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AS-006

Screening of drought tolerant cassava (*Manihot esculenta Crantz*) using morphological and protein characteristics

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ABSTRACT

The availability of superior cassava varieties that resistant to the drought stress is necessary. The drought tolerance varieties will be utilized for cassava cultivation in the drought-prone areas. Here we have conducted the research to identify the cassava clones that show the higher drought tolerance through the analysis of morphological and protein characteristics. The research was carried out in green house and plants laboratory analysis of Agricultural Faculty of Jember University, used group random design with 2 factors by 3 remedial. The first factor was variety (V) consisting 20 clone's number that collected from 20 farmers in some regions in Indonesia. The second factor was field capacity consist of 50% (C1) and C2=100% of field capacity (FC). Every clones showed different response to the drought stress treatment. Analysis of leaf total protein showed that lactate dehydrogenase (35 kDa) and ovalbumin (4.5 kDa) were over accumulated during 50% field capacity rather than 100% of field capacity. The best tolerance response on drought stress based on the fresh weight of storage root were shown by clone number 5, 10 and 11. While the susceptible varieties were clone number 8, 9 and 16.

Keywords: stress, field capacity, Indonesian variety

I. INTRODUCTION

Indonesia imported some commodities, one of which is cassava. To solve the problem of cassava's imports, the government suggest the farmers to increase the production by means of intensification and extensification of agricultural land. Extensification for cassava needs to be directed to use marginal land such as the water stress land. Fertile land more in use for other food plants. Water stress land consisting of dry land which have less water and wet land with excess water. The size of dry land in Indonesia reach 140 million ha.

In general it can be said that drought conditions would change the osmotic pressure and pressure the cell walls from positive to be negative (Bae et al. 2007). Drought stress strongly influenced the morphology of plants. Drought stress resulting in a change of leaves and rooting form on the coffee plant (Pinheiro et al. 2005). While in Soybean drought caused a decline of dry weight (Hanum et al. 2007).

Drought stress also influenced on the physiological characters. Drought stress will reduce the water level of the ground at growing media, potential leaves, the water level on the leaves, the relative water content of leaves and wide leaves relative in the oil palm plant (Toroun-Matius et al. 2001). Drought stress affect the process of forming of prolin, glisin, betaine and glucose. Some plants increased the deposits of ascorbic acid and tocopherol. The existence of those compounds can improve the survival of plants to stress drought in Whitania somnifora (Jaleel, 2009). In the event of drought, some leaves stomata close and decrease the entry of CO2 and decrease the photosynthetic activity. The influence of drought stress is not only pressing growth and the production even cause the death of plants. Now a days we are still not have Indonesian cassava varieties that tolerant to drought stress that have been released. This study was aimed to find superior cassava variety which tolerant to drought stress.

II. MATERIALS AND METHODS

The research was carried out in green house and plants laboratory analysis of agricultural faculty of Jember University, used group random design with 2 factors by 3 remedial. The first factor was variety (V) consisting 20 clone's number that collected from 20 farmers in some regions in Indonesia. The second factor was field capacity consist of 50% (C1) and C2=100% of field capacity (FC). The difference between treatment were tested by Duncan multiple range test (DMRT) with the levels of trust 5 %.

Protein Extraction. The extraction used 0.5 grams leaves sample which crushed use mortar with a mixture of quarsa sand. Then sample already crushed mixed with a buffer phosphate by comparison of weight of sample and buffer 1:3. Then, protein solution in ependorf tube was centrifuged with 10,000 rpm for 10 minutes and supernatant were collected. The levels of a protein determined by Bradford (1976) method. Analysis of band protein leaves using SDS page gel 12%. Staining dye in gel use Comassie Blue R-250. The process of distaining using 40 methanol, 10% acetic acids. The variable observation that used in this experiment were the plant high, number of leaves, the color of leaves, weight of fresh roots, diameter of stem, roots volume, the chlorophyll content, and analysis of protein.

The results of observation can be seen in Table 1. The characters that can determine tolerance plants against water stress indicated by variable of high of plant, number of leaves, stem diameter, the color of leave and the chlorophyll content.

The best parameters that used for selection of cassava variety can be seen from heavy fresh of roots and volume of roots. Using that characters we determined the drought resistant varieties (Table 2). Variety with the highest of fresh roots were clone no 11 (182,3 grams), clone no 10 (112,4 grams) and clone no 5 (108,7 grams). With the root volume 98, 110, and 120 grams respectively. So we chose that the best response of drought resistant indicated by clone no 11, 10 and 5. Variety with the lowest weight of fresh roots were clone no 19, (21,7 grams), clone no 16 (29,4 grams) and clone no 8 (32,1 grams). With the root volume 10, 50, and 90 grams respectively. Thus the most susceptible of drought resistant variety indicated by clone no 19, 16 and 8.

The effect of water stress on the cassava growth parameters can be seen on Table 3. The real influence shown on the variables of plants height and unreal influence shown in variable number of leaves and stem diameter. Data in Table 3 shows that drought stress decrease the plant height, number of leaves, and the diameter of stem.

The electrophoresis results of polyacrylamide gel shown on (Figure 1). The protein band (35 kDa) of clone number 4, 6, 7, 11, 13, 17, 18 thicker compared to other clone. While the protein band (35 kDa) on clone number 1, 2, 3, 5, 8, 9, 10, 12, 14, 15, 16, 19 and 20 were thin. The clone no 11 also have the most thick of protein band with the molecular weight of 45,0 kda. According to the data on Table 2 the best response of clone after drought resistant treatment indicated by clone number 11 which have the most weight of fresh roots.

Every clone of the cassava showed a different response against water stress. This indicates that different clone have difference restricting factor and different tolerance on changes in environment (Solichatun et al. 2005). Phenotype diversity of cassava plant indicates a difference response of the genotype and the environment factors. Variation which is found in an individual plant caused by two factors, genetic and environment factors. Variation of genetic and environment cause the presence of differences in plant phenotypic. Genetic factors is internal factors which are affected by gene such as ages of plants, the high of plants, the yield, the capacity of storing food storage, resistance to disease and others. The external factor is environmental factor, such as climate, land, abiotic and biotic factors. The difference of growth and yield obtained caused by one or both of this factors.

The visibility of phenotype depending on the relationship between genotype and environment. In reality the development of an organism greatly affected by the state of the environment and interactions among gene (Choi and Kim, 2007). Different gene of plant demonstrating different ability of life, and causing inheritance of chromosomes and genes are not the same. This resulted in the irregularities segregation.

The availability of water in the land often altered due to the weather conditions and tend to change from time to time. This can result in the growth of plants disturbed. While drought stress happened because of the evapotranspiration rate higher than the water absorption by the roots (Upadhyaya et al., 2012).

Drought stress can affect all aspects of growth and plants metabolism including membrane integrity, pigment content, osmotic potential, photosynthetic activity (Bhardwaj & Yadav, 2012), the potential of protoplasm (Mundre, 2002), the decline of growth (Suhartono et al. 2008) and decrease in the diameter of stems (Belitz & Sams, 2007). If the water decrease, plants can be stunted, because water dissolving nutrient and help metabolic processes in plants. If the water not met the minimum need of plant the growth will be decreased and stunted, because the water serves dissolving element of nutrition and help metabolic processes in plants including in the cassava plant. (Wayah et al. 2014).

Chlorophyll is the main component of chloroplasts and the chlorophyll content positively correlate with the rate photosynthesis (Li et al. 2006). Chlorophyll synthesized in leaf and receive the sun light with different amount for each species. Chlorophyll synthesis affects by light, sugar or carbohydrate, water, temperature, genetic factors, and elements nutrient such as N, Mg, Fe, Mn, Cu, Zn, S and O (Hendriyani dan Setiari, 2009).

Then, radiant energy will be transferred to central reaction of photosystem I and II that is a place of the occurrence of a change of light energy into the chemical energy (Li et al., 2006). Two mechanisms involved in the formation of a protein chlorophyll complex are the distribution new chlorophyll synthesized and redistribution of chlorophyll existing. Chlorophyll b is the result biosynthesis from chlorophyll a and play an important role in photosystem reorganization for adaptation to the quality and the intensity of light. Therefore loss of chlorophyll a and b have a negative influence on photosynthesis efficiency (Van Der Mescht et al. 1999).

The decline in chlorophyll content when plants suffered with drought stress related with the activity of photosynthesis and decrease the rate of photosynthesis. The chlorophyll content can be used as a trusted indicator to evaluate imbalance metabolism between photosynthesis and production at the time of drought stress (Li et al. 2006). In this study the chlorophyll content not clearly related to the weight of fresh roots and volume roots. Need further research to see the relationship between chlorophyll content with the other parameters.

Variable leaves color in Table 1 showed the highest value was 4 which means dark green. The better growth of plants with high yields requires supply enough nitrogen (N), if the supply of N is not enough, the organ and the whole plants do not normally growth and show the symptoms such as chlorosis that looked at leaves. Leaves turn paler and yellowing. In the condition of a so much lacking of N plant going to die. In addition the symptoms can be informed us when plants really needed an additional N fertilizer.

Leaves color is a indicators which is good for needs fertilizer a plant. Leaves looks pale or yellowish green show that plants lack of N. If a color value leaves lower of the certain critical value, so plants need N fertilizers additional. Symptoms of N deficiency the most easily seen is yellowing of leaves due to the loss of chlorophyll, green pigment participate in the photosynthesis process, distributed somewhat flatten on a whole leaves. Deficient of nitrogen characterized by speed of low growth and dwarf plants (Imas and John, 2013).

Table 1. Morphological and Physiological Character of 20 Varieties Cassava Clone.

Clones	High of plant (cm)		Number of leaves		Diameter of stem (cm)		Leave colour		The chlorophyll content (μmol/m2)	
1	102.6	abcdefg	12	bc	1.22	abcdef	4	a	39.21	bcde
2	114.9	abcd	17	abc	1.06	bcdef	3	b	43.67	abcde
3	70.0	efg	14	abc	1.14	abcdef	4	a	43.48	abcde
4	65.0	g	11	bc	1.22	abcdef	3	b	42.72	abcde
5	110.0	abcde	18	abc	1.38	abc	4	a	46.78	abcde
6	81.8	bcdefg	9	c	1.18	abcdef	4	a	45.54	abcde
7	121.1	ab	14	abc	1.31	abcdef	3	b	43.74	abcde
8	79.2	cdefg	12	bc	1.08	bcdef	3	b	50.81	ab
9	80.4	bcdefg	15	abc	1.16	abcdef	3	a	41.92	abcde
10	92.7	abcdefg	23	ab	1.26	abcde	4	a	47.84	abcd
11	119.2	abc	15	abc	1.34	abcd	3	b	41.34	abcde
12	97.7	abcdefg	17	abc	1.21	abcdef	3	b	49.20	abc
13	109.3	abcdef	12	bc	1.34	abcde	4	a	41.02	abcde
14	101.3	abcdefg	16	abc	1.42	a	4	a	42.57	abcde
15	90.1	abcdefg	20	abc	1.25	abcdef	4	a	47.28	abcde
16	81.0	bcdefg	9	c	0.99	f	4	a	40.20	abcde
17	99.6	abcdefg	13	bc	1.38	ab	4	a	38.89	bcde
18	80.1	cdefg	14	abc	1.06	bcdef	3	b	35.91	de
19	129.2	a	26	a	1.18	abcdef	4	a	52.73	a
20	92.2	abcdefg	12	bc	1.19	abcdef	4	a	46.38	abcde

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

The presence of water it is essential for the protoplasm and forming 80-90% of fresh weights of plant tissue active growing. Water is the solvent in which there is a variety of sorts of salt and gas and other substances of dissolved and move out enter cells of an organ into an organ in the process of transpiration. Water is photosynthesis and reagent hydrolysis and maintain turgidities of them in enlargement and opening stomata cells (Baloch et al., 2013). This allegedly happened because of the availability of enough water will affect cell turgor that will affect enlargement of cells so that will determine the level of growth especially on the stems and leaves.

Water as a solvent of organic element and plant nutrient able to carry out essential element in the soil and can be easily absorbed by plants. As has been seen that the allegation of nitrogenous organic serves to stimulate growth as a whole especially stems, branches and leaves. In addition, nitrogen also play an important role in the formation of green leaves that is very useful in the process of photosynthesis (Tao et al, 2011).

The availability of water will affect growth and development a plant. Plant growth can be measured through the heavy of dry matter and relative growth rate. Heavy dry herbs of biomass total, viewed as a manifestation metabolic processes is happening inside herbs. Biomass herbs covering the results of photosynthesis, absorption element nutrient and water. Heavy of dry matter can show the productivity of plant because most of photosynthesis results contained in the form of heavy dry matter (Mandal and Sinha, 2004).

Table 2. The Effect of Drought Stress Treatment on the Weight of Fresh Roots and Roots Volume

Clones	weight of fresh roots (grams)		Volume of root (ml)	
	50 % of FC	100 % of FC	50 % of FC	100 % of FC
1	62,6	31,4	150	120
2	51,7	22,4	50	57
3	53,5	32,2	90	40
4	56,5	112,6	136	157
5	108,7	185,7	98	86
6	42,5	93,4	96	83
7	64,7	53,3	120	107
8	32,1	41,3	90	50
9	41,6	42,8	150	50
10	112,4	114,6	110	150
11	182,3	101,5	120	250
12	41,8	162,3	110	200
13	62,2	164,2	90	50
14	59,4	191,5	90	230
15	61,4	62,4	50	150
16	29,4	91,4	50	20
17	11,4	51,5	50	50
18	41,8	95,6	10	90
19	21,7	68,1	10	20
20	42,3	39,6	10	25

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

Drought stress on the contrary increases the activity of hydrolysis enzyme such as amylase, can also lower the level of productivity of the plant, due to the decrease of the primary metabolism, and decrease the size of leaves and photosynthetic activity (Yang et al. 2001). Turgor cells affected by availability of water. Availability of water in the 50% field capacity decrease the pressure of cells turgor and cut the ability of a cell to grow and develop. The difference in value of plant high, number of leaves, leaves color and diameter of the stem able to illustrate the tolerance plant when suffered with drought stress (Table 3).

The data showed that drought stress up to 50% of field capacity could reduce the plant high, number of leaves, leaves color and diameter of the stem. Basically growth is the balance between the carbons used in photosynthesis and loose carbon in respiration process. In water stress condition, that balance will change resulting in interference in growth (Brunner et al., 2015). That variables can be used as growth indicators related to the availability of water and nitrogen. The growth of plants more intensified when nitrogen and water enough available. But if the availability of water reduced will cause decreased of growth, showed by the decline in plant height, number of leaves, and diameter of the stem (Table 3).

Table 3. The Effect Drought Treatment on Parameters

Field Capacity	The plant high (cm)	No. of leaves	The diameter of stem (cm)
C1 50%	68.53 c	8	1.15
C2 100%	95.71 b	12	1.16

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

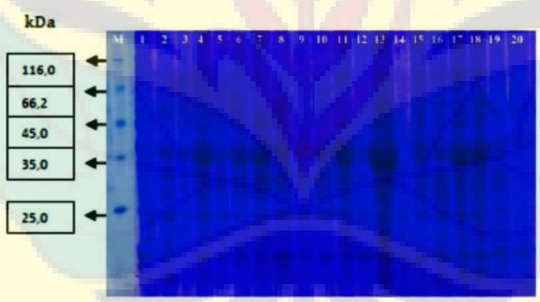

Figure 1. Type of protein band after drought stress treatment (50% of Field Capacity); M = Marker; 1-20 = no of clones. The content of protein marker that used were *Lysozyme* (14,4 kDa), *β-Lactoglobulin* (18,4 kDa), *REase Bsp981* (25,0 kDa), *Lactate dehydrogenase* (35,0 kDa), *Ovalbumin* (45,0 kDa), *Bovine serum albumin* (66,2 kDa) dan *β–galactosidase* (116,0 kDa).

IV. CONCLUSIONS

We concluded that best response of drought tolerant clone base on the weight of fresh roots indicated by clone no. 11, 10 and 5. While the susceptible indicated by clone no 19, 16 and 8. Cassava protein with molecular weight 35,0 kDa (Lactate dehydrogenase) and 4.5 kDa (Ovalbumin) appear clearly and thick in the plant that tolerant to drought stress.

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