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Rootstock selection of physic nut (*Jatropha curcas* Linn.) on drought stress tolerance

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Abstract: *Jatropha has the potential to be developed as biofuel, because of its high seed oil content. The seeds contain up to 35% oil which are convert into biodiesel easily. Superior plant materials had been produced through the composite selection (IP-1 to IP-3), were only able to express the potential yield on land that was in optimal conditions. On the contrary, land available for jatropha development, generally, in the form of dry land with limited water availability. Development of Jatropha in large scale in dryland require plant material with high productivity and able to adapt to drought conditions. In an effort to maintain the stability of jatropha production in dry land, it can be done through grafting technology by combining the advantages of prospective shoot section which has a high yield potential as a scion and rootstock candidates from plant material that is able to adapt to limited water availability. The activity had been carried out viz adaptability study of jatropha rootstock candidates associated with drought stress in glasshouse. The results of the study showed there were three jatropha rootstocks (IP-3M, Sulawesi 117 dan Jatim 045) those were tolerant to drought stress.*

Key word: *Jatropha, drought stress, productivity*

I. Introduction

Urgency of biofuel development in the country was higher with the fact that the government's ability to subsidize the price of oil decreased, and consumption for transportation, electricity and household grew up (Hasnam 2007). The use of plant-based energy sources is the most appropriate choice, given land and agro-climatic conditions that support and most of the population relies on agriculture. In addition, Bioenergy development has purpose to diversify sources of energy to overcome the energy crisis, as well as to support diversify of agriculture product management.

Jatropha (*Jatropha curcas* Linn.) is one species of shrub plants that can produce biofuels. Among the type of oil-producing plants, Jatropha is prefered because the seed oil content is 35%, and is easily converted into biodiesel. Besides Jatropha can also be used for reclaiming back the critical areas that provide a positive impact on the ecological and socio-economic development (Francis *et al.* 2005). Jatropha cultivation on a large scale in Indonesia began in 2005, and in its development, going through ups and downs. Some of the main causes of low success Jatropha cultivation were limited quality plant materials, unsuitable soil character with the growing requirements of the jatropha plant materials (Cholid *et al.* 2006; Swamy and Singh. 2006).

The potential area for the Jatropha development in Indonesia to the criteria of S1 (highly suitable), S2 (as suitable), and S3 (less suitable) was 49.53 million hectares (Mulyani *et al.* 2006). The major constrain of available land for jatropha development was limited water availability. Jatropha development on a wide scale requires a highly productive plant material and is able to adapt to the drought stress conditions.

Superior plant materials of the composite selection that have been produced by Research Centre for Estate Crops were superior provenances (IP-1 to IP-3), were only able to express the potential advantages of yield on optimal land conditions. Research trial about superior provenances (IP-1A, IP-1M, and IP-1P) showed that the highest seed production were achieved with optimum irrigation after soil moisture content reached 65%, while the plants were not irrigated seed yield decreased up to 72.8% (Riajaya *et al.* 2007).

One effort to maintain the stability of jatropha production in the drylands, can be done through grafting technology to combine scion candidate that has a high production potential with a potential rootstock that can adapting to the conditions of limited water availability. The evaluation results of the Jatropha accessions in Indonesia exploration showed that some accessions had tolerant properties of water shortage conditions (Sudarmo *et al.* 2007), so its can be used as a potential rootstock.

Jatropha plants have root systems that far exceed the crop canopy area, both at 1, 2, and 4 years crop ages (Fatah *et al.* 2010). Unfortunetly, there were lack information about roots distribution of jatropha accessions, and their response to drought stress. Diversity rooting characteristics of jatropha accessions allowed to select some accessions as potential rootstocks (Djumali 2010).

The activity will be carried out viz adaptability study of jatropha rootstock candidates associated with drought stress in glasshouse. The Selected rootstocks would used as grafting combinations those are expected to improve drought stress tolerance, and suitable with the characteristic land available for jatropha development, so could support the development of bioenergy.

II. Methodology

The experiment was conducted in a greenhouse at the Cikabayan Research Station (University Farm), Bogor Agricultural University (240 m above sea level), Dramaga Bogor, June - December 2011. The Experiment was arranged in a factorial with two treatments and four replications, using a randomized block design (RBD). The first factor was the water content consists of soil moisture content 80% (control), 60% and 40% field capacity. The second factor was the 10 accessions of jatropha consisted of IP-3M, Jatim-013, Jatim-045, NTT-065, NTT-080, NTB-019, NTB-047, NTB-116, Sulawesi 072 and Sulawesi 117, thus obtained 3 x 11 x 4 = 120 experimental units.

Germination of seeds was done by soaking the seeds first in the water and fungiside (Dithane 45) for 12 hours. Once that seeds were planted in the nursery box for 7 days, then seeds that had germinated seedlings planted one in each polybag size 40 cm x 50 cm which already contained sand and manure with the composition of 3: 1 in 7 kg medium weight. NPK fertilizer was given one week after planting (WAP) at a dose of 40 g urea + 40 g SP-36 + 20 g KCl.

Available water in the soil was determined by finding the difference between the soil moisture at field capacity and permanent wilting point. Determination of field capacity (pF 2:54) using 'pressure plate apparatus' and the permanent wilting point using a 'pressure membrane apparatus'. Soil samples included in the copper ring as deep as 20 cm. Further soil samples were saturated until excess water and left for 48 hours. Then instrument were sealed, and each pressure enforced in accordance with the desired pF (ie 1/3 bar for 2:54 and 15 pF bar for pf 4.20). If it has reached equilibrium (after being given the pressure for 48 hours), soil samples are removed and the water level was determined by the gravimetric method.

Watering was done every day for the first month. Irrigation treatment began at one month after plant seedlings were transpanted in polybags for 3 months. Adjustment of soil moisture content for each treatment was done every day during the experiment was conducted. Data were collected for plant growth variables included: plant height, stem diameter, number of branches and number of leaves. Analysis of the a and b chlorophyll content were done at 2 MAP using the Yoshida *et al.* (1976) method. At the end of the experiment 4 MAP, destructive observations include: leaf stems and roots dry weight. The data obtained were analyzed statistically using analysis of variance (ANOVA). Median difference test between treatments using Duncan's Multiple Range Test Range = DMRT 5%.

III. Results And Discussion

3.1 Morphological characters

The results indicated that all growth characters were affected by both soil water content and accessions, while the interaction between both treatments was not significant. In general, the growth of jatropha accessions look normal in soil moisture content (SMC) 80% field capacity (FC). Plant growth was inhibited at soil moisture content of 60% FC, and the highest inhibition was occured in the treatment of soil moisture content of 40% FC (TABLE 1).

Drought stress inhibited the growth of jatropha, showed by reducing the growth variables those were observed. The growth inhibition was indicated by the decrease in vegetative growth components included: plant height, number of leaves, number of branches and stem diameter. Number of branches were more influenced by genotypes of jatropha accessions which IP-3M (4.78) and Sulawesi 117 (4.67) had more branches than other provenances. Observations indicated some accessions had a prominent character as the shoots character and young leaves color on the likely accession of NTB 116 purplish red, and NTB 019 accessions had broad leaf size.

Reducing of morphological characters when limited water availability, showed that jatropha tried to adapt to drought stress for survived and continued their metabolism. The two main components of plant resistance to drought such as drought avoidance and drought tolerance (Harjadi and Yahya 1988). Drought tolerance refers to the extent to which plants maintain their metabolic functions when leaf water potential is very low. Drought tolerant plants is a feature that involves adaptive plant responses at the cellular level and the whole plant as the synthesis and accumulation of compatible organic solutes, the synthesis of stress proteins, up-regulation of antioxidant enzymes, the development of a deep and dense root system, epicuticular wax, and leaf rolling (Parry *et al.* 2005; Reynolds *et al.* 2005; Neumann 2008).

Table 1. Plant height, number of leaves, number of branches, and stem diameter of ten accession of rootstock candidates at three levels of drought stress.

Treatments	Plant height	Number of leaves	Number of branches	Stem diameter
	(cm)	(cm)	(branches)	(cm)
Soil water contents				
80% (control)	43.30 a	15.80 a	1.97 a	1.37 a
60%	39.32 b	12.83 b	1.47 ab	1.20 b
40%	38.19 b	12.53 b	1.33 b	1.19 b
Accessions				
IP-3M	39.21 bcd	23.56 a	4.78 a	1.38 a
Jatim 013	41.72 abc	12.00 bc	0.56 bc	1.25 ab
Jatim 045	40.06 bcd	11.00 bc	0.67 bc	1.23 ab
NTT 065	34.38 d	10.11 c	0.11 c	1.10 b
NTT 080	41.64 abc	10.89 bc	0.89 bc	1.29 ab
NTB 019	38.19 bcd	11.67 bc	1.00 bc	1.24 ab
NTB 047	47.60 a	13.22 b	1.67 b	1.35 a
NTB 116	41.63 abc	11.67 bc	1.11 bc	1.29 ab
Sulawesi 72	35.89 cd	9.22 c	0.44 c	1.20 ab
Sulawesi 117	42.36a b	23.89 a	4.67 a	1.24 ab

Note: The values in the same column followed by same letters are not significantly different at 5% level based on the DMRT test.

The use of plant dry weight as a determinant of plant tolerance test in line with the opinion of Levit (1980) and Havaux (1992) that a decrease in the level of plant biomass is one form of plant responses to drought stress. Blum (1996) explains that when the water supply is not sufficient for evapotranspiration and plant undergo water stress, transpiration and assimilation tend to start declining. Dry weight of ten accessions of jatropha on three levels drought stress was presented in TABLE 2.

Table 2. Dry weight of ten accessions of jatropha on three levels of drought stress.

Treatments	Dry weight (g/plant)					Rasio root/shoot
	Leaves	Stem	Shoot	Root	Plant	
Soil water contens						
80% (Control)	4.15a	11.68a	15.83a	5.56a	21.40a	0.35b
60%	1.90b	6.91b	8.81b	3.64b	12.45b	0.41a
40%	0.93b	5.86b	6.79c	2.77b	9.56c	0.41a
Accesssions						
IP-3M	2.86	8.15	11.01	3.78	14.79	0.34
Jatim 013	2.53	7.50	10.03	4.88	14.91	0.49
Jatim 045	1.99	9.72	11.71	4.63	16.34	0.40
NTT 065	0.86	5.70	6.56	3.51	10.07	0.54
NTT 080	2.19	8.60	10.78	4.14	14.92	0.38
NTB 019	1.71	6.71	8.42	4.29	12.72	0.51
NTB 047	2.49	10.02	12.51	2.41	14.91	0.19
NTB 116	2.46	8.12	10.58	3.27	13.85	0.31
Sulawesi 072	2.90	8.05	10.95	4.28	15.23	0.39
Sulawesi 117	3.32	8.92	12.24	4.73	16.97	0.39

Note: The values in the same column followed by same letters are not significantly different at 5% level based on the DMRT test.

TABLE 2 showed that the leaf, stem and root dry weight were reduced with increasing drought stress. Increasing drought stress level from 80% FC to 60% FC was reduced dry weight of leaves (54.16%), stem (40.87%) and root (34.57%) respectively. When drought stress level increased to 40% FC, then the decreasing dry weight of leaves (77.52%), stem (49.88%) and root (50.16%) were very sharp. Different accessions had different dry weight as respond to drought stress, which accession of Sulawesi-117 reached the highest plant dry weight, although was not significantly different to the other accessions. This result was in agreement with Esfahani and Mostajeran (2011) stated that drought stress reduces the structural and functional organs such as the canopy dry weight and root dry weight.

The root/shoot ratio illustrated the balance of root and canopy growth. Root/shoot ratio increased with increasing degree of drought stress, the ratio of root/shoot was highest at soil moisture content 40% FC (0.41) but was not significantly different with soil moisture content 60% FC. The proportion of root weight can be changed as a respond to the drought, root/shoot ratio increases in water stress conditions (Nicholas 1998). The degree of inhibition of drought stress increased from leaves, roots, and stems.

When gripped to the drought, jatropha would extend roots, (TABLE 2), reduced leaf area and number of leaves (Table 1), in order to remain absorbed water optimally and reduced water loss through transpiration. Plants use the mechanism to avoid drought has deeper roots and dense root systems, higher root penetration ability, higher stomatal conductance, higher cuticular resistance to prevent water loss, and avoid rolling the leaves for a longer interval (Peng and Ismail 2004).

Other plant species avoid water stress by developing a deep root system and/ or mechanisms involved in the loss of water through lower transpiration rates. Legumes at arid areas such as peanut clusters [*Cyamopsis tetragonoloba* (L.) Taub] and cowpea [*Vigna unguiculata* (L.) Walp] shows the development of the deep root system with slow growth (Kumar 2005).

Fig. 1 showed that drought stress increased with a decrease in soil water content from 80% to 40% FC was not significantly reduced morphological character in the tolerant accession (Sulawesi 117), due to jatropha trying to adapt to drought stress, and kept metabolic processes normaly. In contrast, the sensitive accession (NTT 008), when reduction of soil water content was occurred from 80% to 40% FC significantly decreased the morphological traits such as leaf area and leaf numbers, so the loss of water through transpiration can be reduced.

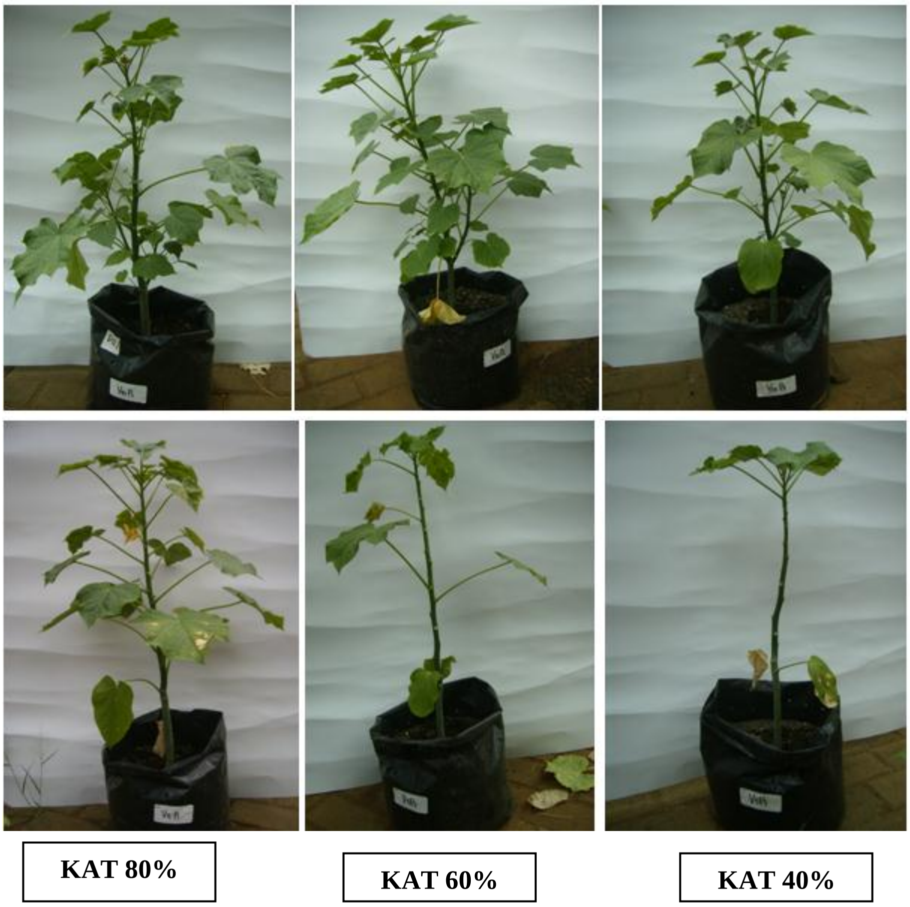

Figure 1. Morphological permormance of Sulawesi-117 jatropha accessions (tolerant) and NTT-080 (susceptible) to drought stress at 80, 60, 40% FC.

3.2 Physiological characters

3.2.1 Chorophyll

Chlorophyll is the photosynthetic pigment found in plants, absorbs red, blue, and purple light, and reflect green light which causes plants to obtain the color characteristics. In the chloroplasts of higher plants, there are two kinds of chlorophyll which is a major energy-absorbent material that are chlorophyll a and chlorophyll b. Thesse chlorophylls utilize the absorbed light energy for the light reactions in photosynthesis. The colour of chlorophyll is a bluish green with the chemical formula $C_{55} H_{72} O_5 N_4 Mg$, whereas chlorophyll b is yellowish green with the chemical formula $C_{55} H_{70} O_6 N_4 Mg$.

Chlorophyll formation is influenced by genetics, the availability of oxygen, light, carbohydrates and some elements such as N, Mg, Fe, and Mn. The results of this study indicated that the content of chlorophyll a, b, and total chlorophyll were not affected by jatropha accessions (Fig. 2).

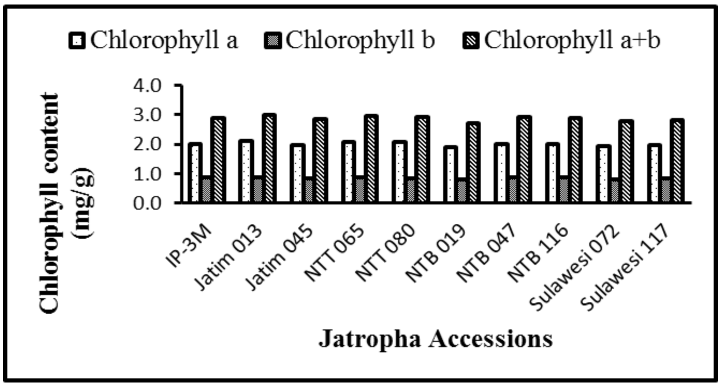

Figure 2. The chlorophyll a and b and total chlorophyll content of ten jatropha accession rootstock candidates

The content of chlorophyll a ranged from 1.92 to 2.12 mg/g, chlorophyll b ranged from 0.82 to 0.89 mg/g, and total chlorophyll ranged from 2.74 to 3.01 mg/g. The content of chlorophyll a, b, and total chlorophyll were decrease with a decreasing in soil water content from 80% to 60% FC (Fig. 3). In drought stress conditions where soil water availability is limited, would reduce of nutrients solubility in the soil and nutrient transport get into the plant, would inhibit the formation of chlorophyll in the leaves. Leaf temperature is increase when transpiration rate is decrease, which transpiration serves as cooling-process, if it lasts long in duration will damage the chloroplast organ as the main location of the chlorophyll.

The decreasing of chlorophyll content will inhibit the photosynthesis process, thus photosynthate formed will decreases, which in turn will reduce the dry weight of the plant. The limited photosynthate produced by the leaves as a source in the process of photosynthesis, resulting in decreasing the number of photosynthate (sink) that can be allocated to plant organs such as leaves, stems and roots. The research proves that when the moisture content reduced from 80% to 40% FC, there was a very sharp decreasing the dry weight of leaves (77.52%), stem (49.88%) and root (50.16%) respectively (TABLE 2).

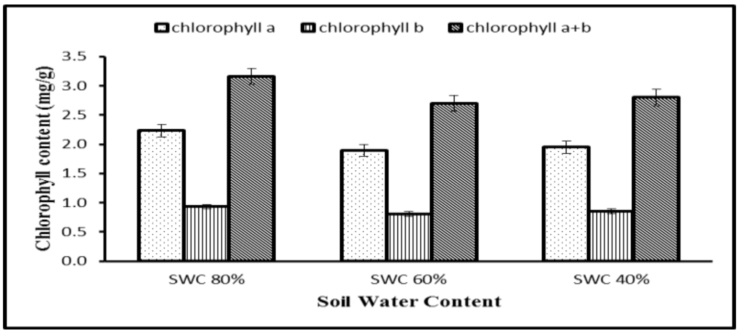

Figure 3. The content of chlorophyll a and b at three levels drought stress.

3.2.2 Open Stomata

Transpiration is the continuously transport process of water and nutrients, so that plant metabolism can take place. Stomata have an important role in transpiration because it serves to prevent excessive water loss, and helps control the leaf temperature. The size and density of stomata can be used as an indicator of water loss (Singh and Sethi, 1995, Venora and Calcagno, 1991, Wang and Clarke 1993), but the width of the stomatal pore and determine the capacity of the stomata to reduce water loss (Aminian *et al.* 2011, Mohammady *et al.* 2005).

Fig. 4 illustrated the number of open stomata in ten jatropha accessions at soil moisture content of 40% field capacity. Accession NTB 019 had the largest number of open stomata compared to other accessions amounting 214.01/mm^2, followed by IP-3M, Jatim 013, Jatim 045, NTB-047, Sulawesi-072, NTB-080, NTB-116, NTT-065. Whereas, Sulawesi-117 had the lowest number of open stomata (71.33/mm^2). According to Lakitan (2007) stated that stomata opening due to turgor pressure by guard cell. Increasing turgor pressure of guard cell caused by water passed into the guard cells. The number of stomata are open in drought stress conditions indicate that the accession is able to regulate the rate of transpiration so that the metabolic process can take place properly.

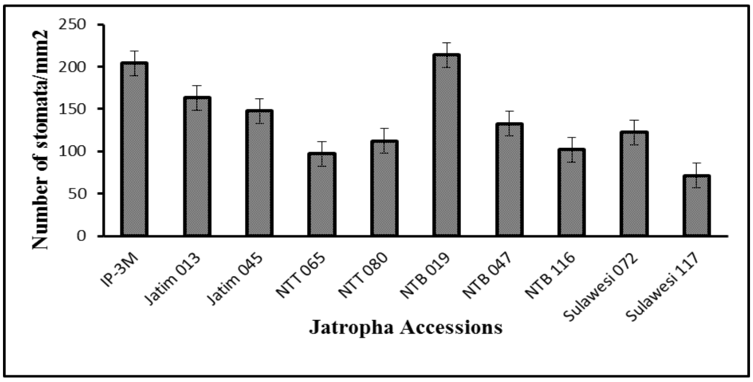

Figure 4. The number of stomata of ten Jatropha accessions at soil moisture content 40% field capacity

Stomatal closure resulted in discontinuation of some or all of the transpiration stream so that the plant can not absorb water and nutrients in the soil. As a result, the rate of photosynthesis decreases reducing the number photoasimilate for plant growth and reproduction. Dehydration root trigger the synthesis of abscisic acid (ABA) which helps remove potassium ions from the guard cells causes stomatal closure and retention of water on the leaves (Gomes *et al.* 2004; Parry *et al.* 1992).

IV. Conclusion

Drought stress by reducing soil moisture content up to 40% field capacity inhibited vegetative growth of jatropha, with a decrease in the size and plant dry weight on average greater than 50%. Jatropha plant responsed to drought stress indicated by morphological changes in the mechanisms that plant height, number of leaves, number of branches, stem diameter, reduction in plant dry weight, and root length. There are three potential accession as drought-tolerant rootstocks namely: IP-3M, Sulawesi-117 and Jatim-045. By obtaining rootstocks are adaptive to drought stress, so it can be used as a source of rootstock which is expected to improve jatropha productivity on limited water availability condition.

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References

[1]. Aminian R, Mohammadi S, Hoshmand S, Khodombashi M. 2011. Chromosomal analysis of photosynthesis rate and stomatal conductance and their relationships with grain yield in wheat (Triticum aestivum L.) under water-stressed and wellwatered conditions, Acta Physiol Plant. Vol. 33 755–764.
[2]. Blum A. 1996. Crop response to drought and the interpretation of adaptation, J. Plant Growth Regul., 20(2): 135-148.
[3]. Cholid M, Romli M, Estiana H. 2006. Shoot regeneration of physic nut (Jatropha curcas L.) via micro cutting. Workshop II Technology Status of Physic Nut (Jatropha curcas L). Bogor, November 29, 2006. Indonesian Center for Estate Crops Research and Development.
[4]. Djumali. 2010. Identification of Jatropha character affecting production and oil content. Mid-year report of Indonesian Tobacco and Fibre Crops Research Institute, Malang.
[5]. Esfahani MN, Mostajeran A. 2011. Rhizobial strain involvement in symbiosis efficiency of chickpea–rhizobia under drought stress: plant growth, nitrogen fixation and antioxidant enzyme activities Rhizobial strain involvement in symbiosis efficiency of chickpea–rhizobia under drought stress: plant growth, nitrogen fixation and antioxidant enzyme activities, Acta Physiol Plant. Vol. 33 1075–1083.
[6]. Fatah GSA. 2010. Studies Jatropha rooting system. Mid-year report of Indonesian Tobacco and Fibre Crops Research Institute, Malang.
[7]. Francis G, Edinger R, Becker K. 2005. A concept for simultaneous wasteland reclamation, fuel production, and socio-economic development in degraded areas in India: need, potential and perspectives of Jatropha plantations. Natural Resources Forum 29, 12–24.
[8]. Gomes AMM, Lagoa AMMA, Medina CL, Machado EC, Machado MA. 2004. Interactions between leaf water potential, stomatal conductance and abscisic acid content of orange trees submitted to drought stress, Braz. J. Plant Physiol. Vol. 16 (No. 3): 155-161.
[9]. Harjadi, S.S dan S. Yahya. 2008. Physiological stress of Envrironment. Pau Biotechnology. Bogor Agricultural University Press. Bogor. p. 191-196.
[10]. Hasnam. 2007. Status of plant material improvement and supply of Jatropha (Jatropha curcas L.). Proceedings of Workshop II: Technology Status Jatropha.
[11]. Havaux M. 1992. Stress tolerance of photosystem 2 in vivo. Antagonistic effects of water, heat and photoinhibition stresses. Plant Physiol., 100, 424-432.
[12]. Kumar D. 2005. Breeding for drought resistance. In: Ashraf M, Harris PJC (eds) Abiotic Stress: Plant Resistance Through Breeding and Molecular Approaches. Haworth Press, New York, pp 145–175.
[13]. Lakitan, B. 2007. Basics of Plant Physiology. Jakarta : Raja Grafindo Persada.

[14]. Levitt J. 1980. Response of Plants to Environment Stresses. Volume 1. Academic Press. New York.
[15]. Mohammady S, Moore K, Ollerenshaw J, Shiran B. 2005. Backcross reciprocal monosomic analysis of leaf relative water content (LRWC), stomatal resistance (SR) and carbon isotope discrimination (D%) in wheat under pre-anthesis water-stress conditions, Aust. J Agric. Res. Vol. 10 1059–1068.
[16]. Mulyani AF, Agus, Allelorung D. 2006. Land resource potential for physic nut (Jatropha curcas L.) development in Indonesia. Journal of Agricultural Research 23 (4): 130-138.
[17]. Neumann PM. 2008. Coping mechanisms for crop plants in drought-prone environments. Ann Bot 101: 901–907.
[18]. Nicholas S. 1998. Plant resistance to environmental stress, Curr. Opin. Biotechnol. Vol. 9. 214–219.
[19]. Parry AD. Griffiths A, Horgan R. 1992. Abscisic acid biosynthesis in roots. II The effects of water stress in wild type and abscisic acid deficient mutant (notabilis) plants of Lycopersicon esculentum Mill., Planta Vol. 187 192-197.
[20]. Parry MAJ, Flexas J, Medrano H. 2005. Prospects for crop production under drought: research priorities and future directions. Ann Appl Biol 147: 211–226.
[21]. Peng S, Ismail AM. 2004. Physiological basis of yield and environmental adaptation in rice. In: Blum A, Nguyen H (eds) Physiology and Biotechnology Integration for Plant Breeding. Marcel Dekker, New York, pp 83–140.
[22]. Reynolds MP, Mujeeb-Kazi A, Sawkins M. 2005. Prospects for utilizing plant-adaptive mechanisms to improve wheat and other crops in drought and salinity-prone environments. Ann Appl Biol 146: 239–259.
[23]. Riajaya PD, Hariyono B, Kadarwati FT. 2007. Physic nut (Jatropha curcas L.) performance on a various of soil water availability. The oral supporting paper of National Workshop on Jatropha III. Jatropha technology innovation to support the DME program. Malang. November 2007.
[24]. Singh S, Sethi GS. 1995. Stomatal size, frequency and distribution in Triticum aestivum, Secale cereale and their amphiploids, Cereal Res. Commun. Vol. 23 103–108.
[25]. Sudarmo H, Heliyanto B, Suwarso, Sudarmadji. 2007. Potential of physic nut (Jatropha curcas L.) accessions. Workshop II Technology Status of Physic Nut (Jatropha curcas L). Bogor, November 29, 2006. Indonesian Center for Estate Crops Research and Development. p. 111-114.
[26]. Venora G, Calcagno F. 1991. Study of stomatal parameters for selection of drought resistant varieties in Triticum Durum DESF, Euphytica Vol. 57 275–283.
[27]. Wang H, Clarke. JM. 1993. Genotypic, intra plant and environmental variating in stomatal frequency and size in wheat, Can. J Plant Sci. Vol. 73 671–678.
[28]. Swamy SL, Lalji Singh. 2006. Strategies for development of quality planting stock of Jatropha curcas for biofuel plantations. Biodiesel Conference Toward Energy Independence-Focus on Jatropha. Rashtrapati Nilayam, Bolaram, Hyderabad India on 9-10 June, 2006. 143-1157.
[29]. Yoshida, S., D.A. Forno, J.H. Cook, and K.A. Gomez. 1976. Laboratory Manual for Physiological Studies of Rice (3rd Ed.). IRRI, Philippines. 83p.