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Combination of Jatropha Cultivars with the Best Dose Fungi Mycorrhizal Arbuscular and Cytokinin Concentrations for Lowland Plant

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ABSTRACT : Indonesia has a very large marginal land area. Marginal land utility for Jatropha growth as an alternative plant of conversion from fuel oil to biofuel is the best solution. One of type marginal soil is inceptisol, with a limiting factor for low soil chemical fertility, especially the P element. The P element can become available to plants with the help of fungi mycorrhizal arbuscular (FMA). To reveal the performance of plant growth through the combination of three Jatropha cultivars with the best dose of FMA and the concentration of cytokinins on photosynthesis results of the lowland plant, an experiment was conducted. The experiment was implemented in 2019 at an altitude of 0-50 meters above sea level. The combination of Jatropha cultivars, the best dose FMA, repeated cytokinin concentrations 3 times were used to evaluate the best composition. The results of the experiment showed that a real effect on each treatment was achieved. From the results of each interaction, leaf chlorophyll content was around 14.43-25.83 CCI, stomatal conduction was around 256.4-474.03 mmol m-2 s-1, plant height around 46.43-65.5 cm, the number of leaves around 13.66-32.33 strands.

KEYWORDS: Photosynthesis, fungi mycorrhizal arbuscular, jatropha, cytokinin, lowland.

I. INTRODUCTION

The current condition many agricultural lands has changed functions settlements and industry encourages efforts to use marginal land, given the vast marginal land area in Indonesia. Inceptisol soil is marginal land that has limited soil chemical fertility, especially the P element, so we need a method to increase P element in the soil. The P element can be available to plants with the help of fungi mycorrhizal arbuscular (FMA). The development of jatropha plant as an alternative crop for conversion of fuel oil to biofuel should not be competent in land use with the national food security agency resilience program. This is because most of the land that is expected to be agricultural land is already used for other commodities or for the non-agricultural sector. Therefore, planting jatropha curcas should be directed at marginal lands, that is, land with limited water availability and nutrients[1], [2]. Recently there has been increased interest promoting bioenergy as an alternate renewable energy source. Biodiesel from Jatropha curcas is an important form of bioenergy and have potensial application in various sector that use fuel for energy[3].

The benefits of FMA can be grouped into three, namely benefits in ecosystems, benefits for plants, and benefits for humans. The benefits of FMA in ecosystems are very important, namely playing a role in nutrient cycling, improving soil structure, and channeling carbohydrates from plant roots to other soil organisms. Benefits for plants can increase nutrient absorption, P especially. The FMA can release phosphatase enzymes and 16 organic acids, especially oxalic acid which can help P free[4]. In addition, The FMA plays a role in improving soil structure, increasing nutrient solubility and weathering of parent materials. The FMA benefits from host plants in the form of carbon compounds from photosynthesis[5]–[7]. The association between mycorrhizae with plant roots is mutualism in that they both benefit from each other. Mycorrhizal fungi can utilize plant root exudates as a source of carbon and energy, while plants more easily absorb nutrients, P nutrients especially[8]. The research result of[3] showed that mycorrhizae propagules stimulated rhizobium and plant growth by increasing plant height, dry weight and nodule weight. Fungi mycorrhizal can also provide hormones such as auxin, cytokines, giberellin, also growth regulators such as vitamins are also reported as a result of mycorrhizal fungal metabolism. The FMA is the type of mycorrhiza that receives the most attention, because it is known to be symbiotic with about 80 % of plant species. Naturally there are mycorrhizal associations between fungi and plants in the form of symbiotic mutualism. Functional obtained benefits with the help of the FMA can be seen from the formation of the structure of arbuskula and vesicles in root cells and spore production which tends to be higher.

The development of the FMA and spore production requires energy obtained through the absorption of organic carbon from the host plant. Meanwhile, host plants can utilize symbiotic fungi in the form of mineral nutrients and water that are absorbed with the help of the FMA so that growth and yield of plants increases[8]–[10]. The symbiosis with the FMA has been widely known to be able to improve the nutrition of host plants through nutrient absorption and water which will ultimately increase plant growth and productivity[11], [12]. The FMA used has the same spore density or the same density of 450 spores / 25 grams or 18 single spores for each genus of FMA, so that in one mix of the FMA consortium there are 54 spores / gram with the germination capacity of at least 80 % consists of the genus Gigaspora sp., Glomus sp., and Acaulospora sp. The FMA (which infect the root system of the host plant) produce external hyphae that can expansively grow and are able to penetrate the subsoil layer. As a result, the capacity of roots to absorb nutrients and water will affect the process, namely: inducing cells, tissues or callus into perfect shoots and plants (organogenesis), accelerating cell division[10], [13]–[15]. The cells that are stimulated will then divide more quickly and the deciduous leaves can inhibit chlorophyll damage. Stem and leaves are part of plants which active in photosynthesis. Jatropha is a compound flowering plant where the fruit will appear at the ends of the armpits of the leaf. The addition of cytokines will increase the number of production branches from 42 to 60 panicles [16]. Cytokines themselves are substances that regulate the process of cell division. That is the main role. The role of cytokines has been seen very clearly for the development and growth of plants, especially in the process of cell division[9]. Using of Jatropha cultivar, best dose of FMA, and Cytokinin concentrations for lowland plant expected to be a promisisng renewable energy source. With This renewable energy is expected to be one solution to the scarcity of fossil fuels from animal fossils in the future.

II. MATERIALS AND METHODS

The materials used in this experiment were cultivated Jatropha seeds IP-3P (Improve Population 3 Pakuwon), IP-3A (Improve Population 3 Asembagus), IP-3M (Improve Population 3 Muktiharjo), FMA Consortium (Glomus sp., Gigaspora sp., and Acaulospora sp.) (Cucu Suherman, 2011), cytokinins (BAP), manure, Chemical fertilizers used are Urea (45% N), SP-36 (36% P2O5), KCl (60% K2O), and Dithane M-45 fungicide. The experiment was carried out in the lowlands (0-50 meters above sea level), having an Inceptisol soil order and C type rainfall [17], [18]. The trial period starts from January to June 2019 as presented in Figure 1.The experimental design used was a factorial randomized block design consisting of : The three factors of the best level of FMA dose, namely cultivar IP-3P with a 5 gram FMA dose, IP-3A with a 5 gram FMA dose, IP-3M with a 10 grams FMA dose (the FMA used has the same spore density or the same density of 450 spores / 25 grams or 18 single spores for each genus of FMA, so that in one mix of the FMA consortium there are 54 spores / gram) and five factors levels of cytokinin concentrations are 0 mg L-1, 100 mg L-1, 200 mg L-1, 300 mg L-1, 400 mg L-1. First factor, the best FMA consortium application combination for each jatropha cultivar (which is symbolized by X), consist of three levels, that : x1 = IP-3P Cultivar + best FMA application; x2 = IP-3A Cultivar + best FMA application; and x3 = IP-3M Cultivar + best FMA application. Second factor, growth hormone concentration (S), consist of five levels, that : s0 = 0,0 mg L-1 cytokinin; s1 = 100 mg L-1 cytokinin; s2 = 200 mg L-1 cytokinin; s3 = 300 mg L-1 cytokinin; and s4 = 400 mg L-1 cytokinin.

The research was conducted in Inceptisol land with indigenous FMA conditions in Sindanglaut, Cirebon Regency as much as 62 spores. 25 grams-1 and the state of FMA indigenous Jatinangor, Sumedang Regency as much as 201 spores. 25 grams-1. The FMA consortium used was obtained from indigenous multiplication with zeolite as a carrier. The application of FMA in plant is when the plants are young (rooted out) because FMA is associated with the host plant roots. FMA in zeolite as it's carrier is given to the planting medium hole around the host plant root rhizosphere according to the treatment dose. Growth hormone cytokinins were given at a dose according to the calibration results, that 25 ml per plant, this is first made a stock solution then given according to the treatment dose. Cytokinin hormones are given only once on growth plant period, that two weeks after pruning. Cytokinins application on Jatropha is by spraying this hormone at the top of Jatropha plant, that on the leaves and stems of the plant.

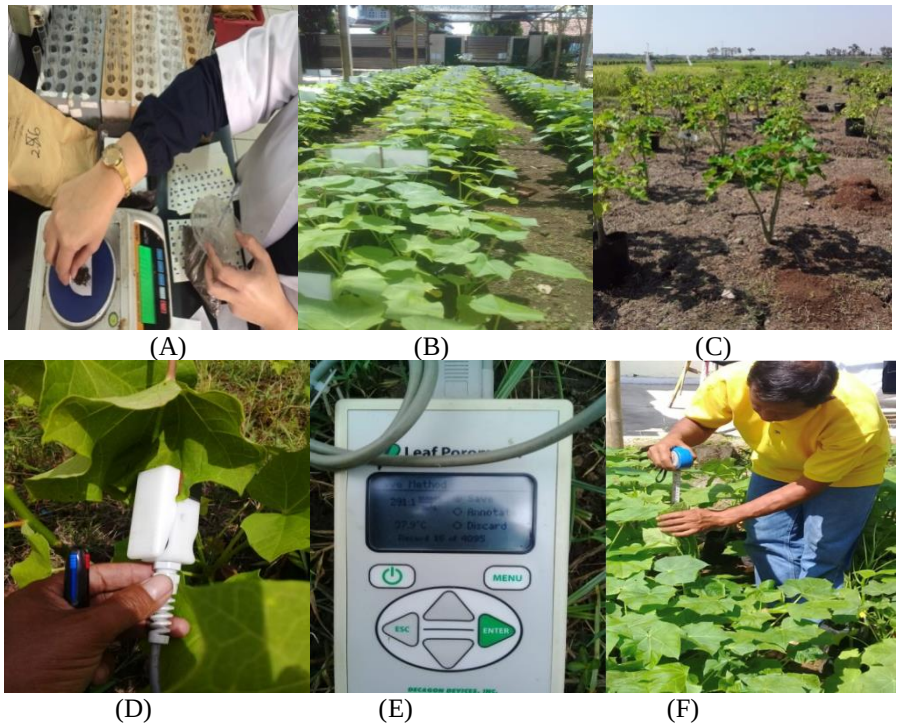

Figure 1. Flowchart of Research: (A) mycorrhizal application, (B) cytokinin application in the nursery, (C) planting in the field, (D) observation of leaf chlorophyll content, (E) observation of stomatal conduction, (F) observation of plant height and number of leaves.

The number of treatment combinations 15 was repeated 3 times each so that the experiment revealed 45 plots. Placement of treatment at each test carried out randomly. Response variable consists of supporting observations and main observations. Supporting observations are observations whose data are used to support the main observations, such as temperature and humidity of the air. The main observation on the response variable. Response variables observed in the field and the data analyzed statistically are as follows: (a). Plant height, observed starting from the base of the stem to the point of growth, observed age 4, 6, 8, and 10 MST; (b). Number of Leaves, all leaves formed were active in photosynthesis at ages 4, 6, 8 and 10 MST; (c). Chlorophyll Leaves, observed on the third leaf which is fully active in photosynthesis at the age of 4, 6, 8, and 10 MST using a chlorometer; (d). Stomata Conductivity, observed on the third leaf which is fully photosynthetic active at ages 4, 6, 8, and 10 MST using a porometer. Then analyzed statistically using analysis of variance with the F test on variables. If there is a significant effect, then followed by Duncan's multiple range test to find out the magnitude of the average difference between treatments.

III. RESULTS

The Based on the statistical analysis results, a real effect on each treatment was found. Interaction occurred between the combination treatment of Jatropha cultivar and the best dose of the FMA with cytokinin concentrations on leaf chlorophyll content, stomata conduction, plant height, and the number of leaves. For more details, each of these influences can be seen in Table 1. In Table 1 it is informed that the average number of treatments is marked with the same lowercase (lane direction) and the same capital letters (row direction) differing insignificantly according to the multiple distance duncan test at 5 % significance level. x1, x2, and x3 are a combination of the best FMA consortium dose with 1P-3P, 1P-3A, and 1P-3M cultivars, respectively. s0, s1, s2, s3, and s4 are the best FMA consortium dosages with concentrations, respectively: 0, 100, 200, 300, and 400 mg L-1. The statement was strengthened by research[2]giving a combination of FMA isolates had a significant effect on vegetative growth (plant height, number of leaves, leaf chlorophyll content and stomata conduction) of Jatropha plants at 4 months after planting.

Table 1. Diversity of plant photosynthesis results: leaf chlorophyll, stomata conduction, plant height, and the number of leaves.

	chlorophyll leaves (CCI)					Stomata Conduction (mmol m^{-2} s^{-1})				
	s_0	s_1	s_2	s_3	s_4	s_0	s_1	s_2	s_3	s_4
x_1	14,86 a A	16,3 a A	23,8 b B	16,56 a A	14,73 a A	298,4 a AB	334,8 a AB	404,3 b B	263,83 a A	357,33 a AB
x_2	14,43 a A	21,66 a AB	18,9 ab AB	16,73 a A	25,83 b B	256,4 a A	350,63 a AB	429,03 b B	283,8 a A	316,83 a AB
x_3	21,73 b A	16,43 a A	16,13 a A	17,26 a A	18,46 a A	311,5 a A	332,1 a A	285,2 a A	474,03 b B	316,83 a A
	Plant Height (cm)					Number of Leaves (sheet)				
	s_0	s_1	s_2	s_3	s_4	s_0	s_1	s_2	s_3	s_4
x_1	49,03 a AB	55,16 a BC	46,43 a A	60,16 b C	61,06 b C	15 a AB	17,33 a AB	12,66 a A	32,33 a C	22 a B
x_2	59,93 b BC	53,6 a AB	48,33 a A	65,5 b C	50,66 ab A	13,66 a A	23,33 a B	29 b B	25,66 a B	26,66 a B
x_3	47,16 a A	51,5 a A	50,33 a A	52 a A	46,83 a A	20 a A	23,33 a A	19 a A	26,33 a A	24 a A

Values followed by similar letters under the same column are not significantly different at p = 0.05 according to Duncan's multiple range test. x_1 = IP-3P cultivar + best FMA application; x_2 = IP-3A cultivar + best FMA application; x_3 = IP-3M cultivar + best FMA application; s_0 = 0 mg L^{-1} cytokinin; s_1 = 100 mg L^{-1} cytokinin; s_2 = 200 mg L^{-1} cytokinin; s_3 = 300 mg L^{-1} cytokinin; s_4 = 400 mg L^{-1} cytokinin.

Based on Table 1, the obtained results are Jatropha curcas IP-3A with a dose of 5 grams with a cytokinin concentration of about 400 mg L-1 (s4) showing the best results for leaf chlorophyll content of 25.83 CCI. Giving the combination of Jatropha cultivar with the best FMA dose and cytokinin concentration showed better results in leaf stomata content when compared without FMA and cytokinin which only ranged from 3.2 CCI, Ince Raden et al (2008). The chlorophyll content of leaves is obtained from the leaves of the third jatropha plant which is active in the process of the photosynthesis. Leaves are both an indicator of growth and a parameter that can describe the ability of plants to carry out photosynthesis. The more chlorophyll content indicates the greener of the leaves. The leaves are used by plants to have photosynthesis.

IV. DISCUSSIONS

The arbuscular mycorrhizal symbiosis between terrestrial plants and FMA is regulated by plant hormones. For cytokinin there are still contradictory reports even tought for most of hormones has been clearly assigned in this mutualistic interaction. [19]concluded that cytokinin have a stimulatory role in arbuscular mycorrhizal colonization because increased active cytokinin level were paralleled with increased arbuscular mycorrhizal colonization while decreased cytokinin levels corresponded to reduce arbuscular mycorrhyzal colonization. Ultra-performance liquid chromatography-based estimation revealed a significant increase in cytokinins in the inflorescence meristem of OsCKX2-knockdown plants [16]. Treatment of plants with 6-Benzyladenine at the beginning of flower formation (stage 1) significantly increased the number of male and female flowers per inflorescence, while [20]explained that to determine the effects of the plant growth regulator 6-benzyladenine (BA) on floral development and floral sex determination of Jatropha curcas. Exogenous application of BA significantly increased the total number of flowers per inflorescence, reaching a 3.6-fold increase (from 215 to 784) at 160 mg/l of BA. Provision of the FMA can increase the supply of plant photosynthates. [11], [21]suggested that the presence of the FMA inoculation could increase the accumulation of amino acids, proteins, chlorophyll and sugar content compared to non-mycorrhizal plants. Increased phosphorus in plants affects photosynthetic activity. Mycorrhizal infection in plants can increase nutrient translocation to the top of the plant resulting in an increase in the rate of photosynthesis and the use of assimilates in the canopy as well as an increase in photosynthate supply from leaves to roots. As a result, mycorrhiza plants have higher biomass

compared to without mycorrhiza. Administration of cytokines in plants has a direct impact on the growth process. Spraying cytokines can reduce damage caused by drought, avoid decreasing the number of secondary tillers, increasing yield and biomass in plants. Table 1 represents the obtained results that Jatropha curcas cultivar IP-3M with a dose of 10 grams with a cytokinin concentration of 300 mg L-1 (s3) showing the best results for stomata conduction of 474.03 mmol m-2 s-1. That is because most of the transpiration occurs through stomata although it can also be through the cuticular. One of mycorrhizal and growth hormone functions as a provider of nutrients by tethering N2 from the air. Nitrogen plays a role in the expansion of leaf strands in plants, so that it affects the process of plant photosynthesis. Giving the combination of Jatropha cultivar with the best FMA dose and the cytokinin concentration showed better results on stomatal conduction when compared without giving the FMA and the cytokinin which only ranged from 228.1 mmol m-2 s-1. The more open stomata will increase its conductivity, so that its transpiration is faster. If the stomata open there will be a link between the cavity between cells and the atmosphere. When the water vapor pressure in the atmosphere is lower tan the intercelular cavity, the water vapor from the cavity will come out. However, there are other factors that also directly or indirectly affect on the rate of transpiration, such as light intensity, humidity and air temperature, wind speed and ground wáter content.

Others obtained resuts on Table 1 are Jatropha curcas IP-3A with a dose of 5 grams with a cytokinin concentration of 300 mg L-1 (s3) showing the best results for plant height of about 65.5 cm. These results were obtained because mycorrhizae were able to infect plant roots and influence the height. Giving the combination of the Jatropha cultivar with the best dose of the FMA and the cytokinin concentration showed better results in plant height after 3 months when compared with out administration of the cytokines and only one single FMA genus Glomus sp. Which is only around 28 cm. Giving BAP 2,4-D can stimulate protein synthesis in plant tissue which can cause increased cell wall permeability, thereby stimulating cell division and elongation which will affecton the increase of the height. Elongation of the stem occurs because of the process of division, elongation and enlargement of new cells that occur in the apical meristem and stem segments that cause plants to grow taller. With the provision of BAP to plants will affect tissue growth. 2,4-D growth regulator has properties that are more easily absorbed by plant cells, not easily decomposed and functions to encourage morphogenesis.

In Table 1 also shows that the Jatropha cultivar IP-3P with a dose of 5 grams with the cytokinin concentration of 300 mg L-1 (s3) showed the best results for the number of leaves as much as 32.33 strands. Addition of growth hormone causes increased on the plant growth that can be seen from an increase in the number of leaves. Fitohormon synergistically works with other growth of hormones in stimulating growth. IAA which is a natural auxin produced in plant tissue synergistically works with 2,4-D BAP. IAA, which is also a synthetic cytokinin, is important in regulating cell division and stimulating leaf number growth. The number of leaves is highly related to the process of photosynthesis and plant metabolism, and nutrient absorption, because the leaves are the main organ of the process of photosynthesis. In addition, 2,4-D plays an important role in the directly formation of the somatic embryogenesis

V. CONCLUSION

The experiment results showed that there was a real effect on each treatment and there was also highly interaction between the treatments. The value of the Jatropha curcas cultivar combination with the best dose of the FMA and the cytokinin concentration on the leaf chlorophyll content between 14.43-25.83 CCI, stomata conduction between 256.4-474.03 mmol m-2 s-1, plant height between 46.43-65.5 cm and the leaves number from 13.66 to 32.33 strands. The best result of jatropha curcas cultivar combination with the best dose of the FMA and the cytokinin concentration is about 400 mg L-1 for the growth variable of the leaf chlorophyll content, while the concentration of the cytokinin is about 300 mg L-1 for the growth variable of stomata conduction, plant height, the leaves number that is novelty of this research. This research is expected to increase the development of agricultural science, including plant ecophysiology. In addition, the results of this study are expected to add information and recommendations in the development of agricultural technology and contribute solutions to the energy crisis problem. Fossil oil with renewable energy that is environmentally friendly and renewable, in determining the biofuels program, especially from Jatropha.

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