkan jenis varietas non-hibrida, namun varietas hibrida interspesifik seperti V2 dapat diduga memiliki potensi yang tinggi sebagai varietas moderat toleran terhadap cekaman garam mengingat adanya tren peningkatan jumlah daun, berat kering, dan total luas daun pada level cekaman garam moderat (8 dS m^{-1}). Potensi ketahanan varietas hibrida interspesifik terhadap cekaman garam dikonfirmasi oleh hasil pengukuran respon fisiologis yang menunjukkan respon adaptif kultivar hibrida interspesifik terhadap cekaman garam, yang juga ditunjukkan oleh kultivar non-hibrida.

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Growth and Physiological Response of Jatropha Interspecific Hybrid (*Jatropha curcas* x *J. integerrima*) under Salt Stress

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Abstract

Interspecific hybrid of *Jatropha curcas* x *Jatropha integerrima* is expected to answer the low oil yield problem of Jatropha (*Jatropha curcas* L.). However, as a novel invention, research concerning on Jatropha interspecific hybrid is still limited especially in the aspect of its adaptability to unfavorable environment such as salt stress condition. It is interesting to know how Jatropha interspecific hybrid responses to salt stress condition due to the moderate salt tolerance ability of its mother plant (*Jatropha curcas* L.). The objectives of this study were to compare the growth and physiological response of interspecific hybrid and non-hybrid Jatropha under salt stress. Three varieties of both interspecific hybrid and non-hybrid Jatropha seedling were exposed with 3 levels of sodium chloride treatment in Hoagland media solution which are 2.0 (control), 8.0, and 16.0 dS m^{-1} for 7 weeks. The parameters measured include plant height, number of leaves, shoot dry weight, SPAD index, total leaf area, specific leaf area, leaf water potential, leaf solute concentration, leaf osmotic potential, and leaf turgor potential. Leaf number of V2 (interspecific hybrid variety) showed a slight increase at moderate salt stress level (8 dS m^{-1}). Shoot Dry weight of V1, V2 (interspecific hybrid), KUBP 35, KUBP 40 (non-hybrid) showed an increasing trend at 8 dS m^{-1} and decreasing trend at higher salinity level. The solute concentration, osmotic potential, and turgor potential of both interspecific hybrid and non-hybrid variety showed an increasing pattern which could be implied as an adaptive response to salt stress. This study has showed that Jatropha interspecific hybrid might have an adaptive physiological response to salt stress and might also be classified as moderate tolerant to salt stress eventhough its salt tolerability is lower than non-hybrid Jatropha.

Keywords : osmotic adjustment; salt tolerance; solute concentration; SPAD; sustainable energy

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I. INTRODUCTION

1.1 Introduction

Energy is one of the basic human needs. The demand of energy in the world is getting higher every year due to the increase of human population. Over last decades, the global energy demand increases around 2% every year (International Energy Agency (IEA), 2010) and is predicted to increase by one third in the next 15 years (IEA, 2013). In the other hand, fossil fuel which supplies majority of global energy demand is an unrenewable energy resource and will be depleted soon. Shafiee and Topal (2009) calculates that the fossil fuel time depletion would be around 35, 107, and 37 years for oil, coal, and gas respectively. Therefore, biofuel as one kind of renewable energy resources is predicted to have more important role in the future. Global biofuel consumption is predicted to increase from 1.3 mboe/d in 2011 to 4.1 mboe/d in 2035 (IEA, 2013). This increasing trend is not only caused by the depletion of fossil fuel, but also by the increase of environmental concerns due to carbon emission and climate change problem caused by fossil fuel use (Basili and Fontini, 2012).

Jatropha (*Jatropha curcas* L.) is known as an oil bearing species and has been used widely as source of biodiesel (Divakara et al., 2009). Lately, Jatropha get a lot of attention because of its potential to be used as main biodiesel resource to replace fossil fuel (Abou Kheira et al., 2009). Jatropha has some superiorities compared to other biodiesel resources. First, Jatropha is a non edible plant (Patil et al., 2009), due to the presence of toxic phorbol esters in its fruit (Basili and Fontini, 2009). This means there is no competition regarding its usage. Second, Jatropha has ability to grow in marginal lands including saline soil which usually is not suitable for growing plant (Basili and Fontini, 2012). Nearly 20% of the world's cultivated land and nearly half of the irrigated land is affected by salinity (Zhu, 2001). High soil salinity is one of the major abiotic stress factors limiting plant growth and productivity (Hishida et al., 2012). Productivity of thirty percent of irrigated lands in the world have declined because of salinity problem (Munns and Tester, 2008). The moderate salt tolerance ability of Jatropha makes it become one of the most potential crops for marginal lands utilization.

Despite its superiorities, Jatropha has some weaknesses compared to other biodiesel resources. First, Jatropha normally has a low oil yield especially compared to oil palm. Karmakar et al. (2010) reported that Jatropha oil yield is only 1,892 liter/hectare, lower than the oil yield of oil palm which can reach 5,950 liter/hectare. Low number of female flowers, reduced branching, and inadequate pollination are the major factors which limit Jatropha seed production and thus oil yield (Carels, 2009). Second, due to its continous flowering characteristic, fruit production of Jatropha occurs for 4 months per year and the fruit should be harvested three times during this period (Carels, 2009). This characteristic makes Jatropha fruits must be harvested manually since it complicates mechanization and this will lead to high labor cost.

Some breeding methods have been applied to improve unfavorable traits of Jatropha. One of the most promising breeding techniques to improve the yield and favorable characteristics of Jatropha is interspecific hybridization. Jatropha has many related species and genera making it possible to be improved through interspecific and intergeneric crosses (Tanya et al., 2011). Among the various crop breeding approaches, interspecific hybridization is an immediate option for genetic enhancement of *J. curcas* (Sujatha, 2006). Interspecific hybridization among Jatropha species which transfers useful traits such as high oil content, maximum number of seeds, more femaleness, and hard stem could create an interspecific hybrid varieties which have combined favorable characteristics.

Jatropha interspecific hybrid variety comes from crossing of *J. curcas* and *J. integerrima* is considered to be one of the most promising interspecific hybrid variety. Previous research by Parthiban et al. (2009) reported that cross between *J. curcas* and *J. integerrima* showed a successful result. Among the interspecific crosses within Jatropha genus, Jatropha hybrid clones between *J. curcas* and *J. integerrima* produced succesful hybrids with more seed set and expressed superiority in terms of early flowering and fruiting coupled with early yield.

Basha and Sujatha (2009) successfully obtained BC_1F_1 hybrid plants from *J. curcas* × *J. integerrima* with increased fruit size and high fruit bearing ability.

However, Jatropha interspecific hybrid variety is a novel invention and there is still limited research concerning in this topic. As a promising Jatropha variety, interspecific hybrid of *J. curcas* x *J.integerrima* is interesting to be studied, especially about its other potential favorable traits such as salt tolerance ability. Salt tolerance ability is important since the main purpose of Jatropha development is to develop Jatropha varieties which not only have high seed yield and oil content but also have ability to adapt well with various planting conditions (Divakara et al., 2009). It is interesting to know how the interspecific hybrid of *J. curcas* x *J.integerrima* responses to salt stress because its mother plant (*J. curcas* L.) is known to has moderate salt tolerance ability. This study tried to compare the growth and physiological response of interspecific hybrid and non-hybrid Jatropha under salt stress and determined which variety has the higher salt tolerance ability.

1.2 Problems Identification

The problem identifications of this research are :

1. How different is the growth performance of interspecific hybrid and non-hybrid Jatropha varieties under salt stress condition ?
2. How do Jatropha interspecific hybrid and non-hybrid varieties show a physiological response during salt stress condition and how are they different ?
3. What is the potential salt tolerant level of the experimental Jatropha varieties ?

1.3 Objectives

The objectives of this research were (1) To compare the growth performance of Jatropha interspecific hybrid and non-hybrid under salt stress condition, (2) To compare the physiological response of Jatropha interspecific hybrid and non-hybrid under salt stress condition, (3) To determine the potential salt tolerance level of the experimental Jatropha varieties.

1.4 Benefits

This study will be useful as source of information about growth and physiological response of Jatropha interspecific hybrid (*J. curcas x J. integerrima*) under salt stress condition and its comparison with the non-hybrid Jatropha (*J. curcas*). This information will help readers, especially breeder who concerns on the improvement of Jatropha, to have a better understanding about salt tolerance ability of Jatropha interspecific hybrid (*J. curcas x J. integerrima*).

II. LITERATURE REVIEW

2.1 *Jatropha curcas* L.

2.1.1 Description of *Jatropha curcas* L.

Jatropha (*Jatropha curcas* L.) belongs to Euphorbiaceae family (Divakara et al., 2009). Jatropha genus contains approximately 175 known species and has two subgenera (Curcas and Jatropha) with 10 sections and 10 subsections, where the *J. curcas* is the most primitive form of Jatropha genus (Dehgan and Webster, 1979). Jatropha is a deciduous, monoecious, perennial shrub or small tree (Da Schio, 2010). Jatropha shows articulated growth, with a morphological discontinuity at each increment. Jatropha leaves have five to seven lobed, hypostomatic, and its stomata are of paracytic (rubiaceous) type (Kumar and Sharma, 2008). Jatropha branches bear 5 to 7 lobed alternately arranged leaves and a terminal inflorescence. The flowers are generally unisexual with occasional hermaphrodite flowers (Singh et al., 2010) and the inflorescence is axillary paniculate polychasial cymes and form a bunch of green trilocular ellipsoidal fruits yielding approximately 10 or more ovoid fruits (Tewari, 2007).

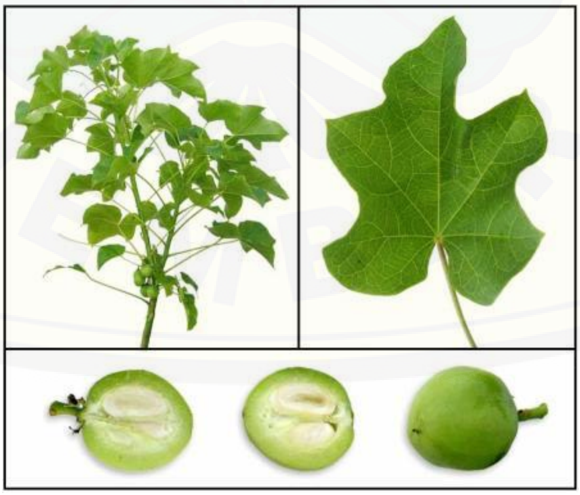

Figure 1. Jatropha Tree, Leaf, and Fruit Morphology (www.carboncapture.us)

2.1.2 Uses of *Jatropha curcas* L.

Jatropha is known as a versatile plant. Historically Jatropha was used as a hedge plant which useful as live fence to keep out grazing live stock, control erosion, and conserve the soil (Xu et al., 2012). It is also used for providing oil for lighting lamps, making soap (He, 2011), and making insecticide or molluscicide (Brittaine and Lutaladio, 2010). Jatropha has also been known to be used as medicine, but are mostly restricted to indigenous areas (Heller, 1996). Soomro and Memon (2007) reported that Jatropha is one of the most valuable drugs of primitive times and is still widely used in modern medicine. Not only its oil, another part of Jatropha like latex produced from its branches is also useful as haemostatic agent (Emil et al., 2009). The by-product of oil extraction, e.g. seedcake, produces organic fertilizer through composting that can eventually be used as organic manure (Basili and Fontini, 2009).

Today, main use of Jatropha is as biodiesel source. *Jatropha curcas* is one of the main non-edible oil yielding species that can be used for biodiesel production in several countries such as India, Tanzania, Madagascar, Cambodia, and Guinea (Basili and Fontini, 2009). Biodiesel is gradually gaining acceptance in the market as an environmentally friendly fuel and the demand is expected to increase sharply as an alternative renewable energy source in the near future (Ong et al., 2010). Jatropha has been considered as one of the most potential crops for main biodiesel production in the future (Xu et al., 2012). The Jatropha oil is considered as a high quality oil (Soomro and Memon, 2007). The oil content is around 25-30% in the whole seeds and 50-60% in the kernel (Shrivastava and Banerjee, 2009). Jatropha produces less carbon dioxide and nitric oxide emission compared to regular diesel oil and therefore it is considered as environmentally cleaner since it creates less greenhouse gasses (Satyawan and Tasma, 2011). Jatropha ranks first among all possible crops after considering the social, economic, and humanitarian aspects (Runge and Senauer, 2007).

2.1.3 Occurence of *Jatropha curcas* L.

Jatropha is thought to originate from Mexico or Central America (Heller, 1996), but the exact origin of Jatropha remains controversial until now (Singh et al., 2010). Generally Jatropha grows in tropical temperatures, with annual precipitation of 200 mm – 1500 mm. It is particularly common in tropical America and Africa, with a few species native to Southwest Asia, Asia, one species in Madagascar, and none are native to Southeast Asia, Australasia or Oceania (Soontornchainaksaeng and Jenjittikul, 2003). Jatropha is widely distributed in Central and South America, Africa, India, and Southeast Asia (Xu et al., 2012) and has spread widely spanning the whole tropics (King et al., 2009). Jatropha has spread beyond its original distribution because of its hardiness, drought endurance, and adoption to wide agroclimatic condition (Kumar and Sharma, 2008). There are more than 85 % of Jatropha plantation, mainly in India, China, Myanmar, and Indonesia (Brittaine and Lutaladio, 2010).

2.2 Jatropha Interspecific Hybrid

Among the various crop breeding approaches, interspecific hybridization is an immediate option for genetic enhancement of *J. curcas* (Sujatha, 2006). The interspecific hybridization in Jatropha species has played a significant role in crop improvement of Jatropha by transferring useful traits such as high oil content, maximum number of seeds, more femaleness, and hard stem for promotion of Jatropha as biofuel crop (Parthiban et al., 2009). Making interspecific hybrid variety from *J. curcas* is very potential since it is the most primitive member of the genus which has ability to interbreed with species from both genera (Dehgan and Webster, 1979). Jatropha genus has many species and each species has its own desirable trait (Table 1) which potential to be used as genetic sources for Jatropha improvement.

Jatropha integerrima Jacq. is a drought tolerant perennial shrub of the family Euphorbiaceae (Sujatha and Dhingra, 1993). It has a rounded or narrow domed form and gets up to 15 ft (4.6 m) tall with a spread of 10 ft (3.1 m) and known to be indigenous to Cuba and West Indies (Roxas et al., 2002).Its leaves have variable shape which are elliptic, oval, fiddle shaped, or have three sharp pointed lobes. *Jatropha integerrima* is known to be frost tolerant, root rot resistant with sturdy stem and with high degree of resistance to leaf eating caterpillars (Dhillon et al., 2009).

Jatropha integerrima is a monoecious shrub which produce flowers in racemose inflorescence with dischasial cyme pattern. Its inflorescence is a terminal dichasial cyme and the flowers of both the sexes are present on the same inflorescence, with ratio of male to female flowers is 9:1 (Gupta et al., 2007). Its flowers are bright red and star-funnel shaped (Figure 2), while its fruit is a capsule. The fruit formation usually starts in the month of October to February (Sharma, 2012). The main use of *J. integerrima* known today is as ornamental plants and medicine. *J.integerrima* Jacq. is commonly grown as an ornamental for its attractive crimson flowers (Sujatha and Dhingra, 1993). Various parts of *J.integerrima* Jacq. like leaves, branches and latex are traditionally used as medicine such as for warts, tumors, rheumatism, herpes, pruritis, toothaches, scabies, eczema, and ringworm and anti-cancer (Sharma and Singh, 2013).

Figure 2. Flower of *Jatropha Integerrima* Jacq. (source : www. floridata.com)

Jatropha interspecific hybrid (*J. curcas* x *J. integerrima*) is one of the most promising Jatropha variety. Interspecific hybridization between *J. curcas* and *J. integerrima* was undertaken with the objective of combining the desirable traits, such as high oil content, resistance to root rot, and frost tolerance (Dhillon et al., 2009). Interspecific hybrids between *J. curcas* and *J. integerrima* have been succesfully developed by Sujatha and Prabakaran (2003). Basha and Sujatha (2009) successfully obtained BC_1F_1 hybrid plants from *J. curcas* × *J. integerrima* with increased fruit size and high fruit bearing ability. Novel study, regarding interspecific hybrid between *J. curcas* and *J. integerrima*, found that this interspecific hybrid variety is potential to be developed in the future because it has some favorable characteristics like high yield and early flowering (Parthiban et al., 2009).

Table 1. Desirable Traits of Jatropha species for Making Interspecific Hybrid Varieties (Parthiban et al., 2009)

Species	Desirable Attributes
Jatrophacurcas	High seed yield and oil content
Jatropha integerrima	Semi-hard wood stem and disease-resistant
Jatropha podagrica	Bigger fruit and resistant to fusarial wilt
Jatropha gossypifolia	Drought-tolerant and profuse fruiting
Jatropha multifida	Bigger fruit size and resistant to diseases
Jatropha glandulifera	Profuse fruiting and drought-tolerant

2.3 Salinity

Salinity can be defined as the total concentration of dissolved salts in a given volume of a soil which is most conveniently measured by the electrical conductivity (EC) of a given soil / water system (White, 2013). A soil is classified as saline when containing a sufficient concentration of soluble salts to give EC values greater than 4 dS m^{-1} (Brady and Weil, 2002). Salinity is one of major abiotic stresses that affect crop growth and yield in agricultural areas (Zhu, 2002). High soil salinity is characterized by the presence of excess levels of soluble salts (saline soil) and / or high amount of sodium (Na^+) in the soil solution (Niu et al., 2012). There are two main types of soil salinity, which are dryland salinity (unirrigated land) and irrigated land salinity. In both cases the development of plants and soil organisms are limited leading to low yields (Douaik et al., 2005). That is why, lands which have high soil salinity is classified as marginal lands.

Based on its salinity level, exchangeable sodium percentage (ESP), and pH level characteristic soil can be classified into three types which are saline soil, sodic soil, and saline-sodic soil. Saline soil is soil which has EC level more than 4 dS m^{-1} and exchangeable sodium percentage (ESP) less than 15 (Juan et al., 2011). Sodic soil is soil which has EC level more than 4 dS/m, exchangeable sodium percentage (ESP) more than 15, and pH level is always above 8.5. Saline-sodic soil is soil which has EC level more than 4 dS m^{-1}, exchangeable sodium percentage (ESP) more than 15, and pH level is usually less than 8.5 (Brady and Weil, 2002).

Salinity can reduce crop yield as much as 50 % (Chaves and Oliveira, 2004) and limit plant growth and productivity, particularly in arid and semi arid areas (Hishida et al., 2012). Plant responds to salt through changes in several morphological, physiological, biochemical, and metabolic processes (Lee et al., 2001). As salinity stress continues to increase, various physiological and chemical processes are damaged (Niu et al., 2012). Detrimental effect of salinity is plant death and decrease in productivity. The earliest response of salinity stress is a reduction in the rate of leaf surface expansion and lower photosynthesis rate (Parida and Das, 2003).

2.4 Rationale of Research

Jatropha has been described as drought tolerant (Zhang et al., 2007; Basili and Fontini, 2009) and capable of growing in marginal and poor soils where most other crops cannot survive (Openshaw, 2000). The major cultivation areas of Jatropha are often characterized by frequent soil water deficit and excessive salinity (Fairless, 2007). Jatropha has remarkable capacity to survive in varied climatic conditions as in areas of low rainfall to almost on any type of soil whether gravelly, sandy, or saline soil (Johnson et al., 2011). Jatropha grows in drier regions with a rainfall of 500 – 600 mm/year and can survive long drought periods of 7 or 8 months, depending on air humidity, and can also withstand light frost (Basili and Fontini, 2009). Jatropha is well adjusted to semiarid climate (Foidl et al., 1996) and can be easily cultivated on marginal land (Johnson et al., 2011) or under diverse environmental growth conditions (Satyawan and Tasma, 2011). Trabucco et al (2010) reported that natural occurence of Jatropha mostly is in the arid and semi arid area (Figure 3). It can be inferred that Jatropha must have a certain natural adaptation to unfavorable condition including salt stress.

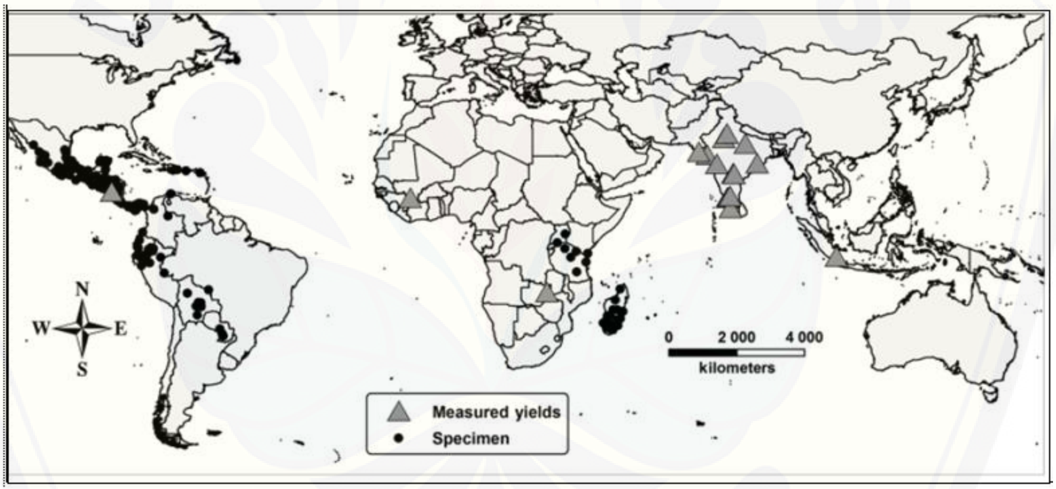

Figure 3. Locations of Jatropha Natural Occurence Used for Model Training ('Herbarium Species') and Locations of On-site Yield Assesment ('Measured Yield') Used for Model Validation (Trabucco et al., 2010)

Jatropha has some defense mechanisms to adapt with salt stress condition. First, previous study reported that to adapt with salt stress condition. Jatropha induce antioxidant enzyme activity like peroxidase (Campos et al., 2012), superoxide dismutase (Kumar et al., 2008), and catalase (Gao et al., 2008). Antioxidant enzyme is an enzyme which is possesed by plant to protect them against potentially cytotoxic reactive oxygen species (ROS) (Parida et al., 2005).

ROS are formed during water deficit condition caused by salt stress (Gill and Tuteja, 2010). Second, Jatropha has mechanism to accumulate compatible solutes (osmolytes) like proline, total soluble sugars, total soluble protein to adapt with salt stress condition (Kumar et al., 2008; Campos et al., 2012). Accumulation of compatible solutes has main purpose to accomodate ionic balance in the vacuoles (Hasegawa et al., 2000). This mechanism is important as part of osmotic adjustment mechanism of plant during water deficit condition caused by salt stress. Third, Jatropha has mechanism to reduce its transpiration rate for avoiding over water loss. Research conducted by Diaz-Lopez et al (2012) reported that Jatropha avoids water loss through strong restriction of leaf transpiration via stomatal closure which will lead to a decrease in the transpiration stream. Jatropha also has ability to reduce leaf growth which will reduce specific leaf area for avoiding water loss.

As a promising Jatropha variety, interspecific hybrid of *Jatropha curcas* x *J.integerrima* is interesting to be studied, especially about its other potential favorable traits such as salt tolerance ability. There are only few researches concerning in Jatropha (Niu et al., 2012), mainly because Jatropha is not a domesticated industrial crop, so researches of Jatropha have just been started (Silip et al., 2010). Research concerning on salt tolerance ability of Jatropha interspecific hybrid (*J. curcas* x *J. integerrima*) is also still limited. It is interesting to know how the interspecific hybrid of *J. curcas* x *J.integerrima* responses to salt stress due to the moderate salt tolerance ability of its mother plant (*J. curcas* L.). This study will be one of the pioneer reports in observing the growth and physiological response of Jatropha interspecific hybrid (*J. curcas* x *J. integerrima*) under salt stress.

III. MATERIAL AND METHODS

3.1 Date and Place

The experiment was conducted at greenhouse of Department of Agronomy, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Nakhon Pathom, Thailand from December, 21st 2013 until May, 21st 2014.

3.2 Plant Material

3.2.1 Varieties Used

Six varieties of Jatropha cuttings consisting of 2 different kinds of Jatropha plant, which are *Jatropha curcas* L. (3 varieties) and interspecific hybrid of *J. curcas* x *J. integerrima* (3 varieties), were used as plant material. The experimental Jatropha cuttings were prepared from the 1 year old Jatropha plants grown in the Land Development Department at Nakhon Pathom, Kamphaeng Saen District, Nakhon Pathom Province, Thailand. All of Jatropha plant materials used in this experiment were supported by KU-Biodiesel Project, Kasetsart University.

3.2.2 Preparation of Cutting

The cuttings were obtained from cut of secondary branch of Jatropha tree with 30 cm length. After obtaining the cutting, the bottom tip side of cutting obtained was immersed in a root stimulator which has traditional name Atonik® (5 ml in 20 L of water) for 30 minutes to stimulate the root initiation. After that, the experimental cuttings were grown in the growth media made from mixture of soil and coconut husk 1:1 (v/v) in plastic bag under greenhouse condition for 10 weeks.

3.2.3 Transplantation of Seedling to Hydroponic System

After 10 weeks, on December 21st, 2013, the experimental plants were transplanted into hydroponic culture. Rice husk ash and coco coir 3:2 (v/v) were used as the artificial soil for supporting the plant root. Plant cuttings were placed in pots containing modified ½ Hoagland solution. For maintenance, the Hoagland solution was renewed every 2 weeks and the nutrient level inside the pots was observed and adjusted everyday. While for electric conductivity (EC) maintenance, the EC level was measured every 3 days to maintain it at desired level. The sodium chloride treatment was planned to be conducted on 4 weeks after transplanting. But, due to some problems which are transferring shock, cool temperature shock during winter season in Thailand (on January 2014 which has minimum temperature 8 ^{o}C), and insect issue, the growth of Jatropha seedling is inhibited. It took 10 weeks (6 weeks recovery period) for seedlings to fully recover from those issues and ready to be treated.

3.2.4 Sodium Chloride Treatment

The sodium chloride (NaCl) treatment was started on March 19th, 2014 with 2 weeks salinity acclimatization period. In this period, NaCl was added gradually every 3 days until reach particular concentration. After that, the salinity tolerant ability test was started and took 7 weeks of exposure time from April 2nd to May 21th, 2014. The Hoagland solution with three different NaCl concentrations; 2.0 (control), 8.0, and 16.0 dS m^{-1} was set and its EC level was measured every time before being used in the hydroponic culture. The treatment nutrient was renewed every 2 weeks for keeping the nutrient and EC level at desired level throughout the experiment period.

3.3 Experimental Design

Thirty six cuttings with 12 inch height and similar diameter size in each variety were selected for the treatment. Factorial in complete randomized design (CRD) with 4 replications was used in this experiment. Three plants were used and sampled in each replication. Factor A was the variety of Jatropha which are interspecific hybrid variety (V1, V2, V4) and non-hybrid variety (India, KUBP 35, KUBP 40). Factor B was the level of saline stress in Hoagland nutrient added with NaCl which are 2.0 (control), 8.0, and 16.0 dS m^{-1}. Two way factorial

ANOVA and Least Significant Difference (LSD) test were used as the method for mean comparison by using R stat program ver 2.13.1

3.4 Measurement

Parameters measured can be classified into two which are growth and physiological parameter. Growth parameter include number of leaves, relative growth rate (plant height), total leaf area, relative chlorophyll content (SPAD index), shoot dry weight. Physiological parameters include specific leaf area (SLA), leaf water potential (Ψt), solute concentration (Cs), leaf osmotic potential ($\Psi\pi$), and leaf turgor potential (Ψp).

Plant height and number of leaves were measured weekly, while specific leaf area and SPAD index were measured every 2 weeks. Leaf water potential (Ψt) and solute concentration (Cs) parameters were measured at sixth week of the experiment by following the previous study of Chaiwut (2010). Leaf water potential (Ψt) and solute concentration (Cs) measurement were only conducted to salt tolerant potential varieties (based on growth performance) which are V2 (interspecific hybrid), KUBP 35, and KUBP 40.

Specific leaf area (SLA) was measured using portable LI-3000A leaf area meter. Leaf samples for SLA measurement were taken from leaf number 4 and 5 from the tip. Total leaf area (TLA) was calculated as TLA = LA x LN, where LA is average leaf area and LN is leaf number. Relative chlorophyll content (SPAD index) was measured using SPAD-50 Minolta Inc. Japan. The leaf samples used for SPAD measurement were leaf number 4 and 5 from the tip.

Leaf water potential (Ψt) was measured with a pressure chamber using technique described by Turner (1981). Leaf water potential (Ψt) was measured during predawn (4.00 am – 5.30 am) from leaf number 5 from the tip. Stalk of leaf number 5 of every plant sample was excised from the plant using knife and then sealed with plastic. Then, sealed leaf sample was partly sealed in a pressure chamber. Next, the pressure chamber was pressurized with compressed gas until