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PROCEEDINGS

THE 6th INDONESIAN BIOTECHNOLOGY CONFERENCE

**“ENHANCING INDUSTRIAL COMPETITIVENESS
THROUGH BIOTECHNOLOGY INNOVATION”**

Surakarta, 6-7 September 2016

Editors:

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Presenter Papers

Topic Field: Agriculture And Forestry Biotechnology 192

1. **Efficiency of Simple Sequence Repeats (SSR) Markers in Estimating Genetic Diversity of Jabon Merah (*Anthocephalus macrophyllus*)** [Restu, Muhammad, Gusmiaty, Larekeng, Siti Halimah] 193
2. **Genetic Diversity of Sulawesi Ebony in *in Situ* Conservation Area Revealed By Microsatellite Markers** [Larekeng, Siti Halimah, Restu, Muhammad, Gusmiaty, Yuni Fitri Cahyaningsih] 199
3. **Pollen Dispersal Distances of a Vulnerable Tropical Tree, Ebony (*Diospyros celebica* Bakh.), in Experimental Forest of Hasanuddin University** [Gusmiaty, Restu, Muhammad, Arsyad, Mirza Arsiaty, Ikhsan La Husen, Larekeng, Siti Halimah]..... 206
4. **Cassava (*Manihot esculenta* Crantz) Tolerance Screening on Wetness Using Morphological, Physiological and Protein Markers** [Sholeh Avivi*, I Gusta Dimas Satyalowa, Didik Pudji Restanto, Tri Agus Siswoyo, Sigit Soeparjono, Sri Hartatik, Achmad Subagio]..... 214
5. **Rejuvenation of Long Term Culture of Embryogenic Callus of Sago Palm (*Metroxylon sago* Rottb.): Effect of Coconut Water and Sucrose in Liquid Medium** [Rizka Tamania Saptari, Imron Riyadi, Sumaryono] 222
6. **The Micro Propagation Strategy of *Phalaenopsis* sp Orchid by Somatic Embryogenesis** [Didik Pudji Restanto, SigitSupardjono and Budi Kriswanto] . 229
7. **Differential Gene Expression in Oil Palm Varieties Susceptible and Tolerant to *Ganoderma*** [Riza Arief Putranto, Indra Syaputra, Asmini Budiani]..... 233
8. **Impact of Batik Industry Waste on Several Rice Varieties (*Oryza Sativa*.)** [B. Suryotomo, Samanhudi, Suwanto, A. Yunus] 244
9. **Production of Biopesticide from Tobacco Leaves (*Nicotiana tabacum*) With Digestion and Reflux Extractions** [Ahmad Fauzantoro, Amirah Amatullah Dalimunthe, Misri Gozan] 250
10. ***Bradyrhizobium japonicum* Plasmid Characterization from Agroforestry System** [S. Idiyah, Hartawati, N.Z. Lutfiyah, and M.P. Mberu] 255
11. **Nutrient Content and Antioxidant of Tomato Under Drought Stress Inoculated with Mychorrhiza** [Amalia T Sakya, Muji Rahayu and Heri Widiyanto] 259
12. **Genetic Diversity of Rice (*Oryza sativa*) Local Cultivated of Boyolali Black Rice Based on Morphological Characters** [Edi Purwanto, Endang Yuniastuti, Meyriza Ayu Hatari] 265
13. **Exploration of Potential Marine Red Macroalgae from the Southern Coast of Java Island, Gunung Kidul Regency, Yogyakarta, Indonesia as Source of Lectins** [Choiroel Anam, Danar Praseptiangga, Ahmad Yunus, Ekowati Chasanah, Nurrahmi Dewi Fajarningsih]..... 273

CASSAVA (*MANIHOT ESCULENTA* CRANTZ) TOLERANCE SCREENING ON WETNESS USING MORPHOLOGICAL, PHYSIOLOGICAL AND PROTEIN MARKERS

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Abstract

Wet land including on the marginal land. The land can be suffered by wet throughout the year as marshland, land tides on the beach or can form from land affected by heavy rainfall in a long time. Thus, in the future will be required plants that tolerance and produce the harvest from wet land. The objectives of study were (1) to identify cassava variety that tolerant of wet, (2) to know the character of morphology, physiology, and protein of clone cassava after tested with wet treatment. The research uses random design factorials consisting of two factors and three replication. The first factor was, 20 cassava varieties (V1-V20) collected from 20 cassava farmers in Indonesia. The second factor was field capacity consisting of C1=100% and C2 = 150% field capacity (FC). The result showed that every variety give different response after wet treatment. Wet treatment on cassava significantly increase parameter of plant height, number of leaves, level of green leaves, and stem diameter. Clone by best wet tolerance indicated by variety number 13, 14 and 17. While most susceptible to wet indicated by varieties code of 2, 6, and 8. Cassava's protein with molecule weight of 66,2 KDa appear thicker in tolerant plant after receiving treatment of 150% field capacity and appearing thinner (even not appear at all) in intolerant plant on wet.

Keywords: cassava varieties, wet, protein band

1. Introduction

Indonesia is an agraris country that most of the country people are farming and rely on agriculture. The population of the fastest growing have seized the use of farm land for the benefit of non-farming, as a result farm land became much smaller and agricultural product decreased (Moniaga [1]). Consequently, country trying to import some commodities as cassava to provide for its inhabitants. In accumulated, on January-april 2016, total of Indonesia cassava import has achieved 7.266 tons \$1,2 million dollars. Higher than the same period last year on 2015, namely 28,4 tons of us \$6.802 (Jefriando [2]).

The Indonesian government has overcome this import by means of intensification and

extension. Agricultural extension done by means of land use marginal. Marginal land in water stress land consisting of land water shortage and land with a surplus water as tidal land, marsh land, and the land often exposed to flood. An area of land marsh in Indonesia estimated 33.393.570 acres consisting of 20.096.800 acres (60,2%) tidal land and 13.296.770 acres (39,8%) marsh land non tidal (lebak) (Anon [3]).

Water stress can cause anaerobic conditions in the rooting area and reduce gas interchange between the ground and air. The availability of O₂ for the roots of plants and micro-organisms in the soil becoming more limited. The air in the soil finally will be encouraged out and inhibit the rate of diffusion, due to limited supplies of O₂ around rooting. This can decrease leaves water potential

resulting in close stomata and wither leaves (Ashraf and Harris [4]). Excess water in plants can damage growth. Declining oxygen resulted in energy for the cells that causes metabolic activities and energy production. By this condition, plant made changes morphology, anatomy, physiology and biochemical to adapting to the environment were fearing the worst (Tetsushi and Karim [5]). Today, still has not been any variety of Indonesian cassava which survive of wet stress. This research want to found the varieties cassava that tolerant to wet stress.

2. Methods

Experiments undertaken in Jember, in a site of green house and laboratory analysis of the Agriculture Faculty of Jember University. The materials and instrument used in this experiment is 20 varieties of cassava (the variety number 1 through 20 derived from various regions in Indonesia), chart colored leaves research IRRI agriculture agency, leaf porometer, digital refractometer, chlorophyllmeter (SPAD-502 Minolta), MINI-PAM, spectrophotometer, electrophoresis and tools that deals with maintenance plants and the harvest. This research using factorials random design group, with 2 factors and 3 times of replication. The first is varieties (V) consisting 20 varieties namely V1 to V20. The second factor is high flooding (c) consisting of 2 field capacity namely: C1 (the water 100% field capacity) and C2 (the water 150% field capacity). The difference between treatments tested with Duncan Multiple Range Test (DMRT) with confidence 5%. Observation was done in the agronomy, physiology, and protein band character closely related to wet resistant (Begum et al. [6]).

Watering various field capacity (FC) condition on media. Watering performed when shoots after planted in a media after wind dried to 14 kg. After was done of sprinkling with the volume of water in accordance with different treatment that is 100% and 150% field capacity. To know the volume of water has reached field capacity by weighing heavily early media after wind dried (14 kg). Then flushing with water until the media in polybag not trickling down under the surface. The volume of water that added to the condition were 1200 ml (condition is established as the condition 100% field capacity). Next counting how much the volume of water that splashed in a media until it reaches the condition field capacity. Determination of the volume of watering water as follows: C1 volume watering water 100% field capacity = $100/100 \times 1200 \text{ ml} =$

1200 ml; C2 volume watering water 150% field capacity = $150/100 \times 1200 \text{ ml} = 1800 \text{ ml}$.

Protein Extraction. This extraction uses 0.5 grams sample leaves crushed use mortar with a mixture of quarsa sand. Then sample that already crushed mixed with a buffer phosphate by comparison heavy sample and a buffer 1:3. The next step protein solution in eppendorf was centrifuged 10,000 rpm for 10 minutes and taken its supernatant. Levels of a protein determined with the Bradford methods. As many as 5 μl protein solution mixed with 45 μl aquades and solution Bradford 950 μl . Solution in vortex until homogeneous. Absorbent solution measured with a wavelength 595 nm. Standard solutions of protein used was BSA solution by concentration of the 0 $\mu\text{g}/\mu\text{l}$, 2 $\mu\text{g}/\mu\text{l}$, 5 $\mu\text{g}/\mu\text{l}$, 10 $\mu\text{g}/\mu\text{l}$, 15 $\mu\text{g}/\mu\text{l}$, 20 $\mu\text{g}/\mu\text{l}$. Analysis pattern ribbon protein leaves SDS PAGE. First, make separating gel 12%: Aquades 3.35 ml, 1.5 M Tris-Cl, pH 8.8 2.5 ml, 10% SDS 0.1 ml, Acrylamide/bis (30% T, 2.7% C) 4.0 ml, 10% ammonium persulfate 50 μl (0.05%), TEMED 5 μl (0.05%). Next, prepared 10 ml stacking gel solution monomers (4% T, 2.7% C) by mixing matter: Aquades 6.1 ml, 0.5 M Tris-Cl, pH 6.8 2.5 ml, stock akrilamid solution (30% T) 1.3 ml, 10% SDS 0.1 ml. Dispose of air absorb in monomers solution with a vacuum in about 15 minutes.

Next, include separating gel in electrophoresis plate to get to the limit of separating gel. And then add aquades gel average and avoid the presence of oxygen. Then let the gel left about 30 minutes until polymerization. Then threw aquades after separating gel polymer. After that include comb slowly into plate (do not formed bubbles). And let it left about 30 minutes until the polymerization after being polymer gel, combs released from gel slowly and move gel slowly into tanks electrophoresis. Next dissolving into each sample of a buffer (0,06 M Tris-Cl, pH 6,8, 2% SDS, 10% Glycerol, 0,25% Bromophenol Blue): Aquades 4,8 ml, 0,5 M Tris-Cl, pH 6,8 1,2 ml, 10% SDS 2,0 ml, Glycerol 1,0 ml, 0,5% Bromphenol Blue (w/v water) 0,5 ml. the process of dissolving/dilution sample done by comparison 1:4. Then heats sample to 950 C temperature for about 4 minutes.

Assemble a series of instrument electrophoresis. Fill a reservoir up and down with a buffer electrodes (0.025 M Tris, 0.192 M Glycine, 0.1% (w/v) SDS, pH 8.3 (0.3 grams Tris Base, 1.4 grams Glycine, 1 ml 10% SDS/100 ml a buffer the electrodes). Admit sample into well by 1 μg /well. Then connecting a tool with power supply and running gel in voltage 200 volt for 45 minutes or up samples has reached that part of the

base gel. After the electrophoresis completed, continued to take off homemade instrument electrophoresis, release gel and do staining (staining dye) in a gel with use Comassie Blue R-250. Staining done by making solution dye: 0.1% Comassie Blue R-250 (w/v) in 40% methanol (w/v), 10% acetic acids (v/v). Filter dye solution after homogenized, next to soak the gel in dye solution for 30 minutes. After that distaining process by using 40% methanol, 10% acetic acids. Do the distaining process to be clear gel, next replace distaining solution with a solution of acetic acid glacial 10%. After that the results photographed and scanned using scanner.

Variable observation used in this experiment includes: plant height, number of leaves, colored leaves, heavy fresh plants, diameter of the stem, roots volume, conductivity of stomata, sugar content (brix), chlorophyll content, the rate of photosynthesis, protein analysis. Observation against the root of was also made of a heavy wetness and heavy dried root (without fingerprint). The real difference between treatments analyzed

by test Duncan's Multiple Range Test (DMRT) on 5% standard.

3. Results and Discussion

Any plant having different water needs, similarly of the cassava plant. Even any kind of plant varieties of cassava show different response also against water stress. This shows that every plant having a limiting factor and tolerance power to environment (Solichatun et al. [7]). The result of the observation can be seen on Table 1a & 1b shows the morphology and physiology character of 20 different varieties of cassava. The characters that can determine tolerance plants to water stress indicated by variable plant height, number of leaves, chart colored leaves, diameter of the stem and the chlorophyll content and all significant impact on varieties tested. The highest score on the variables of higher plants and number of leaves found in clone code 19 with the 129,2 cm and 26 compared to other varieties.

Table 1a. The Morphological and Physiological Characters on the 20 Cassava Varieties

| Varieties | Plant Height (cm) | Number of Leaves | Colored Leaves | Diameter of the Stem (cm) | |
|-----------|-------------------|------------------|----------------|---------------------------|------------|
| 1 | 102.6 | abcdefg | 12 bc | 4 abc | 1.2 Abcdef |
| 2 | 114.9 | Abcd | 17 abc | 3 c | 1.1 Bcdef |
| 3 | 70.0 | Efg | 14 abc | 4 a | 1.1 Abcdef |
| 4 | 65.0 | G | 11 bc | 3 abc | 1.2 Abcdef |
| 5 | 110.0 | abcde | 18 abc | 4 ab | 1.4 Abc |
| 6 | 81.8 | bcdefg | 9 c | 4 abc | 1.2 Abcdef |
| 7 | 121.1 | Ab | 14 abc | 3 abc | 1.3 Abcdef |
| 8 | 79.2 | Cdefg | 12 bc | 3 abc | 1.1 Bcdef |
| 9 | 80.4 | bcdefg | 15 abc | 3 abc | 1.2 Abcdef |
| 10 | 92.7 | abcdefg | 23 ab | 4 abc | 1.3 Abcde |
| 11 | 119.2 | abc | 15 abc | 3 abc | 1.3 Abcd |
| 12 | 97.7 | abcdefg | 17 abc | 3 abc | 1.2 Abcdef |
| 13 | 109.3 | abcdef | 12 bc | 4 abc | 1.3 Abcde |
| 14 | 101.3 | abcdefg | 16 abc | 4 abc | 1.4 A |
| 15 | 90.1 | abcdefg | 20 abc | 4 abc | 1.2 Abcdef |
| 16 | 81.0 | bcdefg | 9 c | 4 abc | 1.0 F |
| 17 | 99.6 | abcdefg | 13 bc | 4 abc | 1.4 Ab |
| 18 | 80.1 | cdefg | 14 abc | 3 abc | 1.1 Bcdef |
| 19 | 129.2 | a | 26 a | 4 abc | 1.2 Abcdef |
| 20 | 92.2 | abcdefg | 12 bc | 4 abc | 1.2 Abcdef |

Note: The same letters on the same column shows that markedly similar according to test DMRT 5%

Varieties hold wet (150% field capacity) can be seen in Table 2. The value of varieties with the highest value of fresh root weight are 601,47 grams (variety 14), 390,36 grams (variety 13) and 378,14 grams (variety 17). While the highest value of the volume root 480 mls (variety 17), 350 mls (variety 14) and 300 mls (variety 5). Thus best response selection variety which retains its wet

indicated by variety with the code 14, 13 and 17. Variety does not hold in wet (150% field capacity) can be seen in Table 2. The values of the lowest root volume are 37,26 grams (variety 8), 51,66 grams (variety 6) and 53,41 grams (variety 2). While the value varieties with the lowest value of the fresh weight of roots are mls (20 variety 16), mls (50 variety 15) and 70 mls (variety 18). Thus

most varieties do not hold in wet indicated by varieties with the code 8, 6 and 2. Table 3 indicates the influence of the availability of water on the growth of the cassava plant. Wetness treatment in cassava raise plants height, number of

leaves, green color leaves level, and diameter of the stem. Field capacity 150% have high value plant (123,36 cm), number of leaves (25), chart colored leaves (4) and diameter of the stem (1,34 cm) higher than the control 100% field capacity.

Table 1b. The Morphological and Physiological Characters on the 20 Cassava Varieties

| Varieties | Chlorophyll Content ($\mu\text{mol}/\text{m}^2$) | Heavy Fresh Roots (gram) | Heavy Fresh Stem (gram) | Heavy Fresh Leaves (gram) | Root Volume (ml) | Brix (%) | |
|-----------|--|--------------------------|-------------------------|---------------------------|------------------|----------|-----|
| 1 | 39.2 | bcde | 72.4 | 195.2 | 25.9 | 123.3 | 0.9 |
| 2 | 43.7 | abcde | 42.5 | 161.0 | 20.0 | 64.3 | 0.5 |
| 3 | 43.5 | abcde | 56.7 | 137.5 | 45.9 | 88.3 | 0.7 |
| 4 | 42.7 | abcde | 116.8 | 100.4 | 57.0 | 174.3 | 1.3 |
| 5 | 46.8 | abcde | 169.0 | 160.8 | 71.0 | 161.3 | 1.6 |
| 6 | 45.5 | abcde | 62.5 | 163.3 | 54.5 | 99.7 | 1.5 |
| 7 | 43.7 | abcde | 110.0 | 133.3 | 52.0 | 119.0 | 0.9 |
| 8 | 50.8 | ab | 36.9 | 133.1 | 42.5 | 83.3 | 0.5 |
| 9 | 41.9 | abcde | 65.2 | 119.0 | 38.0 | 100.0 | 1.0 |
| 10 | 47.8 | abcd | 169.4 | 155.5 | 69.4 | 153.3 | 0.7 |
| 11 | 41.3 | abcde | 198.3 | 255.7 | 107.8 | 173.3 | 0.0 |
| 12 | 49.2 | abc | 168.9 | 172.8 | 68.7 | 141.7 | 0.4 |
| 13 | 41.0 | abcde | 205.6 | 308.8 | 37.7 | 140.0 | 0.2 |
| 14 | 42.6 | abcde | 284.1 | 390.7 | 57.4 | 223.3 | 0.0 |
| 15 | 47.3 | abcde | 93.4 | 182.5 | 54.4 | 83.3 | 0.1 |
| 16 | 40.2 | abcde | 63.5 | 130.4 | 83.7 | 30.0 | 0.2 |
| 17 | 38.9 | bcde | 177.0 | 273.6 | 50.1 | 193.3 | 0.1 |
| 18 | 35.9 | de | 80.7 | 115.0 | 40.0 | 56.7 | 0.1 |
| 19 | 52.7 | a | 77.5 | 248.8 | 51.1 | 51.7 | 0.1 |
| 20 | 46.4 | abcde | 76.9 | 213.3 | 64.2 | 58.3 | 0.1 |

Note: The same letters on the same column shows that markedly similar according to test DMRT 5%

Electrophoresis on the results of a gel polyakrilamid on tape protein pattern cassava leaves with treatment 100% of the water the feild capacity shown in Figure 1. As for the content in marker used the lysozyme protein (14,4 kDa), β -lactoglobulin (18,4 kda), REase Bsp981 (25,0 kDa), lactate dehydrogenase (35,0 kDa), Ovalbumin (45,0 kda), Bovine serum albumin (66,2 kDa) and β -galactosidase (116,0 kDa). The results of electrophoresis in polyakrilamid gel about pattern protein bands leaves cassava with water treatment 150% field capacity shown in

Figure 2. Pattern a protein band on variety with code 2, 5 and 8 shows band formed seemed the most thin and the possibility of degraded because all molecular weight do not appear in figure. Corresponding in Table 2 wetness resistant varieties (150% field capacity) can be seen best selection response of wetness resistant variety indicated by variety with code 14 in which variety has thickness a protein band on molecular weight 66,2 kDa. Expected this protein band influenced variable plants height because variety height with code 14 was higher value than the other varieties.

Table 2. Comparison of 100% and 150% field capacity treatment for 20 varieties cassava on Fresh root weight and Root Volume

| Varieties | Fresh root weight (gram) | | Root Volume (ml) | |
|-----------|--------------------------|---------|------------------|---------|
| | FC 100% | FC 150% | FC 100% | FC 150% |
| | 1 | 31.42 | 123.11 | 120 |
| 2 | 22.41 | 53.41 | 57 | 86 |
| 3 | 32.21 | 84.37 | 40 | 135 |
| 4 | 112.56 | 181.48 | 157 | 230 |
| 5 | 185.69 | 212.56 | 86 | 300 |
| 6 | 93.43 | 51.66 | 83 | 120 |
| 7 | 53.26 | 212.14 | 107 | 130 |
| 8 | 41.25 | 37.26 | 50 | 110 |
| 9 | 42.79 | 111.25 | 50 | 100 |
| 10 | 114.56 | 281.42 | 150 | 200 |
| 11 | 101.46 | 311.26 | 250 | 150 |
| 12 | 162.34 | 302.51 | 200 | 125 |
| 13 | 164.15 | 390.36 | 50 | 280 |
| 14 | 191.51 | 601.47 | 230 | 350 |
| 15 | 62.43 | 156.26 | 150 | 50 |
| 16 | 91.42 | 69.74 | 20 | 20 |
| 17 | 51.47 | 378.14 | 50 | 480 |
| 18 | 95.61 | 104.56 | 90 | 70 |
| 19 | 68.11 | 142.65 | 20 | 125 |
| 20 | 39.64 | 148.51 | 25 | 140 |

The difference response of cassava varieties to water stress

Some genotype of plants capable of adapting to the environment water stress through adaptation using physiological and genetic mechanisms. All varieties indicating the nature of genotip and different phenotype. The properties of is in line with character each variety. Growth and development an organism supported by the interaction of genes and environment influence it. The genes that several of each variety expressed in various character too. According to Matasci et al. [8] the visibility of a phenotype of hanging from the nature and relations between genotip and environment.

Chlorophyll is a component of a main chloroplast and chlorophyll content relatively positively correlate with the rate of photosynthesis

(Li et al. [9]. Chlorophyll synthesized in leaves which are useful to catch the light of the sun which numbers are different for each species. Which affects the chlorophyll synthesis is light, sugar or carbohydrate, water, temperature, genetic factors, disturbances such as elements of the N, Mg, Fe, Mn, Cu, Zn, S and O (Hendriyani and Setiari [10]. The radiant energy will be transferred to the center reaction fotosistem I and II which is the occurrence of light energy change into the chemical energy (Li et al. [9]). Two mechanisms involved in the formation of chlorophyll complex protein is the new chlorophyll distribution synthesized and redistribution of chlorophyll existing. Chlorophyll b is the result of biosynthesis of chlorophyll a and play an important role in reorganization fotosistem for adaptation to the quality and the intensity of light. Therefore loss of chlorophyll a and b have a negative influence on efficiency photosynthesis (van der Mescht et al. [11]).

Table 3. Effect of wet stress treatment on several parameters

| Field capacity (FC) | Plants height (cm) | Number of leaves | Leaves color | Stem diameter (cm) |
|---------------------|--------------------|------------------|--------------|--------------------|
| FC 100% | 95.71 b | 12 b | 3 b | 1.16 b |
| FC 150% | 123.36 a | 25 a | 4 a | 1.34 a |

Note: The same letters on the same column shows that markedly similar according to test DMRT 5%

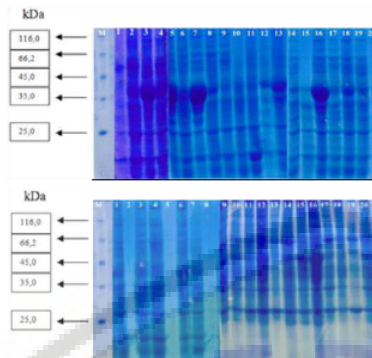


Figure 1 The proteins band on cassava leaves on 100% field capacity (upper) and 150% field capacity (bottom). Note: M = marker and varieties 1-20 encode by numbers.

Chlorophyll content can be used as an indicator of being credible to evaluate an imbalance metabolism between photosynthesis and producing stuff at the time of water shortage (Li et al. [9]). In Table 1 shown on the variables of the highest variable colored leaves chart shown on codes variety 3 with a value of 4 which means dark green. The growth of plants which is good and offer high yields requires supply nitrogen enough. If the supply of N is not enough then looked the growth of an organ and the whole plants that do not normally like symptoms from chlorosis that looked at leaves. Leaves turn paler, yellowing of the and in the condition of a lack of N become dead.

The difference in plant height value, number of leaves, chart colored leaves and diameter of the stem able to illustrate one of the types tolerance of the existence of wetness (Table 3). Basically growth is the balance between the carbons in photosynthesis and found in respiration. The variables is an indicator growth pertaining to the availability of water and nutrient element especially nitrogen in land.

The changed characters of plant after wet stress.

Generally flooding stress will cause a decline in morphological character growth, the decline in the production and even death. The loss of crops production due to the wetness stress on

plant depending on genotype, environmental conditions, the growing phase when the wetness happened, the long of wetness, the depth of wetness, and the water movement on lands, and the plant growth (Tetsushi and Karim [5]; Susilawati, et al. [12], Islam MS et al. [13]). The higher of wetness that occurred during active growth phase will influence to the weight of the sugarcane stem and reduced the production in line with an increase in the wetness (Islam MS et al. [13]). Wetness could decrease plant height and leaves number (Avivi, et al. [14]).

According to Glaz, et al. [15] the reduction of the production as 8.3% on nine cultivars of sugarcane after treatment high wetness -15 cm. The increase in wetness time in generative phase significantly decrease the capacity of plants survive, the quantity of a crop life, the plant high and the number of branches of chili plant (Susilawati, et al. [12]). Jagadisha [16] reported that a decline in growth parameters as high of plant, the number of green leaves, the broad leaf index, the chlorophyll content and the total production dry weight after wetness treatment. Begum et al. [6] reported that wetness decrease the green leaves of seven clone of sugarcane while the number of leaves yellowing increase (Malik and Tomer [17]; Tetsushi & Karim [5]).

Some physiological characters that changed after wetness stress were: (1) the decrease of transpiration, (2) the decrease of photosynthesis but the stomata conductance increased, (3) the decrease of growth, (4) the respiration to be higher. The effect of wetness stress to respiration depends on varieties and physiology phase of plant, The young phase growing plants are getting worse the stress effect (5) change the metabolic processes from aerobic to be anaerobic, (6) the nutrients absorption by the roots declining (Malik and Tomer [17]; Tetsushi & Karim [5]). Also reported that wetness could change the morphology, anatomy, physiology, and biochemistry of plant. The damage due to the wetness stress influenced by several factors, those are high of wetness, the duration or long wetness, and the speed of water flow (Tetsushi & Karim [17]). The crops character that tolerance to wetness stress present in Table 1.

The wetness stress on root will lower the amount of available oxygen. The decrease in oxygen under optimal level, called hypoxia. This is the most common form of wetness stress and occurs when the roots were submerged in water but the shoot remain in the atmosphere (Parent et. al. [18]). The maximum number of dissolved oxygen when wetness happened only 3% compare with normal air. That condition caused the change

of respiration from aerobic to fermentation anaerobic that induces glycolysis and fermentation genes (Dat et al. [19]).

Direct consequences from hypoxia is ATP's reduction as a result of reduced availability of O₂. Oxygen plays a role in produce energy for the cells so the presence or absence of oxygen determine the metabolic activity and the production of energy. Oxygen serves as electron acceptor in oxidative phosphorylation line that produces ATP, the major source of energy to cell metabolism, to regenerate NAD as cofactor of NADH (Dennis, et. al., [20]). The increase of glycolytic flux in line with production of NAD⁺ by pyruvate fermentation become ethanol through pyruvate decarboxylase and alcohol dehydrogenase (ADH).

Then ethanol diffuses out of the cell into external environment reduce reserves carbon of plants. Hence, the metabolism of pyruvic become alanine provide an alternative product, including 2-oxoglutarate that can be change to succinic, through a cycle of TCA by Succinate CoA ligase (SCS). This process give additional ATP per sucrose molecule. NADH oxidation in a mitochondria matrix guaranteed by the reduction of oxaloacetate through the opposite of TCA reaction cycle who catalyzed by malic dehydrogenase. Malic then converted into fumaric and succinic that can be carried out from a hypoxia tissue to the aeration tissue (Bailey-Serres, et. al. [21]).

4. Conclusion

Based on the research done can be concluded that response best selection varieties hold wet indicated by varieties with code 14, 13 and 17. While the most variety does not hold wet indicated by varieties with code 8, 6 and 2. Potein cassava with molecular weight 66,2 kDa appear more thick on condition excess water.

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