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Abstract

The availability of drought tolerant rootstock becomes one of solution to obtain tolerant cocoa clone at drought condition. The production of rootstock is easier and faster by using prope-legitimate seeds as plant materials. Seed germination selection phase can accelerate the obtainment of tolerant cocoa rootstocks. The tolerance trait of prope-legitimate seeds can be determined by seed germination on media induced by polyethylene glycol (PEG) solution. To determine the tolerant seeds, it is effective by using a specific character as selection criteria. This study aimed to obtain criteria and selection character to determine drought tolerant characteristic of prope-legitimate seed through PEG 6000 induction in the germination phase. The research was conducted at the Agronomy Laboratory of the Indonesian Coffee and Cocoa Research Institute, Jember, East Java, Indonesia, used split plot design with five replications as experimental design. The main plot was solution of 0% and 6% PEG 6000, while the subplot were prope-legitimate seeds of 13 cocoa clones. The result showed that germination ability and sprout fresh weight were suitable as selection criteria to determine tolerant characteristic of prope-legitimate seeds to drought stress. Based on the value of stress susceptible index and clustering analysis, the tested prope-legitimate seeds were divided into three groups. The group of tolerant genotype consisted of Sca 06, while the group of medium tolerant were ICCRI 03, Sulawesi 02, KW 641, TSH 858, KEE 02, Sulawesi 03, and KW 617. The susceptible group consisted of six genotypes, namely ICS 60, KKM 22, KW 516, Sulawesi 01, and MCC 02.

Keywords: Cocoa, prope-legitimate seed, selection criteria, drought tolerance, PEG

INTRODUCTION

Cocoa is a very susceptible plant to drought stress (Amos & Thompson, 2015; Gateau-Rey et al., 2018). Drought affects cocoa seedling mortality, decreases the fruit and seeds size and weight, and increases damage due to pest and disease attacks (Ofori et al., 2015). Drought threatens the future of cocoa production, therefore drought-tolerant cocoa clone are needed to solve the problems caused by drought (Cazares et al., 2010).

Efforts to obtain drought-tolerant cocoa plants have been carried out by Dos Santos et al. (2016); De Almeida et al. (2016); Setyawan et al. (2018); Zakariyya & Indradewa (2018) through seed selection using the method of limiting water input to

growing media. Selection is more difficult and less efficient when the number of seedlings is large enough on a large area (Alban *et al.*, 2015; Dos Santos *et al.*, 2016). The early-phase screening technique for growth is highly prospective for obtaining drought-tolerant species (Boopathi *et al.*, 2013). Seed germination phase selection is conducted to obtain drought-tolerant plants easily, quickly, and accurately and can potentially grow well in the drought stress area (Petrovic *et al.*, 2016).

The production of tolerant cocoa seedlings under dry growing conditions can also be done through a continuous technique approach using tolerant rootstock. This method is very prospective related to the role of the rootstock which is directly related to water conditions, nutrition, and various biotic and abiotic stresses in the growing environment (Susilo *et al.*, 2005; Warschefsky *et al.*, 2016). Cocoa rootstocks are usually obtained from multiplication of seeds from controlled crosses, but prope-legitimate seeds are more likely to be used as sources of rootstock propagation material (Susilo, 2015). Prope-legitimate seeds are obtained from a female parent whose the identity is known with certainty that receives random pollen from other clones that grow close together in the parent garden. This seed can be used as propagation material to obtain tolerant root-stock.

Seed tolerance can be studied by germinating seeds on a homogeneous water deficit medium in a laboratory or greenhouse (Baloch *et al.*, 2012). Water stress control carried out by using polyethylene glycol (PEG) solution induction is considered to be able to present the homogeneity of selection pressure (Khodarahmpour, 2011; George *et al.*, 2013; Muscolo *et al.*, 2014). Osmotic stress due to PEG 6000 is considered to be able to stimulate equivalent drought conditions in the field. In the field capacity condition, the soil is considered to have an osmotic potential of around -0.33 bar while at the permanent wilting point reaches -15 bar (Mirbahar *et al.*, 2013). PEG 6000 at concentrations of 5-20% produces osmotic potentials ranging from -0.3 bar to -6.7 bar (Michel & Kaufman, 1973; Mexal *et al.*, 1975), therefore, they are often used for seed selection (Shao *et al.*, 2015). PEG treatment on cocoa seeds has been carried out by Rahayu *et al.* (2014) and Hutabarat *et al.* (2018) which uses PEG to maintain seed quality. According to Rahardjo (1986), PEG was used to maintain the viability of cocoa seeds during seed storage and delivery.

Testing the tolerance of prope-legitimate against drought have not been studied. To estimate the genetic potential of tolerant prope-legitimate seeds requires selection criteria based on the genetic variability, heritability (Mayo, 1980), and correlation, that refer to the seed germination character in dry germination media (Sevik & Ertruk, 2015; Widyastuti *et al.*, 2016; Akbar *et al.*, 2018). Drought tolerance could be determined based on the drought susceptibility index (Fischer & Maurer, 1978), there is still limited studies regarding this issue. This study aimed to determine the tolerance properties of prope-legitimate seeds to drought stress through induction of PEG solution.

MATERIALS AND METHODS

The study was conducted at the Agronomy Laboratory of the Indonesian Coffee and Cocoa Research Institute, Jember, East Java, Indonesia in November-December 2018. The temperature and humidity of the germination chamber during the study were recorded at about 29.8°C and 80.2%, respectively. The genetic material used were prope-legitimate seeds from bulk cocoa clones consisting of KW 516, KW 617, KW 641, ICCRI 03, TSH 858, Scavina 06, KEE 02, KKM 22,

MCC 02, ICS 60, Sulawesi 01, Sulawesi 02, and Sulawesi 03. The genetic material is a superior germplasm collection from the Indonesian Coffee and Cocoa Research Institute, Jember, Indonesia which has the potential to be developed as a drought tolerant rootstock.

The experiment was carried out using a randomized complete design. PEG 6000 solution (6% and without PEG) induction treatment was the main plot and prope-legitimate seed was a subplot. Determination of 6% PEG 6000 concentration was based on a preliminary experiment using random prope-legitimate seeds. The treatment was applied in 5 replications and in each treatment unit 25 seeds were sown on filter paper in a (30 x 20 x 15) cm³ sized box. The seedling media was moistened with 40 mL of the treatment solution then 20 mL solution was added every 2 days until the seeds were 14 days old. In the next step, PEG solution was applied as much as 40 mL until the germination was 21 days old.

Seed tolerance evaluation was carried out until the 21st day by observing several components. These components were germination ability (%) that was calculated based on the number of normal germinated seeds characterized by the appearance of two leaves in the 14th and 21st days; vigor index (%) that was calculated based on the number of normal germinated seeds characterized by the appearance of two leaves on the 14th day; seed growth potential (%) that was calculated based on the number of seeds germinated on the 14th and 21st days. The number of sprouted leaves was measured on 21st day; root length (cm) was measured from base to root tip on 21st day; shoot length (cm) is measured from the base to the tip of the shoot on 21st day; root/shoot ratio; fresh weight of the sprout/dry sprout (g) was measured by weighing the sprout wet/dry weight without cotyledon on 21st days.

Data were analyzed to obtain the average value and standard deviation. Homogeneity variance was tested using the F-test at α 5% confidence level using R software version 3.44. Relative decrease and heritability were calculated as done by Susilo *et al.* (2005); also for genetic variability (Anderson & Bancroft, 1952); and phenotype correlation (Singh & Chaudhary, 1979). Genotype tolerance was determined using stress susceptibility index (Fischer & Maurer, 1978). The stress susceptibility index was calculated by the formula: $SSI = (1 - Ys/Yp) / (1 - \bar{Y}s/\bar{Y}p)$ (Fisher & Maurer, 1978), Ys = average specific genotype on drought stress conditions, Yp = average specific genotype on optimum condition, $\bar{Y}s$ = average of all genotypes on drought stress and $\bar{Y}p$ = average of all genotypes on optimum condition. The grouping of seed genotypes was performed by using a heatmap clustergram analysis.

RESULTS AND DISCUSSION

Selection Criteria

Seed selection is an alternative on improving plant genetic quality. Prope-legitimate cocoa seeds were obtained from female parents that receive pollen randomly in the same plot of land. The availability of prope-legitimate tolerant seeds can accelerate the efforts to obtain drought-resistant seeds. The tolerance properties estimation of prope-legitimate cocoa seeds in drought stress seeds can be conducted through the identification of the germination character in the osmotic solution (Farooq *et al.*, 2012).

Seed selection required criteria that refer to the germination characters which showed significant interaction variance. Characters with significant interaction of variance indicate that there were the differences in responses between sensitive and tolerant genotypes to stress (Eeuwijk *et al.*, 2016). Based on the

results of the analysis of variance (Table 1), the character of germination ability, number of sprout leaves, and the sprout fresh weight showed a significant interaction variance. This indicates that these characters can be selected as selection criteria for drought stress tolerance seed.

The tolerance level on plants to drought stress are estimated based on the relative decrease of various growth characters. The relative decrease analysis indicated the level of genotype response to stress condition (De Leon *et al.*, 2015; Anshori *et al.*, 2018b). The analysis results (Table 2) showed that the character of germination ability, number of sprout, leaves and fresh weight of the sprouts decreased varied and that values were reached more than 50% due to drought stress. Prope-legitimate Sca 06 seeds showed the lowest relative decrease at character of germination ability, the number of sprout leaves, and the sprout fresh weight. The less relative decrease of those characters indicated that the genotype was tolerant to drought stress. Tolerant genotypes are characterized by the ability to survive and growing under stress conditions (Rukundu *et al.*, 2014).

Heritability estimation was analyzed to evalute genetic potential with the phenotypic performance estimation of the selected plants. The inheritance character is important in plant selection (Islam *et al.*, 2015). There are two types of heritability values which consist of broad heritability which define as ratio between genetic variance and phenotypes variance of character (h_{bs}^2). The second is the narrow heritability defining as ratio between additives variance and the phenotypes variance (h_{ns}^2). The narrow heritability represented the role of additive genes to the phenotypic aspect, so it is often used to determine the selection character (Susilo, 2005; Rubiyo & Sudarsono, 2011).

Genetic parameters estimation (Table 3) showed that the genetic variance of the germination ability was 11.85 greater than the genetic variance of number of sprout leaves and sprout fresh weight, which were 0.00 and 0.06. However, the character of germination ability have an individual inheritance value (h_{ns}^2) was 0.29 and the average family inheritance (h_{nsf}^2) was 0.39. The individual inheritance value of sprouts fresh weight character was 0.88 and the family inheritance (h_{nsf}^2) was 0.99. Both of the inheritance value on germination ability character in present study was categorized as low level, while the sprout fresh weight was high level (Standfield, 1983). The lower value of narrow heritability on germination ability indicated that the lower effect of additive genetic to the phenotypic expression of germination ability. The phenotype expression of germination character is influenced by genetic factors and environmental condition.

In general, the average family inheritance value (h_{nsf}^2) was not different from the individual inheritance value (h_{ns}^2), so that the heritability values were effectively used in determining selection criteria. Mean value-based selection (population) is the most suitable form of family selection for annual plants (Mayo, 1980; Susilo, 2005).

Table 1. Analysis of variance of germination characters of prope-legitimate cocoa seeds to PEG induction

Variance source	GA	VI	MGP	NLS	HL	RL	RHR	SFW	SDW
PEG	0.00**	0.00**	0.00**	0.00**	0.00**	0.57tn	0.00**	0.00**	0.00**
Genotype	0.00**	0.03*	0.19tn	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**
PEG x Genotype	0.00**	0.13tn	0.13tn	0.00**	0.20tn	0.09tn	0.14tn	0.02*	0.25tn
CV (%)	14.87	24.3tr1	4.90	14.77	13.04	12.72	14.99	16.94	6.73tr2

Notes: *significant at level of α = 5%, ** significant at a level of α = 1%, tn, not significant, tr1 = transformation data [$\sqrt{(X+1.5)}$], tr2 = transformation data [$\sqrt{(X+0.5)}$], GA = germination ability, VI= Vigor Index, MGP = maximum growth potential, NLS = number of sprout leaves, HL = hypocotyl length, RL = radicle length, RHR= radicle/ hipocotyl ratio, SFW = sprout fresh weight, SDW = sprout dry weight.

Table 2. The relative decrease of germination ability, number of leaf sprouts, and sprouts fresh weight of prope-legitimate cocoa seeds to PEG induction

Genotype	Germination ability(%)				Number of sprout leaves (leaf)				Sprout fresh weight(g)			
	Environment			RD	Environment			RD	Environment			RD
	PEG 0%	PEG 6%	delta	(%)	PEG 0%	PEG 6%	delta	(%)	PEG 0%	PEG 6%	delta	(%)
KW 516	89.6	38.4	51.2	57.1	2.92	0.97	1.95	66.9	1.59	0.80	0.79	49.9
KW 617	84.8	56.8	28.0	33.0	2.91	1.30	1.62	55.5	1.72	0.58	1.14	66.2
KW 641	93.6	70.4	23.2	24.8	3.69	1.65	2.04	55.3	1.70	0.71	0.99	58.3
ICCRI 03	84.0	60.0	24.0	28.6	2.73	1.19	1.54	56.3	1.29	0.66	0.63	49.1
TSH 858	89.6	59.2	30.4	33.9	3.28	1.26	2.02	61.5	1.82	0.76	1.06	58.2
Sca 06	93.6	72.8	20.8	22.2	2.70	1.81	0.89	32.9	0.99	0.60	0.39	39.5
KEE 02	88.0	29.3	58.7	44.6	2.62	1.12	1.50	57.2	1.08	0.48	0.60	55.3
KKM 22	92.0	48.0	44.0	47.8	3.34	1.25	2.10	62.7	1.53	0.66	0.88	57.2
MCC 02	81.6	36.0	45.6	55.9	2.86	0.83	2.02	70.9	1.92	0.76	1.16	60.5
ICS 60	87.2	44.4	42.8	49.1	3.01	1.09	1.93	63.9	1.67	0.75	0.93	55.3
Sul 01	96.0	39.2	56.8	59.2	3.19	1.30	1.89	59.2	1.62	0.73	0.89	54.8
Sul 02	83.2	53.6	29.6	35.6	2.84	1.35	1.49	52.4	1.39	0.68	0.71	51.2
Sul 03	88.8	52.8	36.0	40.5	2.79	1.33	1.46	52.4	1.36	0.54	0.82	60.2

Notes: PEG 0% = without induction of polyethylene glycol, PEG 6% = induction of polyethylene glycol 6%, RD = the relative decrease, delta = the difference of mean obtained from the results of optimum condition minus the stressed condition.

Table 3. Components of variance, heritability, and deviation standard of genetic variance in prope-legitimate cocoa seed germination to PEG induction

Characters	Variance			Heritability			$2\sigma_{\sigma_g}$
	σ^2_g	σ^2_i	σ^2_f	σ^2_A	h^2_{ns}	h^2_{nsf}	
Germination ability	11.85	164.36	130.25	47.42	0.29	0.39	67.05
Number of sprout leaves	0.00	0.11	0.08	0.00	0.00	0.00	0.03
Sprout fresh weight	0.06	0.28	0.25	0.24	0.88	0.99	0.15

Notes: *Inheritance value that showed more than one was considered the same as one, σ^2_g = variance of genetic, σ^2_i = variance of individual phenotype, σ^2_f = variance of family phenotype, σ^2_A = variance of additive, h^2_{ns} = The narrow-sense individual heritability, h^2_{nsf} = The narrow-sense family heritability, σ_{σ_g} = deviation standard of genetic variance.

Genetic variability describes the genetic background of the genotype tested. The broad and narrow of genetic diversity of a character is determined based on genetic variance (σ^2_g) and the standard deviation of genetic diversity (σ_{σ_g}). It can be seen that the genetic variance of germination ability and sprout fresh weight was smaller than twice of standard deviation of genetic variances (Table 3). Thus, the variability germination ability and sprout fresh weight characters in present study was categorized as narrow. High heritability and wide genetic diversity are important factors in choosing selection criteria. However, the narrow genetic diversity was not directly followed by the narrow phenotypic diversity, because the phenotypic diversity was affected not only by genetic but also environment (Syukur *et al.*, 2011).

Correlation analysis was done to determine the relationship among the observed characters and it was used to obtain selection criteria as carried out by Anita-Sari & Susilo (2013). The results of the correlation analysis (Table 4) showed that germination has a positive and high correlation with the character of the sprout fresh weight. Positive correlated characters can be used in the selection process as character selection for drought tolerance.

The selection process considered the characters that have wide genetic variability, but character selection should be determined according to the objectives of breeding (Susilo *et al.*, 2005; Devy *et al.*, 2018). Prope-legitimate cocoa seeds that have high germination ability and sprout fresh weight character are considered as tolerant to stress. Thus, they are the most appropriate characters

Tabel 4. Pearson correlation of germination characters of prope-legitimate cocoa seeds to PEG induction

	GA	VI	MGP	NLS	HL	RL	RHR	SFW
VI	-0.90 **							
MGP	-0.82 **	0.84 **						
NLS	-0.63 **	0.76 **	0.60 **					
HL	-0.95 **	0.95 **	0.88 **	0.64 **				
RL	-0.98 **	0.92 **	0.83 **	0.66 **	0.96 **			
RHR	-0.66 **	0.65 **	0.50 **	0.58 **	0.69 **	0.70 **		
SFW	0.90 **	-0.82 **	-0.77 **	-0.49 *	-0.85 **	-0.88 **	-0.31 tn	
SDW	-0.90 **	0.79 **	0.69 **	0.48 *	0.90 **	0.89 **	0.67 **	-0.77 **

Noted: *significant at level of α = 5%; **significant at a level of α = 1%, tn, not significant, GA = germination ability, VI = Vigor Index, MGP = maximum growth potential, NLS = number of leaf sprout leaves, HL = hipocotyl lenght, RL = radicle length, RHR = radicle/hypocotyl ratio, SFW = sprout fresh weight, SDW = sprout dry weight

chosen as a selection criteria for prope-legitimate cocoa seeds to drought stress. The estimation of tolerance characteristic of genotypes in the germination phase can be done by using the percentage of germination and sprout weight as a selection criteria (Sumartini *et al.*, 2013; Akbar *et al.*, 2018).

Tolerance Index

Evaluation of drought tolerance characteristic of genotypes can be predicted using susceptible stress index (SSI). The SSI value is used to measure the yield stability due to changes in the environment related to the mechanism of resistance and genotype susceptibility (Mardeh *et al.*, 2006; Khayatnezhad & Gholamin, 2012; Anshori *et al.*, 2018b). Genotype selection for drought tolerance was effective if the test is done under optimum and stress conditions.

The results of analysis based on the value of SSI germination ability and sprouts fresh weight (Table 5), showed that the prope-legitimate seed genotypes were divided into three groups, namely tolerant, medium tolerant, and susceptible to drought stress. Based on the value of SSI germination ability showed that tolerant seed genotype consisted of Scavina 06; the medium tolerant seed genotypes consisted of KW 641, ICCRI 03, KW 617, TSH 858, Sulawesi 02 and Sulawesi 03; while the susceptible seed genotypes consisted of KEE 02, KKM 22, ICS 60, MCC 02,

Sulawesi 01, and KW 516. Based on the value of SSI sprout fresh weight showed that the seed genotypes were medium tolerant, except the seed genotypes of KW 617, Sulawesi 03, and MCC 02 were susceptible to drought. Genotypes that had a SSI value of $0.5 < SSI \leq 1$ indicated to be medium tolerant, while groups of genotypes that had a SSI value of >1.00 were susceptible (Fischer & Maurer, 1978).

Grouping is done to determine the relationship among plant genotypes. The characteristics of the group of plants need to be known so that the selection process is more effective. One of the method of grouping is clustergram analysis which is a multivariate analysis by combining several cluster analyzes in a flat dimension (Schonlau, 2002). Cluster analysis visualization was simple and easy to understand make this analysis aplicable for selection (Yuan *et al.*, 2016). Color intensity indicates the level of strength or degree of genotype in each character. The differences in color contrast make it easy to determine the characteristics of each genotype group (Lee *et al.*, 2016; Anshori *et al.*, 2018).

Clustergram analysis results showed that the prope-legitimate cocoa genotype dendrogram was divided into three groups (Figure 1). The first group consisted of Sca 06; the second group was divided into subgroup 1 consist of three genotypes, namely ICCRI 03, Sulawesi 02, KW 641 and TSH 858; while subgroup 2 consist of KEE 02, Sulawesi 03, and KW 617. The third group was divided

Table 5. Tolerance characteristic of prope-legitimate cocoa seeds to PEG induction based on the susceptible stress index of germination ability and sprout fresh weight characters

| Genotipe	Germination ability (%)		Sprout fresh weight (g)	
	Susceptible stress index	Criteria	Susceptible stress index	Criteria
Sca 06	0.5	Tolerant	0.7	Medium tolerant
KW 641	0.6	Medium tolerant	1.0	Medium tolerant
ICCRI 03	0.7	Medium tolerant	0.9	Medium tolerant
KW 617	0.8	Medium tolerant	1.2	Susceptible
TSH 858	0.8	Medium tolerant	1.0	Medium tolerant
Sulawesi 02	0.9	Medium tolerant	0.9	Medium tolerant
Sulawesi 03	1.0	Medium tolerant	1.1	Susceptible
KEE 02	1.1	Susceptible	1.0	Medium tolerant
KKM 22	1.2	Susceptible	1.0	Medium tolerant
ICS 60	1.2	Susceptible	1.0	Medium tolerant
MCC 02	1.4	Susceptible	1.1	Susceptible
Sulawesi 01	1.4	Susceptible	1.0	Medium tolerant
KW 516	1.4	Susceptible	0.9	Medium tolerant
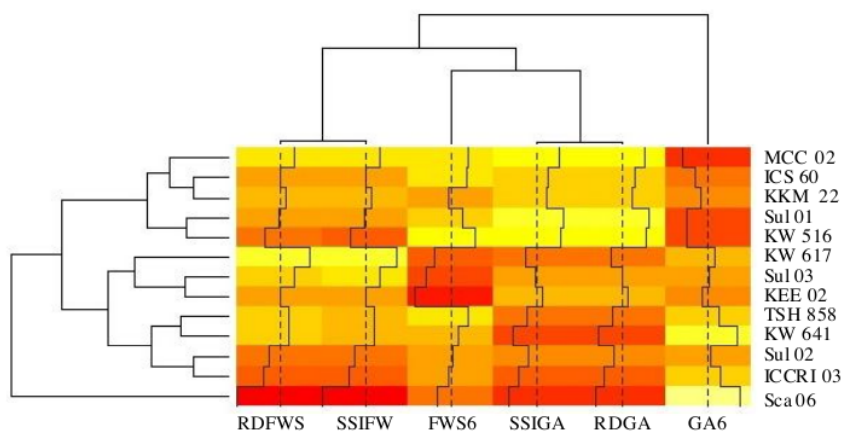
Figure 1. Clustergram heatmap of prope-legitimate cocoa seeds based on 5 observed variables, i.e RDSFW = relative decrease of sprout fresh weight, SSISFW = stress susceptibility index of sprout fresh weight, SFW= sprout fresh weight of PEG induction, SSIGA = stress susceptibility index of germination ability, RDGA = relative decrease of germination ability, GA6 = germination ability of PEG induction

into subgroup 1 consisted of two genotypes, namely KW 516 and Sulawesi 01, while subgroup 2 consisted of KKM 22, ICS 60, and MCC 02. This grouping was based on the pattern of color intensity on the germination characters supported by the value of stress susceptibility index. The relationship between genotypes was explained by a dendogram that divide groups into each subgroup.

The first and second group were groups that were indicated to be tolerant prope-legitimate seed genotypes. The third group was indicated to be susceptible prope-legitimate seed genotypes. In general, the tolerant seed genotype group have a high germination ability and a low of the relatively decrease on germination ability. The prope-legitimate seed genotypes of Sca 06

showed the highest degree of tolerance in the group. The sensitive genotypes have a low germination ability and a high of the relatively decrease on germination ability. The relative decrease of sprout fresh weight in all prope-legitimate seed genotypes were various and can be predicted not different.

PEG induction in germination media shows its role in suppressing water imbibition which causes number of seeds to germinate was low. The effect of PEG on the decrease in germination was also reported by Lahay *et al*. (2018) that the decrease in water content due to PEG induction correlates with the level of viability of recalcitrant cocoa seeds. Sufficient water in the growing environment requires cocoa seeds to soften the seed coat, dissolve food reserves, and hormone activity (Yuliasmara, 2015). Diversity of the character of prope-legitimate seed germination against drought is influenced by genotypes related to seed quality (viability and vigor) and environmental conditions of germination.

CONCLUSION

Germination ability was the most appropriate selection criterion for determining the tolerance properties of prope-legitimate seeds in drought to PEG induction. The group for tolerant genotype was Sca 06, while the group for medium tolerant consisted of ICCRI 03, Sulawesi 02, KW 641, TSH 858, KEE 02, Sulawesi 03, and KW 617. The susceptible group consisted of five genotypes, namely KW 516, Sulawesi 01, KKM 22, ICS 60, and MCC 02.

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