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Genetic Components Estimation of F1 Population of Cocoa (*Theobroma cacao* L.) in Drought Stress Condition

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Abstract

Drought stress is a major constraint in cocoa production. The use of drought tolerant clone is the most efficient tool to overcome drought problem in cocoa production. North Carolina II (NCII) mating-design was used to identify parental and progeny performance in drought stress. The crossing consisted of three female parent clones namely KW 516, Sulawesi 3, and TSH 858, while male parent clones were ICCRI 09 and Scavina 6 then produced 6 combinations crosses. Genotypes used were 11 genotypes consisted of 5 parent clones and 6 cross genotypes. Drought treatment was applied to cocoa seedlings at 6 weeks after sowing with 25% and 100% available water content. The plants were maintained without water for five days to modulate the drought intensity. Variables observed were stem diameter, root length, root volume, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, and root/shoot ratio characters at 16 weeks after sowing. Based on the estimated general combining ability (GCA) value, TSH 858 was the best female parent, while Scavina 6 was the best male parent. Based on the estimated specific combining ability (SCA) value Sulawesi 3 x ICCRI 09 and TSH 858 x Scavina 6 were the best crosses. Based on the estimation of its genetic components, characters of drought tolerance stress were affected by additive genes. The dominant gene only affected the root fresh weight and root/shoot ratio. Based on SSI values, TSH 858 and Sulawesi 3 clones were drought-resistant clones, ICCRI 09 was moderate clone, and KW 516 and Scavina 6 were susceptible. Some of the findings were in contrast with earlier study.

Keyword: Drought stress, GCA, SCA, North Carolina II (NCII), genetic component, *Theobroma cacao*

INTRODUCTION

Cocoa is one of the leading agriculture and industrial crops in Indonesia. Indonesia is the third largest cocoa producer in the world after Ivory Coast and Ghana (ICCO, 2017). Cocoa production in Indonesia in the last 5 years has decreased by 32 thousand tons (Ditjenbun, 2017). According to BPS (2018), national cocoa production in 2016 reached 658 399 tons and in 2017 national cocoa production decreased by 1 349 tons. One factor that causes low cocoa production is abiotic factors such as drought stress (Alban *et al*., 2016).

Drought can affect decreasing of plant growth and development both in the seedling phase and in mature plants. The impact of drought stress can be overcome by the development of superior drought resistant hybrid clones. Development of superior hybrid clones require a good plant breeding strategy. Monsterin & Verteuil (1956) have found heterosis in cocoa plants so that almost all plant breeding programs in cocoa plants refer to the development of inter-clonal hybrids (full-sib families) (Lopes *et al*., 2011). The development program also adopted a "single-generation breeding" strategy where new hybrids will be developed from existing germplasm, not from selected progenies in the advanced generation (Lopes *et al.*, 2011).

According to Lopes *et al*. (2011), one of the crossing designs that can be used to develop a cocoa plant population is North Carolina II (NCII). Each member of the male parents used is crossed with each member of the female parents in NCII mating-design (Nduwumuremyi *et al*., 2013). According to Hallauer (2007), NCII mating-design is suitable for use in a large number of parent but only produces fewer crosses. The design of NCII is similar to the line x tester mating-design. This is because the two mating-designs both calculate the influence of the variance of female parents and the variance of male parents, as well as the influence of the interaction of female x male parents (Fasahat *et al.*, 2016).

Through analysis combining ability at the parents used in the mating-design, the parent combination will be obtained with the best combined ability value. Combining ability is the ability of parents to be used to combine with other parents through a crossing process (Fasahat *et al*., 2016). According to Sprague & Tatum (1942), there are two types of combining ability: general combining ability (GCA) and specific combining ability (SCA). General combining ability (GCA) is the average performance value of the parent used in a cross. Specific combining ability (SCA) is the average performance value of a combination compared to the parent used (Acquaah, 2012). The GCA value is related to the additive gene, while the SCA value is related to non-additive gene (Fasahat *et al.*, 2016).

Aims of this research were to obtain estimated values of genetic components through NCII mating-design. The best parent were expected to drought tolerance based on GCA and SCA values also the best genotypes with the best tolerance levels.

MATERIALS AND METHODS

This research was conducted from August 2018 until May 2019. The research was conducted at Kaliwining Experimental Station, Indonesian Coffee and Cocoa Research Institute. Planting activity after crossing and produced genotypes F1 was carried out at Greenhouse Kaliwining Experimental Station, Indonesian Coffee and Cocoa Research Institute.

This research started with crossing using North Caroline II (NCII) mating-design (Lopas *et al.*, 2011). Female parent clones were TSH 858, KW 516, and Sulawesi 3, while male parent clones were Scavina 6 and ICCRI 09. While, KW 516 classified in drought tolerant clone (Zakariyya *et al*., 2017), Sulawesi 3 and Scavina 6 were moderate clone (Towaha & Wardiana, 2015), and TSH 858 was susceptible clone (Iryono, 2010; Kurniawan, 2017). Total combination of crosses produced through the design were 6 combinations of crosses (Table 1). Planting materials in this research consisted of 6 crosses (offspring) and 5 parent clones.

Table 1. Cross combination used in the research

Male \ Female	TSH 858 (T3)	Sulawesi 3 (T4)	KW 516 (T5)
ICCRI 09 (T1)	T1 x T3	T1 x T4	T1 x T5
Scavina 6 (T2)	T2 x T3	T2 x T4	T2 x T5

Soil analysis used the pF curve to determine the permanent wilt point and field capacity of the soil used in first research preparation. Polybag size was 15 x 25 cm. Drought stress treatment was given to plants at 1.5 months old (6 week after sowing). Drought treatments were of 100% and 25% available water content (AWC). The plants were maintained without water for 5 days to modulate the drought intensity.

Observations were made on plant morphological characters such as: stem diameter, root length, root volume, shoot fresh and dry weight, root fresh and dry weight, and root/shoot ratio. This observation was finished at 4 months old (16 week after sowing).

Data which had been collected (25% AWC treatment) would be analyzed using NCII mating-design. R 3.4 version was used as software analysis. Linear additive models for analysis of variance using NCII is:

$$Y_{ijk} = \mu + m_i + f_j + (m \times f)_{ij} + \varepsilon_{ijk}$$

Y_{ijk} = observation value k on cross combination i × j
μ = overall data mean Y
m_i = male parent influence to i
f_j = female parent influence to j,
$(m \times f)_{ij}$ = interaction between male parent to i and female parent to j
ε_{ijk} = error

NCII mating design results consisted of GCA on the parents used, value of SCA on the cross combination produced, variance of GCA (σ^2_{GCA}), variance of SCA ($_{SCA}$), variance additives ($_{A}$), and variance dominant ($_{D}$). This value was then used to estimate the value of broad sense heritability (h^2_{bs}) and narrow sense heritability (h^2_{ns}).

Clones grouping used their tolerance ability to drought based on the value of the Stress Susceptibility Index (SSI) (Ali & El-Sadek, 2016; Khan & Dhurve, 2016) with the formula:

$$SSI = \frac{(1 - \frac{Y}{Y_p})}{(1 - \frac{X}{X_p})}$$

Y = observation value of variable on the genotypes with drought stress
Yp = observation value of variable on the genotypes with optimum environment
X = the mean value of variable on the genotypes with drought stress
Xp = the mean value of variable on the genotypes with optimum environment

Cocoa plants with SSI value of <0.5 are classified as drought tolerant plants, plants with 0.5 <SSI <1 are classified as moderate to drought stress (Setyawan *et al*., 2018), and plants with SSI>1 are classified as susceptible to drought stress (Helmi, 2017).

RESULTS AND DISCUSSION

Research about drought stress in plants has been carried out. Some studies suggest that the characters associated with the root system have important role in response to drought stress in plant (Alban *et al*., 2016; Medina & Laliberte, 2017; Setyawan *et al*., 2018). These characters include stem diameter, root length, root volume, shoot and root fresh weight, shoot and root dry weight, and root/shoot ratio.

Based on the results analysis of variance, genotypes used in this study were significantly different. Analysis of variance based on NCII found that all observed characters were significantly different in crossing genotypes, except for root dry weight. Female parents and male parents showed significant differences performance in root dry weight. This means that on the character of root dry weight there is an effect produced both from female parents and male parents. The female parents used only showed significant differences in character shoot fresh weight, and shoot dry weight

(Table 2). This indicates that the influence of female parents is very large on the characters of shoot fresh and dry weights.

The results of general combined ability analysis (GCA) show that TSH 858 was the best female parent because it had the highest GCA values in almost all characters, except root length and root/shoot ratio. Scavina 6 was the best male parent that had highest GCA value in almost all characters, except stem diameter, root fresh weight, and root dry weight (Table 3). TSH 858 clone is commonly used for parent material because it has high productivity (Kementan, 2013). Scavina 6 clone is also widely used for the formation of superior hybrids in cocoa plants. According to Zakariyya (2017), Scavina 6 clone has high seedling root length. The highest estimated value of specific combining ability (SCA) was Sulawesi 3 x ICCRI 09 in all observed characters except the root/shoot ratio. TSH 858 x Scavina 6 crosses also showed high SCA values in all observed characters except shoot dry weight character (Table 4). According to Hossein & Aziz (1998), the high value of a GCA in a character does not always produce high value of SCA.

Positive values indicate a contribution to a higher phenotype value. The high value of combining ability was needed on the character associated with tolerance to drought, especially in the plant root system. This shows that a good root system will determine genotypes that are tolerant to drought. The growth of a good stem diameter is related to the physiological mechanism for translocation and assimilate storage, especially in stressed conditions (Santos *et al*., 2016). Good root development will also

Table 2. Variance analysis of the stem diameter and root system characters with NCII mating-design in 25% AWC treatment

Source of variation	df	Mean square (MS)							
		SD	RL	RV	SFW	RFW	SDW	RDW	R
Replication	4	0.0154 *	57.87 *	22.36 *	17.94 *	12.34 *	1.55 *	0.177 *	0.013 *
F1	5	0.0030 *	4.20 *	1.09 *	4.46 *	0.91 *	0.53 *	0.023 ns	0.014 *
Female parent	2	0.0025 ns	9.04 ns	1.74 ns	10.14 *	1.07 ns	1.25 *	0.04 *	0.028 ns
Male parent	1	0.0001 ns	1.38 ns	1.27 ns	0.89 ns	1.38 ns	0.003 ns	0.033 *	0.0006 ns
Female * Male	2	0.0049 *	0.76 ns	0.34 ns	0.57 ns	0.52 *	0.063 *	0.00028 ns	0.0058 ns
Error	40	0.0002	0.38	0.17	0.20	0.16	0.016	0.013	0.0028

Notes: *significantly different, ns not significantly different (α = 5%); SD = stem diameter, RL = root length, RV = root volume, SFW = shoot fresh weight, RFW = root fresh weight, SDW = shoot dry weight, RDW = root dry weight, R = root/shoot ratio.

Table 3. Estimated values of general combining ability (GCA) in female dan male parents in 25% AWC treatment

Female	Character							
	SD	RL	RV	SFW	RFW	SDW	RDW	R
KW 516	-0.006	-0.54	-0.4	-0.522	-0.368	-0.091	0.005	-0.003
Sulawesi 3	-0.12	1.098	-0.034	-0.638	0.115	-0.3	-0.067	0.54
TSH 858	0.018	-0.557	0.433	1.161	0.254	0.39	0.061	-0.052
SE GCA Female	0.005	0.19	0.13	0.14	0.13	0.04	0.04	0.017

Male	Character							
	SD	RL	RV	SFW	RFW	SDW	RDW	R
ICCRI 09	0.001	-0.215	-0.206	-0.172	0.215	-0.01	0.033	-0.004
Scavina 6	-0.001	0.215	0.206	0.172	-0.215	0.01	-0.033	0.004
SE GCA Male	0.004	0.16	0.11	0.12	0.105	0.03	0.03	0.014

Note: SD = stem diameter, RL = root length, RV= root volume, SFW = shoot fresh weight, RFW = root fresh weight, SDW = shoot dry weight, RDW = root dry weight, R = root/shoot ratio.

Table 4. Estimated values of specific combining ability (SCA) in cross combination in this research in 25% AWC treatment

Genotypes	Character							
	SD	RL	RV	SFW	RFW	SDW	RDW	R
KW 516 x ICCRI 09	0.015	0.05	-0.011	-0.177	0.093	-0.046	0.002	-0.027
KW 516 x Sca 6	-0.015	-0.05	0.011	0.177	-0.093	0.046	-0.002	-0.027
Sul 3 x ICCRI 09	0.011	0.248	0.189	0.271	0.123	0.046	0.004	-0.01
Sul 3 x Sca 6	-0.011	-0.248	-0.189	-0.271	-0.123	-0.046	-0.004	0.01
TSH 858 x ICCRI 09	-0.025	-0.297	-0.178	-0.094	-0.217	0.001	-0.006	-0.018
TSH 858 x Sca 6	0.025	0.297	0.178	0.094	0.217	-0.001	0.006	0.018
SE SCA	0.006	0.27	0.18	0.2	0.32	0.056	0.051	0.024

Note: SD = stem diameter, RL = root length, RV= root volume, SFW = shoot fresh weight, RFW = root fresh weight, SDW = shoot dry weight, RDW = root dry weight, R = root/shoot ratio.

support plant growth, biomass formation, and drought resistance (Santos *et al*., 2016). Roots will respond to drought conditions by extending their roots to more easily access deeper water. The high ratio of plant roots and shoot is needed. According to Beets *et al*. (2007) the higher root/shoot ratio will result in stronger plants (especially the seedling phase).

The effect of gene action on a plant character can be seen through range of GCA($_{GCA}$) and SCA ($_{SCA}$). This is indicated by the ratio value of variance GCA($_{GCA}$) and variance SCA ($_{SCA}$) ($_{GCA}/_{SCA}$). According to Istipliler *et al*. (2015), if the value of $_{GCA}/_{SCA}$ is smaller than the value of dominance ratio degree $(_D/_A)^{1/2}$ in a character, it can be said that the character is controlled by the dominant gene. This is in line with the research conducted by Constantine (2017) on oil palm, where if the ratio of $_{GCA}/_{SCA}$ is smaller than the value of dominance ratio degree $(_D/_A)^{1/2}$ then the character is influenced by non-additive factors.

The results genetic component values in this research can be seen in Table 5. Root length, root volume, shoot fresh weight, and shoot dry weight characters showed ratio of $\sigma^2_{DGU}/_{DGK}$ is higher than the value of dominance ratio degree $(_D/_A)^{1/2}$ (Table 5). This result indicated that root length, root volume, shoot fresh weight, shoot dry weight characters were influenced by additive genes. The root fresh weight and root/shoot ratio characters showed the opposite value. Ratio value of $_{DGU}/_{DGK}$ was smaller than the value of dominance ratio degree $(_D/_A)^{1/2}$. Root fresh weight and root/shoot ratio characters were influenced by non-additive genes (dominant genes).

Root fresh weight and root/shoot ratio character showed a low value of narrow sense heritability (h^2_{ns}) (Table 5). Low value of narrow sense heritability (h^2_{ns}), in line with $\sigma^2_{DGU}/_{DGK}$ value which is smaller than the value of $(_D/_A)^{1/2}$ indicating that the character is affected by the effect of the dominant gene (Istipliler *et al*., 2015). Broad sense heritability (h^2_{bs}) showed a high value in stem diameter, root length, shoot fresh weight, shoot dry weight. This result indicated that genetic effect was greater than environment effect. Heritability values can be classified in three classification. High if H value is> 50%, moderate 20% <H <50%, and low H <20% (Syukur *et al.*, 2012).

This result showed that characters which related to drought stress tolerance were strongly influenced by the additive gene rather than the dominant gene. Drought stress tolerance in plant character is influenced by many genes and interactions between genes and their environmental effect (Tardieu & Tuberosa, 2010; Luke *et al*., 2015). In corn, additive gene action is more prominent in drought stress conditions. Conversely, under normal conditions, the effects of additive and non-additive genes will affect controlling a character in plants (Derera *et al*., 2008).

The contribution proportion shows how much contribution of female parents, male parents, and their interactions for each character (Istipliler *et al*., 2015; Constantine, 2017). The results of the analysis found that all characters were strongly influenced by female parents except for stem diameter character. This result showed that female parents had high influences in almost all observed characters. The stem diameter character showed the highest proportion value derived from the interaction between female x male parents (65.59%). The contribution of female parents was higher in the character of root length (86.15%), root volume (64.15%), shoot fresh weight (90.09%) root fresh weight (46.87%), shoot dry weight (95.09%), root dry weight (71.25%), root/shoot ratio (82.32%) compared to the proportion of male parents (Table 6).

Mean values of 11 genotypes showed the best genotypes in each observed character. KW516 genotype was the best genotype for stem diameter character. The highest value for root length was showed in ICCRI 09, but this value did not significantly different with Sulawesi 3, TSH 858, Sulawesi 3 x ICCRI 09, and Sulawesi 3 x Scavina 6. ICCRI 09 also showed the highest value in root volume character and root dry weight character. The highest mean values of shoot fresh and dry weight characters were showed in TSH 858 x Scavina 6 with 6.43 g and 2.30 g, respectively. The highest values of root fresh weight and root/shoot ratio, were found in genotype TSH 858 and Sulawesi 3 (Table 7).

Selection of tolerant genotype to drought stress cannot only be seen through mean value. The mean value shown can only rank which genotypes are best for a particular character. Some studies on tolerance to stress indicate that determining a tolerant genotype requires a calculation such as stress susceptibility index (SSI) (Helmi, 2017; Setyawan *et al*., 2018).

The sensitivity index of drought stress is reflected through the value of SSI. SSI values

Table 5. Estimated genetic component value of the root characters in 25% AWC treatment

Genetic component	Character							
	SD	RL	RV	SFW	RFW	SDW	RDW	R
σ^2_{GCA}	0	0.164	0.04	0.185	0.019	0.022	0.001	0.0004
σ^2_{SCA}	0.00094	0.076	0.034	0.074	0.072	0.0094	0	0.0006
σ^2_{A}	0	0.33	0.071	0.37	0.037	0.044	0.0022	0.0008
σ^2_{D}	0.00093	0.08	0.034	0.073	0.07	0.009	0	0.0006
$\sigma^2_{GCA}/_{SCA}$	0	2.158	1.176	2.5	0.264	2.340	0	0.67
$(\sigma^2_{D}/\sigma^2_{A})^{1/2}$	0	0.492	0.692	0.44	1.375	0.452	0	1
h^2_{ns}	0	0.418	0.258	0.58	0.139	0.639	0.143	0.19
h^2_{bs}	0.823	0.519	0.382	0.69	0.401	0.769	0.143	0.33

Table 6. The contribution rates of female parents, male parents, and female x male parents interaction for hybrid generation (%) in 25% AWC treatment

Contribution rates	Character							
	SD	RL	RV	SFW	RFW, g	SDW, g	RDW, g	R
Female	34.05	86.15	64.15	90.09	46.87	95.09	71.25	82.32
Male	0.36	6.59	23.42	2.18	30.4	0.12	28.27	0.82
Female x Male	65.59	7.26	12.42	7.73	22.73	4.79	0.48	16.86

Note: SD = stem diameter (nm), RL = root length (cm), RV = root volume (mL), SFW = shoot fresh weight, RFW = root fresh weight, SDW = shoot dry weight, RDW = root dry weight, R = root/shoot ratio.

below 0.5 indicate tolerant of drought stress genotype, the SSI value between 0.5 and 1 indicate moderate to drought stress genotype, whereas if the SSI value is greater than 1 indicate susceptible to drought stress (Helmi, 2017; Setyawan *et al*., 2018). Genotypes classified as tolerant to drought stress were Sulawesi 3, TSH 858, and KW 516 x ICCRI 09 (Figure 1). In previous studies showed that TSH 858 was the susceptible clone to drought (Iryono, 2010; Kurniawan, 2017). Moderate genotypes were ICCRI 09, KW 516 x Scavina 6, Sulawesi 3 x ICCRI 09, and TSH 858 x ICCRI 09. Genotypes KW 516, Scavina 6, Sulawesi 3 x Scavina 6, and TSH 858 x Scavina 6 were susceptible to drought stress, this in contrast with many prior trials. Based on SSI values, drought tolerant parents were Sulawesi 3 and TSH 858, moderate parents were ICCRI 09, and susceptible parents were KW 516 and Scavina 6 (Figure 1).

Table 7. Mean values of observed characters on 11 genotypes in 25% AWC treatment

Genotypes	Character							
Parents	SD	RL	RV	SFW	RFW	SDW	RDW	R
KW 516	0.55a	17.48bc	3.73ab	4.73cd	2.90ab	2.25a	0.77b-d	0.34e-g
ICCRI 09	0.53b	18.60a	4.10a	5.93ab	2.77ab	1.93b	0.94a	0.48ab
Scavina 6	0.45f	16.90c-e	3.20ab	4.07e	2.60a-c	1.59de	0.75b-e	0.46a-c
Sulawesi 3	0.48de	18.49a	3.50ab	4.57de	2.67a-c	1.62de	0.82a-c	0.51a
TSH 858	0.51bc	17.73a-c	3.13b-d	5.27bc	3.03a	2.23a	0.64e	0.28g
F1								
KW 516 x ICCRI 09	0.50cd	16.58de	2.17e	4.13e	2.17cd	1.71cd	0.70c-e	0.41c-e
KW 516 x Scavina 6	0.46ef	16.91c-e	2.60de	4.83cd	1.27e	1.85bc	0.71c-e	0.37d-f
Sulawesi 3 x ICCRI 09	0.49d	18.41a	2.73cd	4.47de	2.40b-d	1.65de	0.72b-e	0.43b-d
Sulawesi 3 x Scavina 6	0.46ef	18.35ab	2.77cd	4.27de	2.00d	1.49e	0.69de	0.46a-c
TSH 858 x ICCRI 09	0.48de	16.21e	2.83cd	5.90ab	2.33b-d	2.22a	0.71c-e	0.32fg
TSH 858 x Scavina 6	0.53ab	17.24cd	3.60ab	6.43a	2.34b-d	2.30a	0.84ab	0.36d-f
Mean	0.49	17.54	3.12	4.96	2.41	1.89	0.75	0.40

Note: The numbers followed by the same letter in the same column are different based on the DMRT test at a 95% confidence level; SD = stem diameter (cm), RL = root length (cm), RV= root volume (ml), SFW = shoot fresh weight (g), RFW = root fresh weight (g), SDW = shoot dry weight (g), RDW = root dry weight (g), R = root/shoot ratio.

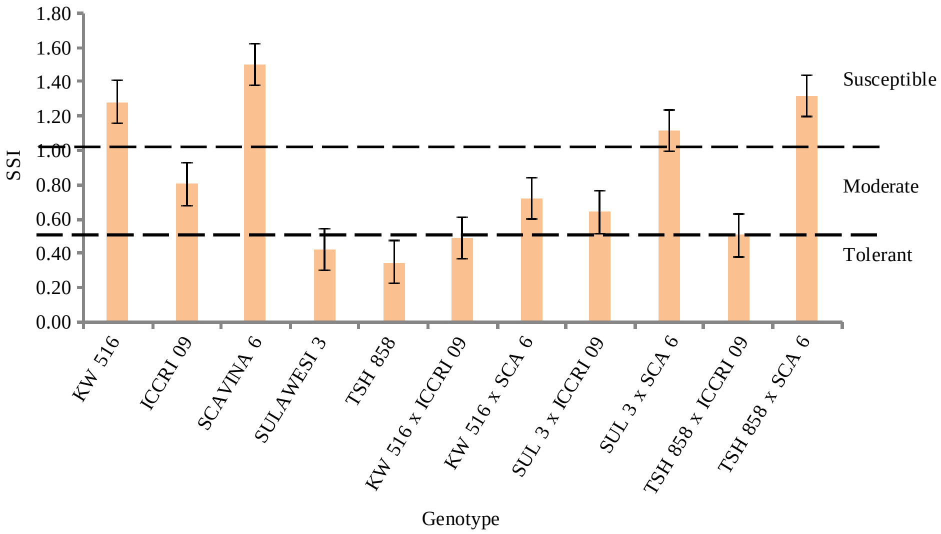

Figure 1. Drought susceptible index for 11 genotypes

CONCLUSIONS

Based on general combining ability (GCA) and specific combining ability (SCA) value from analysis NCII mating-design, we found that TSH 858 was the best female parent, Scavina 6 was the best male parent, and Sulawesi 3 x ICCRI 09 and TSH 858 x Scavina 6 were the best crossing genotypes in this research. Characters which associated with drought stress were mostly controlled by additive genes. In this research we found that dominant genes influenced root fresh weight and root/shoot ratio. Based on SSI value we found that Sulawesi 3 and TSH 858 were tolerant parent, ICCRI 09 was moderate parent, and susceptible parent were KW 516 and Scavina 6. Some of the findings are in contrast with previous studies where TSH 858 was susceptible clone while Sca 6 was tolerant clone to drought. Crossing genotype classified as tolerant to drought stress was KW 516 x ICCRI 09, moderate genotype were KW 516 x Scavina 6, Sulawesi 3 x ICCRI 09, TSH 858 x ICCRI 09, and susceptible genotypes were Sulawesi 3 x Scavina 6 and TSH 858 x Scavina 6.

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