

Multiple Tolerances of Cassava Germplasm to Drought Stress and Red Spider Mite Attacks

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Abstract. Uncertain climate change encourages the assembly of cassava varieties with multiple tolerances to both abiotic and biotic stress. The research aimed to evaluate the multiple tolerances of cassava germplasm to drought stress and red spider mite attacks. The research was held at Installation for Research and Assessment of Agricultural Technology of Muneng from February to December 2019 using a randomized block design nested in two environments with two replicates. The treatments consisted of 50 cassava accessions from the Indonesian Legumes and Tuber Crops Research Institute collection and two irrigation environments, i.e. a normal environment and a drought environment. Drought stress caused a decrease in plant height, tuber yield; on the other hand, increased red spider mite attack. Eight accessions have Stress Tolerance Index values reaching above 1.00, and two of them also have resistance to red spider mites. Accessions MLG 10361 and MLG 10362 had a high level of tolerance to drought stress as well as resistance to red spider mites so that both accessions may be used as a source of multiple resistance genes for biotic and abiotic stresses.

Key words: cassava accessions; drought; red spider mite; multiple tolerance

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INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is an important tuber commodity not only in Asia, but also in Africa and Latin America. Cassava is widely adapted to tropical areas with air temperatures above 18°C, has good tolerance to drought conditions, and can grow well on less fertile land compared to other food crops (Aye & Howeler, 2017). The inherent mechanism of cassava to adapt the changes in air humidity as well as soil water deficits, makes this crop suitable for a wider range of climatic conditions (Pushpalatha & Gangadharan, 2020). Cassava, which is a source of carbohydrates, has also been used commercially as raw material for the animal feed industry, bioethanol and all-starch-based industries (food, feed, medicine, cosmetics, biopolymers, and biofuels) (Li et al., 2017).

Cassava farmers on dry land generally start planting at the beginning of the rainy season, so they are prone to experiencing drought stress in the generative phase (tuber filling phase). If cassava is planted at the beginning of the rainy season, then when the dry season comes, the plants are 5-7 months old. At that age the plants have passed a critical growth period and are relatively more tolerant to drought stress. The critical period occurs at the age of 0-150 days after planting

(DAS) with the availability of water until the age of 120 DAS. This phase is characterized by intensive shoot growth and leaf area index reaching its peak at the age of 150 DAS, while the initiation phase of tuber formation occurs at the age of 120-150 DAS (Pipatsitee et al., 2019). According to Daryanto et al. (2017) the initiation phase of tuber formation is more sensitive to drought stress than the development phase of the vegetative part.

Drought stress caused a decrease in cassava biomass in all growth phases. However, the greatest decline was if drought stress started at the beginning of the growth phase (2 months after planting), whereas tuber production was less affected by drought stress except for stress at the beginning of growth. Plants with short performance are more efficient in using nutrients and form tubers earlier (El-Sharkawy, 2012), however, according to Okogbenin et al. (2013), high plant performance and leaf area index support high tuber yield. An increase in tubers in a stressful and non-stressed condition is associated with an increase in total biomass production and harvest index, so that increased leaf longevity is a way to increase cassava production (de Oliveira et al., 2021). Cassava selection in drought conditions

generally uses tuber yield parameters (de Oliveira et al., 2015). Evaluation of the genetic source of cassava showed that accessions that were tolerant of drought were indicated by a higher harvest index, yield of fresh tubers, number of tubers, and dry matter compared to less tolerant accessions (Turyagyenda et al., 2013).

Season anomalies often occur as the effect of global warming which have an impact on the decline in agricultural commodity production, even crop failure. After the beginning of the rainy season, there is often a long dry period during which young plants experience drought stress. The response of cassava plants to drought stress depends on the duration and level of water deficiency as well as cultivar factors (Zhu et al., 2020). Efforts to deal with drought stress are the use of superior varieties that are tolerant to drought stress, have high potential yields in optimal water conditions, and experience a slight yield decrease in drought stress. For this reason, an evaluation of genetic resources is needed to obtain a genotype of drought tolerant cassava, especially the stress that occurs in the critical plant phase (<150 DAS) with high yield and tuber quality.

Plant production cannot be separated from biotic and abiotic factors so climate change as abiotic stress certainly affects the biology of pests and diseases of cassava plants. One of the most important and dominant pests of cassava in the Asian region is the red spider mite (*Tetranychus urticae* Koch), whose development is triggered by dry and hot environmental conditions (Bellotti et al., 2012). In Indonesia, the attack of red spider mites can cause a decrease the yield of up to 50%. Evaluation of the accession response to biotic and abiotic stresses has been carried out on characters whose impacts are economically detrimental; however, those are separate activities. These

important characters include the accession response to drought stress (Oliveira et al., 2017; Turyagyenda et al., 2013; Wahyuni, 2014), evaluation of accession resistance to *Mononychellus tanajoa* (Bondar), *Aleurotrachelus socialis* Bondar, and *Frankliniella williamsi* Hood (Parsa et al., 2015), evaluation the response of accessions to mealybugs attacks (Indiati et al., 2014), evaluating the resistance of accessions to green mites (Ezenwaka et al., 2018). The evaluation of plant tolerance to biotic stress usually has been implemented in soil moisture optimal environment as is done in soybean (Krisnawati & Adie, 2018). Therefore, the development of cassava varieties that have multiple resistance both to biotic and abiotic stress needs to be developed (Pushpalatha & Gangadharan, 2020). The research aimed to evaluate the multiple tolerances of cassava germplasm to drought stress and red spider mite attacks.

METHODS

The research was held at Installation for Research and Assessment of Agricultural Technology of Muneng (East Java, Indonesia) from February to December 2019 using a randomized block design nested in two environments with two replicates. The treatments consisted of 50 cassava accessions of Indonesian Legumes and Tuber Crops Research Institute collection (Table 1) and two irrigation environments, i.e.: L1=normal environment (optimal irrigation since planting to 8 months ages), and L2=drought environment (irrigation was stopped since the age of 3 MAP/months after planting).

Each cassava accession was planted on a 2 x 6 m plot with a spacing of 1 m x 0.5 m. Plants were fertilized with 700 kg of Phonska + 100 kg of

Table 1. Cassava accessions used for evaluation.

Plot number	Number of MLG	Plot number	Number of MLG	Plot number	Number of MLG	Plot number	Number of MLG	Plot number
1	10009	11	10031	21	10043	31	10054	41
2	10010	12	10032	22	10044	32	10055	42
3	10011	13	10033	23	10045	33	10056	43
4	10012	14	10034	24	10046	34	10057	44
5	10015	15	10035	25	10048	35	10058	45
6	10019	16	10036	26	10049	36	10060	46
7	10020	17	10037	27	10050	37	10061	47
8	10024	18	10038	28	10051	38	10062	48
9	10025	19	10039	29	10052	39	10063	49
10	10029	20	10041	30	10053	40	10064	50

urea/ha. Irrigation relied on rainfall until 3 MAP, then irrigation for normal environments used irrigation water once a month until the age of 8 months, while the drought environment was not irrigated until harvest. Soil samples were taken at a layer of 10-20 cm before planting for analysis of soil moisture content at various pF values (0 = saturated; 2.5 = field capacity; and 4.2 = permanent wilting point).

Parameters observed included plant height at 3, 6, and 9 MAP, number and weight of tubers per plant, the intensity of red spider mites attack at 3 and 6 MAP, and tuber yield per hectare. As supporting data, observation of soil moisture content was carried out before and after irrigation since 3 MAP.

The intensity of the attack of red spider mites is calculated according to the formula:

$$I = \sum \frac{nxv}{N \times V} \times 100\%$$

I = intensity of red spider mite attack

N = number of leaves in a plant

V = highest score (score 5)

n = number of leaves in each score

v = score category (0 to 5)

The criteria for the resistance of each accession are determined based on the Standard Deviation method developed by Doreste et al. (1979) as follows:

Very resistant (VR)	$= I < (\bar{X} - 2\delta)$
Resistant (R)	$= (\bar{X} - 2\delta) < I < (\bar{X} - \delta)$
Slightly resistant (ST)	$= (\bar{X} - \delta) < I < (\bar{X} + \delta)$
Susceptible (S)	$= I > (\bar{X} + \delta)$
\bar{X}	= the average of intensity of red spider mite attack

The Stress Tolerance Index (STI) was used to identify the tolerance of cassava accessions to drought stress. Accession is classified as tolerant if it has an STI value >1. STI was calculated by adopting the equation used by Fernandez (1992), namely:

STI	$= (Y_p \times Y_s) / (\bar{Y}_P)^2$
STI	= Stress tolerance index
Y _p	= yield without salinity stress (t/ha)
Y _s	= yield under salinity stress (t/ha)
\bar{Y}_P	= average yield without salinity stress (t/ha)

Descriptive statistic was used to describe the conditions of the observed variables. Correlation analysis was used to examine the correlation of plant characteristics which had a significant effect on tuber yield and cassava resistance to drought stress red spider mite attack.

RESULTS AND DISCUSSION

Soil Moisture Content

The physical analysis of the soil resulted that the type of soil in IP2TP Muneng is classified as a dusty loam with a saturated moisture content of 51% (pF 0), a field capacity of 35% (pF 2.5) and a permanent wilting point of 12% (pF 4.2). During the study, cassava plants were rained during the first four months when drought stress had not been applied.

Soil moisture content since drought stress was applied is shown in Figure 1. Soil moisture in both environments during the study, both before and after irrigation, was in the range of available water (between permanent wilting point and field capacity), except at 143 days after planting (DAP) and 235 DAP, which showed value above field capacity. Soil moisture at those times was the moisture after irrigation, meaning that it was probably related to the excess discharge of irrigation, causing the soil water content to pass through field capacity but have not reached the saturation point. Watering at 142 DAP was carried out by flooding so that the soil was saturated until one day later. Flooding was applied because of the very hot weather and limited irrigation water.

Soil moisture in drought stress environment decreased as drought periods increased; however, soil moisture from 3 months (92 DAP) to 7 months (235 DAP) was still at the available water level (between field capacity and permanent wilting point). The slope of the moisture was low near the point of permanent wilting point. When the plants are in available water moisture content, the plants can still survive even though irrigation has been stopped. Soil moisture in both environments decreased at 9 MAP (278 DAP) where soil moisture in the normal environment started to approach the permanent wilting point, while soil moisture in stressful environments reached below the permanent wilting point. Burns et al. (2010) stated that cassava plants have a good root system that can penetrate to a depth of 2 meters, therefore, although soil moisture at 20 cm depth in this study showed a permanent wilting point, the plants were still alive because the cassava roots could still reach deeper water sources.

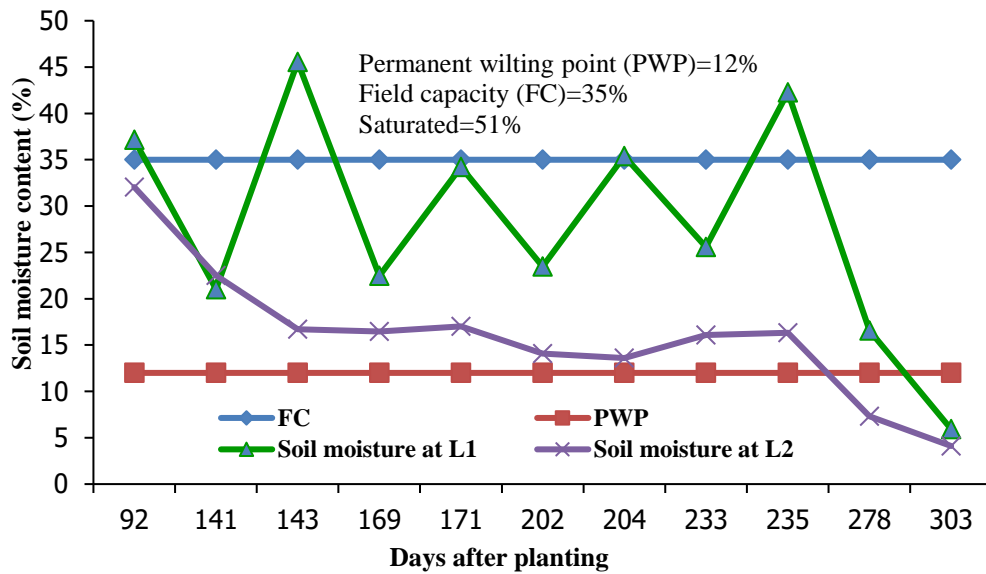


Figure 1. Soil moisture content during study at two environments (L1= normal, L2= drought stress)

Descriptive Statistic of Parameters

Descriptive statistics of all observed parameters are presented in Table 2. From the standard deviation value, it is shown that the greatest variation occurs in the plant height parameters, which means that there were accessions that were far above or below the average plant height of all cassava accessions.

Plant height continued to increase throughout its growing period and slowed down when above 6 MAP. The average plant height at 3 MAP was higher at L2. At that time the plants had not been

given drought stress, so there was no decrease from L1 to L2. After being given drought stress, the average plant height decreased by 9.77% at 6 MAP and 14.15% at 9 MAP. Plant growth is very sensitive to drought stress. Plants that are affected by drought stress become shorter, and have a smaller leaf area index, which reduces the amount of phosphynthetic active radiation absorbed by leaves, decreases the rate of photosynthesis, and ultimately decreases yield (Ullah et al., 2018). In pots research, the reduction in plant height due to drought stress could be greater up to 47.4% when

Table 2. Statistic descriptive all of parameters

Parameter	L1			L2			The reduction of L2 to L1 (%)
	Range	Average	SD	Range	Average	SD	
Plant height at 3 MAP (cm)	98.33–186.00	124.76	18.21	93.33–214.33	134.70	23.25	-7.97
Plant height at 6 MAP (cm)	158.67–254.33	208.37	23.54	123.33–267.33	188.02	27.24	9.77
Plant height at 9 MAP (cm)	209.67–308.33	254.15	24.30	154.67–273.33	218.19	27.78	14.15
Intencity of red spider mite attack at 6 MAP (%)	12.29–21.97	17.24	2.34	9.08–59.58	25.12	7.08	-45.71
Intencity of red spider mite attack at 9 MAP (%)	7.41–20.18	13.18	2.96	4.67–23.50	15.91	3.81	-20.71
Number of tuber per plant	3.00–18.33	9.56	2.91	0.66–13.33	7.66	2.47	19.87
Tuber weight per plant (kg)	0.77–3.77	2.23	0.79	0.17–3.2	1.30	0.70	41.70
Tuber yield (t/ha)	12.67-79.58	37.70	13.62	8.00–51.25	23.29	9.23	43.29

Notes: L1= normal, L2= drought stress, SD= Standart of Deviation

the plants were 5 months old (Koundinya et al., 2018).

Drought stress increased the attack of red spider mites by 45.71% at 6 MAP and 20.71% at 9 months of age. The increased of the red mites attack was supported by the climate changes in drought environment. According to Ngongo et al. (2022), the intensity of mite damage generally increases during the dry season because environmental conditions during the dry season greatly help increase the mite population. Vongcharoen (2018) reported that the non-irrigated environment of cassava plants had lower humidity and higher temperatures. According to Dhooria (2016), dry and hot weather supports the reproduction and survival of red spider mites, especially when plants experience drought stress.

Drought stress reduced the number of tubers per plant by 19.87% and tuber weight per plant by 41.70%. The decrease in the number of tubers was not as big as the reduction in tuber weight, meaning that in drought-stressed conditions, the tubers were still formed but the filling was not optimal. According to Adjebeng-Danquah et al. (2016) in drought-stressed conditions, the distribution of dry matter will be allocated more to tuber lengthening than to tuber size or thickness. Drought stress greatly affected tuber production per hectare. Drought stress that was given since 3 MAP caused a decrease in tuber yield by 43.29%.

Tuber Yield and the Tolerance of Cassava Germplasm to Drought Stress and Red Spider Mite

The yields showed that cassava plants in drought stress environment could still produce tubers however the quantity and quality were lower than the yield in a normal environment. Accession MLG 10072 showed higher tuber yield or relatively the same as the normal environment. Therefore, the stress tolerance level of cassava germplasm is not only determined by the yield of tubers in drought-stressed environments but through the stress tolerance index (ITC) (Table 3).

The average of tuber yield at L1 (normal environment) was 38.70 t/ha with a yield range of 18.50-78.17 t/ha, while at L2 (stress environment) was 23.29 t/ha with a yield range of 9.33-43.67 t/ha. The high yield potential in normal environments was up to 78.17 t/ha supported by sufficient water availability during the growth period. Drought stress reduced the average yield of cassava accessions by 38.45% with a decrease in the range of -2.61-63.01%. The negative value indicates that there was one accession that gave

higher or relatively the same yield than the normal environment, namely accession MLG 10072. However, even so, the potential yield was very low (21.29 t/ha). The average reduction in tuber yield due to drought stress was almost the same as the yield obtained by Laban et al. (2013) which shows a decrease of 37.04%.

The high yield of tubers in a drought stress environment and a low percentage of yield reduction is not the main measure to determine the tolerance level of cassava accessions. The stress tolerance index (STI) also takes into account the potential yield of an accession under normal conditions because a high yield potential increases the STI value. Cassava accessions are more tolerant if the STI value is higher than 1. Table 3 shows that from the 50 accessions tested, eight accessions had STI values above 1.00. Those accessions were MLG 10032 (STI 1.59), MLG 10034 (STI 1.94), MLG 10052 (STI 1.29), MLG 10054 (STI 1.02), MLG 10060 (STI 1.08), MLG 10063 (STI 1.10), MLG 10361 (STI 1.43) and MLG 10362 (STI 1.64). Of the eight tolerant accessions, one accession was sensitive to red spider mites, namely MLG 10052, and two accessions classified as resistant to red spider mites, namely MLG 10361 and MLG 10362, while the other five accessions were classified as mild.

Accessions classified as tolerant to drought stress had tuber yields >30 t/ha (30.83–43.67 t/ha). Tolerant cassava has physiological adaptations to withstand drought stress such as high control of stomata opening, leaf movement to maximize light interception, and stimulation of root growth (Pushpalatha & Gangadharan, 2020). According to More et al. (2020) varieties that maintain photosynthetic activity followed by a high number of leaves, leaf area index, leaf retention index, and harvest index will produce high yields under drought stress conditions.

Canopy growth (leaves and stems) is controlled by genotype and environment (Phoncharoen et al., 2019) and plant height is one indicator of cassava tolerance to drought stress (Laban et al., 2013). The results of the evaluation of cassava accessions against drought stress in previous studies showed that accessions classified as tolerant had higher plant performance than accessions classified as vulnerable in both optimal and stressed environments and plant height was positively correlated with tuber yield (Wahyuni, 2014). Plant height will be useful for plants as planting material for subsequent planting. The drought tolerant cassava genotype was also indicated to be resistant

to red spider mites and had the genetic ability to maintain as many green leaves as possible during the dry season (Pramudianto & Sari, 2016).

Correlation Between Parameters

The correlations between parameters are presented in Table 4. In a normal environment, plant height at 3 months was positively correlated with plant height at 6 and 9 months and vice versa. On the other hand, plant height was correlated with the number of tubers per plant, meaning that the higher the plant, the less number of tubers formed. Parameters that were positively correlated with tuber yield were the number and weight of tubers per plant.

In a drought stress environment, plant height at 3 months was positively correlated with plant height at 6 and 9 months, meaning that plants with high performance would still provide high performance after drought stress. In stressed conditions, plant height was positively correlated with red spider mite attack, especially at 9 MAP. This means that the higher the cassava plant, the more susceptible it is to attack by red lice. The yield of cassava, when stressed by drought was negatively correlated with plant height and attack of red spider mites at the age of 6 months, and positively correlated with the number and weight of tubers per plant. Meanwhile, drought stress tolerance index was negatively correlated with red spider mite attack at the age of 6 months and positively correlated with the number, weight of tubers per plant, and tuber yield.

Cassava tubers under normal and stressed conditions were influenced by the number and weight of tubers per plant, however, in drought conditions the tuber yield is also affected by the intensity of red spider mite attack at 6 MAP ultimately affecting the cassava tolerance index to drought. The high yield potential under normal conditions is also linear with the tolerance level in drought conditions, meaning that if cassava has a large yield potential, it will also have relatively high yields under stressed conditions. Given that there were accessions that were declared tolerant in this study but were classified as sensitive to red spider mite attacks, control needed to be optimized under stressed conditions, especially at 6 MAP. This study has provide a new gene resource of cassava with multiple resistance to drought stress and red spider mite. Having a such cassava with multiple resistance is useful for efficiency in plant management.

CONCLUSION

Drought stress significantly decreased plant height, and tuber yield, however, increased the intensity of red spider mites attack. Eight accessions had drought tolerance index scores above 1.00, namely MLG 10032 (STI 1.59), MLG 10034 (STI 1.94), MLG 10052 (STI 1.29), MLG 10054 (STI 1.02), MLG 10060 (STI 1.08), MLG 10063 (STI 1.10), MLG 10361 (STI 1.43) and MLG 10362 (STI 1.64). The drought tolerance index was negatively correlated with red spider mite infestation at 6 MAP and positively correlated with the number, the weight of tubers per plant, and tuber yield. Accessions MLG 10361 and MLG 10362 had a high level of tolerance to drought stress as well as resistance to red spider mites so both accessions could be used as a source of multiple resistance genes for biotic and abiotic stresses.

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