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Physiological Responses of *Jatropha* to Drought Stress in Coastal Sandy Land Conditions

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Abstract

Jatropha curcas L., an important tropical biofuel crop, is reputed for its drought resistance, however, its ability to perform in dry conditions has still hardly been investigated. Changes in leaf water status, chlorophyll content, leaf surface temperature, stomatal conductance, proline and abcisic acid (ABA) content, transpiration and photosynthetic rate were studied in four *Jatropha* genotypes (IP-1A, IP-2M, Local superior and Yellow leaf) and subjected to drought stress in coastal sandy land conditions in Central Java, Indonesia. Drought stress significantly decreased the leaf water status, leaf chlorophyll content, stomatal conductance, transpiration and photosynthetic rate, and increased leaf temperature, proline and ABA content. Resistant genotypes (IP-1A and IP-2M) had significantly higher leaf water status, chlorophyll content and photosynthetic rate than susceptible genotypes (Local superior and Yellow leaf). There were no differences between the *Jatropha* genotypes on leaf temperature, stomatal conductance and transpiration rate.

Abstrak

Tanggapan Fisiologis Tanaman Jarak Pagar terhadap Cekaman Kekeringan di Lahan Pasir Pantai. Jarak pagar (*Jatropha curcas* L.), salah satu tanaman sumber bahan bakar nabati tropis, telah terkenal karena ketahanannya terhadap cekaman kekeringan, akan tetapi, kemampuannya untuk tumbuh pada lingkungan yang kering masih jarang diteliti. Perubahan status air daun, kadar klorofil, suhu permukaan daun, konduktansi stomata, kadar prolin dan *abcisic acid* (ABA), laju transpirasi dan fotosintesis dikaji pada empat genotip jarak pakar (IP-1A, IP-2M, Unggul lokal dan Daun kuning) yang diperlakukan cekaman kekeringan pada lahan pasir pantai di Jawa Tengah, Indonesia. Cekaman kekeringan menurunkan secara signifikan status air daun, kadar klorofil daun, konduktansi stomata, laju transpirasi dan fotosintesis, dan meningkatkan suhu daun, kadar prolin dan ABA. Genotip tahan (IP-1A dan IP-2M) memiliki status air daun, kadar klorofil dan laju fotosintesis lebih tinggi secara signifikan dibandingkan dengan genotip yang peka (Unggul lokal dan Daun kuning). Tidak terdapat perbedaan yang nyata di antara genotip jarak pagar terhadap suhu daun, konduktansi stomata dan laju transpirasi.

Keywords: ABA, biofuel, photosynthetic rate, proline, stomata, water status

1. Introduction

Dwindling sources of fossil fuel is forcing many countries in the world to begin looking for alternative biofuels. Researching biofuel substitutes for fossil fuels should focus on biofuels extracted from perennials grown on abandoned agricultural or degraded lands, as these do not cause a carbon debt at land use change [1-2].

Coastal sandy soil is abandoned agricultural but potential land, especially in Indonesia. As an archipelagic country, Indonesia has about 81,000 km of coastline [3]. With the assumption that half of the coastline has beaches of sandy soil of about 1 km wide, there is around 4.05 million ha of coastal sandy soil. The main problems encountered in utilizing coastal sandy soil to become agricultural land are its low organic matter and nutrients content, very loose soil structure, low water holding capacity and high salinity stress. In addition to that, extreme sunlight, high surface temperature, and high salt condense winds make it difficult to cultivate plants [4]. In this context, *Jatropha curcas* L. promises to be a sustainable biofuel option. With its seed containing up to 35% oil that is easily convertible into biodiesel, its potential for reclaiming land, its positive effects on ecology and socio-economic development [5] no

competition with food production or depleting natural carbon stock and ecosystem services [6], and with its reputation of being a drought-resistant and easy to establish species, it is now planted worldwide on wastelands in the tropics [7-8].

As an easy to establish species, *Jatropha* grows relatively quick and is hardy and drought tolerant. It is well adapted to semi-arid conditions, although more humid environmental conditions result in better crop performance. It has low nutritional requirements, can grow up to pH 9, even 11, but as its growth is restricted it does lead to poor yields. In very acidic soil, *Jatropha* might require some Ca and Mg fertilization [9]. It can also handle dryness very well and can survive almost entirely from the humidity in the air. Differences are expressed in its required optimum rainfall range as some readings say 600 mm and some say 800 mm [10], but generally it has been observed to grow in a rainfall range between 250 and 3000 mm. *Jatropha* can tolerate high-temperature extremes, but generally fears frost, which causes immediate damage. The plant is not sensitive to day length [8].

The resistance of some *Jatropha* genotypes to drought stress during the seedling period in coastal sandy land based on the growth rate of stem and leaf, and the plant's dry weight showed that, IP-1A and IP-2M were resistant genotypes, while Local Superior and Yellow Leaf were susceptible genotypes. Watering intervals of once in three days and once every nine days were the respective drought stress watering interval and optimum watering interval [11]. The aim of this study was to investigate the physiological responses of selected *Jatropha* genotypes to drought stress in coastal sandy land conditions. The parameters observed included leaf water relation, leaf temperature, leaf chlorophyll content, stomatal conductance, proline and abcisic acid (ABA) content, transpiration and photosynthetic rate.

2. Methods

The trial was one of a series of experiments conducted from October 2008 to November 2009 at Gadjah Mada Coastal Sandy Land Research Station at Purworejo sub-province, Central Java, Indonesia. Some analyses were carried out in The Crop Science and LPPT Laboratories, Gadjah Mada University, Yogyakarta, Indonesia.

A split plot experiment with genotypes and watering intervals as experimental factors was conducted. Genotypes were put as a sub plot, while watering intervals were put as the main plot. Four *Jatropha* genotypes (IP-1A, IP-2M, Local superior-NTB and Yellow leaf) and two watering intervals (optimum and drought stress) were used in this experiment, based on previous research results [11]. The experimental plot size was 6 x 6 m2, and planting space was 1.5 x 1.5 m2, so there were 16 plants/plots. The plants were grown from two month old *Jatropha* seedlings in coastal sandy land at the beginning of the rainy season (October 2008) and maintained as a standard *Jatropha* cultivation. At the beginning of the dry season (June 2009), when the plants were 7 months old, drought stress treatment was begun. A group of plants from each genotype was watered in optimum conditions (once in three days) until field capacity–determined using Time Domain Reflectometry, through-out the experiment. In the other plant groups from each genotype, their soil water content were imposed to drought stress by withholding water for 9 days. Measurements of all parameters were conducted at the end of the trial, i.e. after 5 months of drought stress treatment.

Measurements of leaf relative water content (RWC) were determined using the Equation (1) [12]:

$$\text{RWC (\%)} = 100 \text{ x } \left(\frac{Fw - Dw}{Tw - Dw} \right) \quad (1)$$

Where, F_w is the fresh weight, D_w is the dry weight, T_w is the turgid weight of leaf samples. Leaves were excised, weighed fresh (F_w) and placed in distilled water to rehydrate in the dark for 24 hours. The following day, leaf turgid weight (T_w) was measured and then leaves were dried at 80 ^oC for 24 hours and dry weight (D_w) was determined.

Leaf water potential (ψ_w) was also measured in different leaves but from the same plant using Scholander Arimad-3000. Leaves for both measurements were handled carefully to minimize water loss by enclosing them in plastic bags and putting them in a container immediately after excision. Chlorophylls were extracted from fresh leaves with 80% acetone and centrifuged at 10,000 g for 5 minutes. The absorbance of cleared extract was read at 645 and 663 nm [13] in Spectronic 21 D for chlorophyll a and b respectively, and calculated according to Lichtenthaler [14]. Total chlorophyll was expressed in mg/g fresh weight. Determination of proline content followed the procedure proposed by Bates [15], and modified by Magna and Larher [16]. The proline content was measured from the fifth leaf from the shoot of the main stem. Abcisic acid leaf content was measured following the procedure proposed by Yokota *et al.* [17] and modified. Leaf temperature, stomatal conductance, transpiration and photosynthetic rate were measured using Portable Photosynthesis System Li-6400 (LI-COR Biosciences Inc., Nebraska, USA). All parameters were observed between 9 to 12 am.

Data collected were analyzed using two-way analysis of variance (ANOVA) at a significant level of $p \leq 0.05$. The model was defined as split plot design on the basis of fixed effects. Effect of main plot (watering intervals),

sub plot (*Jatropha* genotypes) as well as their interaction were considered. When the ANOVA was significant at $p \leq 0.05$, Duncan Multiple Range Test was used for comparison of means.

3. Results and Discussion

Jatropha is a plant traditionally used for medicines, pesticides, cosmetics and hedges. But recently, its potential as an energy plant was realized: tests of its oil indicate that it is a potential substitute for diesel fuel. As a fuel wood substitute, it has far reaching and positive implications in forest conservation. Since it can be cultivated on poor soil and low rainfall areas, *Jatropha* may be a solution to make wastelands and abandoned agricultural or degraded lands productive [10].

Based on the observation and data analysis conducted, the watering interval affected all parameters observed; leaf water relation, leaf surface temperature, stomatal conductance, leaf chlorophyll content, transpiration and photosynthetic rate. *Jatropha* genotype only affected leaf water potential, leaf chlorophyll content and photosynthetic rate and interaction between them did not affect on all parameters observed (Table 1).

Leaf water status was significantly influenced by watering intervals and *Jatropha* genotype as well. The plants with optimum watering had significantly higher RWC (79.528%) and water potential (-0.345 MPa) than those under drought conditions (75.609% and 0.409 MPa). The water relation of resistant genotypes (IP-1A and IP-2M) was significantly higher than that of susceptible genotypes (Local superior and Yellow leaf). The leaf relative water content and potential were 80.273, 78.660, 75.408, and 75.933%; and 0.330, 0.356, 0.420, and 0.402 MPa, respectively (Table 2). There was strong positive correlation between RWC and ψ_w ($r = 0.84$). Drought, like other environmental stress conditions, affects many physiological and metabolic processes within plants. The suppression of watering considerably reduced the soil water content, and in severe drought conditions, the soil water availability induced a decrease in leaf RWC and water potential. The utilization of Leaf RWC as an indicator of plant water status is usual [18-19]. Data presented in this study is in agreement with previous findings. The RWC in leaves of drought stressed plants decreased significantly (Table 2). Many investigations have shown that when subjected to drought, leaves exhibit a large reduction in RWC and water potential [20-25].

Significant differences were found in both cell and surface leaf temperatures. Under drought conditions, leaf temperature increased significantly. The temperatures were 37.879 and 39.144 ^oC in the leaf cell and surface, respectively. Leaf cell temperature was always lower than leaf surface temperature. Although there was no significant difference between genotypes, leaf temperatures of resistant genotypes (IP-1A and IP-2M) were likely to be lower than susceptible genotypes (Local Superior and Yellow Leaf) (Table 2). Leaf temperature, or its depression below atmospheric

Table 1. Result of Variance Analysis for all Parameters Observed

Treatments	Parameters									
	RWC	ψ_w	T_{leafs}	T_{leafc}	g_s	Chl	E	Ab	Pc	Pr
Watering Interval	S	S	S	S	S	S	S	S	S	S
Jatropha genotype	S	S	NS	NS	NS	S	NS	S	S	S
Interaction	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

RWC: leaf relative water content, ψ_w: leaf water potential, T: temperature, leafs: leaf surface, leafc: leaf cell, g_s: stomatal conductance, Chl: chlorophyll content, E: transpiration rate, Pr: photosynthetic rate, S: significant at $p = 0.05$, NS: non significant at $p = 0.05$

Table 2. Leaf Water Relation (RWC in % and ψ_w in MPa) and Temperature (T_{leafc} and T_{leafs} in ^oC), and Stomatal Conductance (g_s in mol m^{-2} s^{-1}), of *Jatropha* Genotypes with Different Watering Intervals

Treatment	Level	RWC	ψ_w	T_{leafc}	T_{leafs}	g_s
Watering interval	Optimum	79.528 a	-0.345 a	36.138 b	36.866 b	0.098 a
	Drought stress	75.609 b	-0.409 b	37.879 a	39.144 a	0.038 b
Genotype	IP-1A	80.273 a	-0.330 a	36.728	37.483	0.103
	IP-2M	78.660 a	-0.356 a	36.740	37.905	0.079
	Local Superior	75.408 b	-0.420 b	37.132	38.470	0.060
	Yellow Leaf	75.933 b	-0.402 b	37.435	38.162	0.028

Different letter in the same column and treatment indicated significant differences at 0.05 based on Duncan Multiple Range Test (DMRT)

temperature, is considered to be a potential and proven indicator of plant water stress [26-31], and is based on the principle that an increase of plant water stress leads to decreased leaf transpirational cooling, and consequently increased leaf temperature relative to well-watered plants. Even, when stomata are fully closed, there is some transpiration, termed residual transpiration [32], and some plants with a high stomatal density showed high residual transpiration rates and cool canopies [33]. These observations support the temperature data presented in Table 2 that both cell and surface temperatures of the drought-stressed plants were significantly higher than temperatures of the well-watered plants.

Stomatal conductance was significantly affected by water deficit. The plants with optimum watering had significantly higher stomatal conductance (0.0975 mol m^{-2} s^{-1}) than those under drought conditions. Different genotypes did not affect stomatal conductance, however, resistant genotypes (IP-1A and IP-2M) tended to be higher than the resistant genotypes (Local Superior and Yellow Leaf) (Table 2). Stomata are the biological channels for gas movement between the plant and the surrounding atmosphere. Under lowered leaf RWC and water potential, plants wilt from a loss of cellular turgidity, especially in the leaves and concealing stomatal aperture thus causing stomatal closure [34]. Stomatal closure is a drought avoidance mechanism and is one of the first steps in a plant's adaptation to water deficit, allowing the water status to be maintained [35]. The closure is then followed by stomatal conductance reduction [36].

Water deficit significantly reduced leaf chlorophyll content. The plants with optimum watering had higher chlorophyll content, namely 0.0419 mg/g. Different genotypes resulted in different chlorophyll content. The drought resistant genotypes (IP-1A and IP-2M) had significantly higher chlorophyll content compared with susceptible genotypes (Local superior and Yellow leaf). The contents were 0.0429, 0.0445, 0.0369 and 0.0368 mg g^{-1}, respectively (Table 3). Limited water supply in a stressed plant causes the plant to take up insufficient water and mineral nutrients from the soil, and many biochemical and physiological activities are arrested, thus resulting in a reduction of leaf chlorophyll concentrations [37]. Water deficit reduces N uptake from the soil. As N is an essential ingredient for chlorophyll formation, drought stress significantly reduced the concentration of chlorophyll pigments [38]. The reduction of chlorophyll during drought conditions may refer to photoinhibition [37] and has been considered to be a typical symptom of oxidative stress and may be the result of pigment photo-oxidation, chlorophyll degradation and or chlorophyll synthesis deficiency [39]. Previous studies have found a reduction in chlorophyll concentration in water-stressed maize [40], wheat [41], phaseolus [42] and rice [43]. Such information is not, however, available for *Jatropha*.

Transpiration rate was only influenced by watering intervals. The plants with optimum watering intervals had significantly higher rates compared with drought-stressed plants. The rates were 3.2158 and 1.9367 mmol m^{-2} s^{-1}, respectively. Transpiration rate was influenced by some parameters including RWC, water potential, leaf temperature, stomatal conductance and closure. Data presented in Table 2 and 3 showed that there was a strong correlation between reduced RWC caused by drought conditions and the temperature of leaf cells and surface (0.83 and 0.84), stomatal conductance (0.81), and transpiration rate (0.87). These data indicate that reduction in RWC caused by drought decreased the transpiration rate. This condition also occurred with the *Jatropha* genotype. The higher value of the parameters affecting the resistant genotypes caused a higher transpiration rate.

Water deficit increased ABA and proline contents. The plants watered under optimum conditions had lower ABA (218.58 ppb) and proline contents (5.24 μmol/g) compared to the plants under drought stress (1196.50 ppb and 6.28 μmol/g, respectively). Resistant genotypes (IP-1A and IP-2M) had lower ABA and proline contents, compared to susceptible genotypes (Local superior and Yellow leaf). There was a negative correlation between RWC and proline content (r = -0.48).

Table 3. Leaf Chlorophyll Content (Chl in mg g^{-1}), Transpiration (E in mmol m^{-2} s^{-1}), ABA (Ab in ppb) and Proline Contents (Pc in μmol g^{-1}), and Photosynthetic Rate (P_r in μmol m^{-2} s^{-1}) of *Jatropha* Genotypes with Different Watering Intervals

Treatment	Level	Chl	E	Ab	Pc	P_r
Watering interval	Optimum	0.042 a	3.216 a	218.58 b	5.24 b	59.275 a
	Drought stress	0.039 b	1.937 b	1196.50 a	6.28 a	52.167 b
Genotype	IP-1A	0.043 a	3.638	335.17 b	5.59 bc	59.617 a
	IP-2M	0.044 a	2.928	206.50 b	4.62 c	58.900 a
	Local Superior	0.037 b	2.285	1183.33 a	6.09 ab	52.017 b
	Yellow Leaf	0.037 b	1.453	1105.17 a	6.74 a	52.350 b

Different letter in the same column and treatment indicated significant differences at 0.05 based on Duncan Multiple Range Test (DMRT)

Abcisic acid (ABA) is defined as a stress hormone because of its rapid accumulation in response to stresses and its mediation of many stress responses that helps plant to survive [44]. The involvement of ABA in mediating drought stress has been investigated by many researchers. ABA plays an important role in controlling plant water status through guard cells and also controlling growth by inducing gene encoding enzymes and proteins involved in cellular dehydration tolerance [45-46]. The previous research showed that ABA can act as a long-distance drought signal to indicate water deficiency in the soil [47]. Other studies support that ABA plays a double role in regulating plant physiological processes [48-49]. The role as an inhibitor, when accumulated in a huge amount during stress, can help a plant to survive through regulating the opening and closing processes of stomata and increasing plant size. Its role to promote plant growth in low concentrations under normal conditions has an important role in the vegetative phase of some plant organs, like primary root growth [50-51] and seedling growth after germination [47].

ABA is synthesized in both roots [52] and leaves, but not much is known about the precise location of this synthesis in roots which may influence how plants perceive and monitor soil water content [53]. In some plant species, root ABA content correlated with soil water and relative root water content [54-55]. Data in this research showed that there was a significant negative correlation between plant relative water content and ABA content ($r = -0.65$) indicating that the decrease in plant relative water content followed the increase in ABA content significantly.

The increase of a plant's ABA content due to drought stress plays an important role in the opening and closing of stomata [44,54,56-57). The high content of ABA promotes stomata closing. The result of this study showed that there was a significantly negative correlation between the ABA content and the width of the stomata opening (-0.71). It means that the increase of ABA content followed the narrowing of stomata opening significantly.

Proline is an amino acid formed from the degradation of amino acid/protein as a plant response to stress. The formation and increase of proline in a plant is an indicator that the plant is under stress [58-61]. Plants under stress increase proline content to manage the stress. In addition to its role as an osmolyte for osmotic adjustment, proline contributes to stabilizing sub-cellular structures (e.g. membranes and proteins), scavenging free radicals, and buffering cellular redox potential under stress conditions. It may also function as a protein-compatible hydrotrope, alleviating cytoplasmic acidosis, maintaining appropriate $NADP^+$/NADPH ratios compatible with metabolism, and also supporting mitochondrial oxidative phosphorilation and generation of ATP for recovery from stress and repairing of stress-induced damages [62-63]. The same results were also reported in other plants, such as mulberry [13], wheat [64], canola [65], and sun flower [66].

Leaf proline content had a close correlation with ABA. There was a significantly positive correlation between the proline and ABA content ($r = 0.76$). That means that an increase in ABA content will be followed by a significant increase in proline content. The increase of a plant's proline in response to drought stress was initiated by an increase of ABA [67]. ABA were needed to promote the increase of proline in low water potential conditions [68], and showed that proline accumulation in plants was mediated by free ABA marker pathways [64].

Significant differences were found in the photosynthetic rate between watering intervals and among genotypes. The photosynthetic rate of the plants with optimum watering was significantly higher than that of water-stressed plants. The rates were 59.275 and 52.167 μmol/ m^2s, respectively. The drought resistant genotypes (IP-1A and IP-2M) had a significantly higher photosynthetic rate compared with susceptible genotypes (Local superior and Yellow leaf). The rates were 59.617, 58,900, 52.017, and 52.350 μmol /m^2s, respectively. Plants under severe drought conditions had small but significant decreases in leaf RWC and a greater decrease in the gas exchange parameter. As stated before, stomatal closure is a drought avoidance response that allows leaf water content maintenance and the consequent decrease of internal CO_2 concentration limits photosynthesis [69,36]. The concomitant decrease in stomatal conductance (gas) CO_2 internal concentration and photosynthesis and linear relationships observed in plants under severe drought conditions indicated that a significant lessening of CO_2 internal concentration in severe drought conditions was mostly induced by stomatal closure [70], and varied between cultivars. This was in agreement with the data that drought stress decreased the photosynthetic rate and varied among genotypes. Resistant genotypes had a higher photosynthetic rate than with susceptible genotypes.

4. Conclusions

Drought stress in coastal sandy soil decreased water status, stomatal conductance, chlorophyll content, transpiration and photosynthetic rates, and increased proline and ABA content, and leaf temperature of *Jatropha*. The different genotypes resulted in different physiological responses. Resistant genotypes, IP-1A and IP-2M, were higher in water status, chlorophyll content and photosynthetic rate, compared with susceptible genotypes, Local superior and Yellow leaf.

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