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Physiological Adaptation of Soybean Genotypes Induced with Gamma Irradiation against Drought

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Abstract: ***Dry land is characterized particularly by the limited water availabiliy and decreasing land productivity and the selection of drought tolerant genotypes is one of the strategies to optimize the productivity of agricultural crops on dry land. The study aims to classify soybean genotypes tolerant and sensitive to drought stress, determine the tolerance limits of the soybean genotypes to drought stress (the percentage of water content of soil) in growth and production on the dry land, and determine the parameters of observations that can be used as indicators for the selection of soybean genotypes tolerant to drought stress. The results are expected to give a contribution in the development of soybean on dry land. The research was carried out as experimental study using a Split Plot Design (SPD) with soil moisture levels as main plot (MP) and soybean genotypes as sub plot (SP). The soil moisture levels were set based on the percentage of soil water content consisted of four levels e.g. 100% field capacity (k0), 80-100% field capacity (k1), 60-80% of field capacity (k2) and 40-60% of field capacity (k3). Soybean genotype mutants of the 4th generation used as the sub plot were Menyapa genotype, 50 Gy; Orba genotype, 25 Gy; Tanggamus genotype; Tanggamus genotype, 25 Gy; Tanggamus genotype, 50 Gy; Orba genotype, 50 Gy; Menyapa genotype; Orba genotype. A total of 32 treatment combinations were obtained and planted on acid dry land. The data was analyzed using statistical software (Excel). The result shows that six genotypes found to be tolerant namely: genotype Menyapa, 50 Gy; Orba genotype, 25 Gy; Tanggamus genotype; Tanggamus genotype, 25 Gy; Tanggamus genotype, 50 Gy; Orba genotype, 50 Gy and 2 genotypes moderate, namely Menyapa genotype and Orba genotype. The percentage of soil moisture content of 40-60% field capacity can be used as an indicator for selecting the soybean genotypes tolerant to drought, parameters of root length, root fresh weight and canopy, Al uptake in roots and canopy, proline content and the date of flowering can be used as indicators for selection of soybean genotypes to drought stress.***

Keywords: Drought, genotype, accumulation, proline

1. Introduction

Nutritional properties of soybean are unique compared to other types of bean crops characterized by its high protein and fat and lower carbohydrate content. In most other nuts, protein content ranging between 20-30%, while soybean. comprises about 35-38% protein. In addition, soybeans also contain some vitamins (Vitamin A, E, K and some B vitamins) and minerals (K, Fe, Zn and P), as well as low in the content of saturated fatty acids, with 60% of unsaturated fatty acid content consists of linoleic acid and linolenic, both of which are known to help heart health (eBookPangan.com. 2006). Therefore, soybean is a source of vegetable protein that is essential in order to improve public nutrition because it is cheap and good for health. This causes soybean demand continues to increase as the development of food processing industries that use soybean as a raw material for making tofu, tempeh, soy sauce, snacks and more. The type of industry is relatively small and medium scale industries, but the number is very likely to cause high levels of soybean consumption requirements (Agency for Agricultural Research and Development, 2012). On the other hand, domestic production can not meet the consumption needs.

To fulfil the needs of the domestic soybean consumption, the government continues to increase production mainly by utilizing marginal lands, such as dry-acid land, dry land. Mulyani et al., (2009) have identified the portion of acid dry land based on the data of land resource exploration scale of 1: 1,000,000, ie of the total about 148 million ha of the dry land, it can be classified into dry-acid land of 102.8 million ha and non acid dryland covering an area of 45.2 million ha (Widjaja-Adhi et al., 2000). However, extension of crops in these new openings area of often face ecological limiting factor.

The main characteristic stands out in the drylands is the limited water and decreasing land productivity. Dry land in Indonesia is dominated by red-yellow podzolic acid soil categorized in the ultisol. Low soil pH (4.6 to 5.5) and cation exchange capacity (CEC) are the constraints in this area causing it susceptible to erosion and poor biotic elements (Mulyani, 2006), as well as high levels of aluminum (Al) (Utama, 2008), therefore implies to the risk of toxicity in plants that can damage the roots of the plants hence the ability of water and nutrients absorption are blocked, element content of macro and micro nutrients like N, P, K, Ca, Mg, and Mo is low, which causes plant growth stunted and die (Ma et al ., 2001; Sutjahjo, 2006).

Jaleel et al. (2009) stated that the lack of water lowers the growth of plants which give effect to some of the physiological and biochemical processes, such as photosynthesis, respiration, translocation, ion absorption, carbohydrate, nutrient metabolism and hormone production. Plant growth in a stressed state responded by accelerating in entering the generative phase (Widiatmoko, 2012). Lack of water during the flowering phase causes the flowers to fall or failure in the process of pollination. Lack of water during the formation of pods and pod filling will reduce the weight of the pods and grains (Lopez, 2008).

One agronomically effective and economically efficient approach, is increased tolerance of high yielding varieties on dry land. This is an appropriate method because the results are permanent and the character is derived to the next generation. Mutations that lead to a positive character and are passed to the next generation is the aim desired by plant breeders in general (Soeranto, 2003).

Plant breeding by induced gamma ray irradiation of the seeds may cause changes in morphology, anatomy and genetics expected to be useful to obtain soybean genotypes that tolerant to dry land. Hanafi, et al., 2011 stated that the gamma-ray irradiation at a dose of 200 Gray effectively cause the genetic diversity in plants. Mulyana (2006) states that testing with mutant strains of M.220 have the advantage advantages such as: the production of soybeans, the average yield of 19.32 ql / ha with a potential yield of 32.07 ql / ha, can adapt well in 19 tolerant testing locations and can adapt both lowland dry land with average yield of 1.9 tonnes / ha and a production potential of 3.2 tons / ha.

This test is a fourth generation mutant (M4) test which is based on agronomic characters, and physiological adaptation of soybean against the percentage of soil moisture level. Based on the above it is necessary to study the adaptation of soybean genotypes induced gamma ray irradiation against drought.

2. Materials and Methods

The study was conducted from July to September 2015 on acid-dry land in the village Baku, district Tanralili, regency of Maros with a pH of 4.7 and Al-dd 1, 75 (cmol (+) kg^{-1}) / 100 g soil.

Research was set as experimental study using Split Plot Design (SPD), as follows: The main plots (MP) were the percentage of soil water content (K) ie field capacity (fc) (k0), 80-100% of field capacity (k1), 60-80% of field capacity (k2), 40-60% of field capacity (k3) .The sub plot (SP) were 8 are soybean genotypes of 4^{th} generation mutants (G) consisted of g1 (genotype Menyapa, 50 Gy); g2 (genotype Orba, 25 Gy); g3 (genotype Tanggamus); g4 (genotype Tanggamus, 25 Gy); g5 (genotype Tanggamus, 50 Gy); g6 (genotype Orba, 50 Gy); g7 (genotype Menyapa); g8 (genotype Orba) resulted in 32 treatment combinations planted on acid dry land. Data was analyzed by analysis of variance followed by Least Significance Difference (LSD) test at 5% or 1%. The analysis of variance performed follows Gomez and Gomez 2007.

Morpho-physiological characters of each M_4 plant was identified to determine the mutant tolerant to drought stress. Observation variable that observed in the experiment were root length (cm), canopy and root fresh weight (g), days to flowering (days), the content of Al in the canopy and roots (ppm) and the accumulation of proline (µg/g).

3. Results and Discussion

Root Length

The growth of soybean genotypes grown on acid dry land taken from Maros regency, sub-district Tanralili, Baku villages, indicating that g_6 provide the longest root length (37.50 cm) and not significantly different with g_3 and with g_1, g_2, g_4, g_7 and g_8 on the 40-60% fc of soil water content treatment (Table 1). This is consistent with research of Robert (2004) which stated that the growth of plant roots are inhibited of in the plant experiencing drought stress, this inhibition of the growth is due to the plant is unable to regulate its normal growth. According to Wu and Cosgrove (2000), intensive growth of root length is a determinant of the ability of plants to adapt to the conditions of drought stress.

Table 1: Average Root Length (cm) of Different Genotypes on Various Percentage of Soil Water Content

Genotypes (g)	Soil water Content Level (K)				LSD_g
	k_0	k_1	k_2	k_3	
g_1	$43{,}60^{ab}_{v}$	$34{,}30^{ab}_{w}$	$30{,}80^{b}_{w}$	$30{,}30^{bc}_{w}$	
g_2	$39{,}40^{bcd}_{v}$	$25{,}80^{c}_{w}$	$29{,}20^{b}_{w}$	$28{,}50^{bc}_{w}$	
g_3	$48{,}70^{a}_{v}$	$33{,}10^{ab}_{w}$	$28{,}50^{b}_{w}$	$33{,}60^{ab}_{w}$	
g_4	$36{,}50^{d}_{v}$	$25{,}40^{c}_{w}$	$32{,}50^{ab}_{vw}$	$30{,}20^{bc}_{vw}$	5,35
g_5	$42{,}20^{bc}_{v}$	$38{,}10^{a}_{vw}$	$33{,}80^{ab}_{wx}$	$28{,}60^{bc}_{x}$	
g_6	$41{,}20^{bc}_{v}$	$31{,}50^{b}_{w}$	$37{,}20^{a}_{vw}$	$37{,}50^{a}_{vw}$	
g_7	$38{,}40^{bcd}_{v}$	$25{,}30^{c}_{w}$	$31{,}60^{b}_{vw}$	$28{,}00^{c}_{w}$	
g_8	$37{,}00^{cd}_{v}$	$23{,}20^{c}_{w}$	$37{,}50^{a}_{v}$	$31{,}30^{bc}_{v}$	
LSD_k	7,34				

Note: numbers followed by the same letter in the column (vwxyz) and the row (abcd) are not significantly different at the level of 5%

Canopy and Root Fresh Weight

The mean of the heaviest roots fresh weight on the percentage of soil moisture content of 40-60% fc was 10.30 g and not significantly different from the treatment g_6, g_8 and significantly differed with g_1, g_2, g_4, g_5 and g_7 (Table 2). While Table 3 shows that on the 40-60% fc percentage of soil moisture content, genotype g_3 showed no significantly difference with g_4 and g_5, and highly significant differed with g_1, g_2, g_6, g_7, g_8. The decline in average of canopy fresh weight with increased drought stress on the percentage of soil water content from 80-100% fc to 40-60% fc. This is consistent with the opinion of Melo et al., 2014 which states that the increase in the number of cells contained in the cortex increases the tolerance of plants to drought stress. Drought tolerant genotypes have root dry weight were greater than the sensitive genotype either in drought stress conditions as well as optimum, so the roots of the character selection can be done only at the optimum environment (Effendi, 2009).

Rosawanti et al, 2015 stated that drought stress treatment with PEG simulation leads to changes in cortex thickness, stele and xylem diameters. Under conditions of drought stress, PG 57-1 and Wilis reduced the thickness of the cortex, stele diameter and the diameter of the xylem to reduce the influence of drought stress. In contrast, increased cortical thickness, xylem and stele diameter were observed in the genotype SC 39-1. Response of root anatomy when exposed to drought stress various between genotypes. The anatomical changes in roots of soybean plants were studied by Makbul et al., 2011. In conditions of drought stress the growth of the plant canopy is inhibited more than the growth of the root (Wu & Cosgrove 2000; Hamdy 2002). Adaptation of plants to sustain growth is more use of energy for root growth compared to canopy growth (Rengel, 2000; Utama et al., 2009). The average weight of the canopy/root $.plant^{-1}$ decreases with decreasing levels of groundwater correlated with grain $weight.plant^{-1}$ of the soybean genotypes (Widiati et al., 2014)

Table 2: Average of Root Fresh Weight (g) of Different Soybean Genotypes with Various Percentage of Soil Water Content

Genotypes (g)	Soil Water Content (K)				LSDg
	k_0	k_1	k_2	k_3	
g_1	$10,20^{abc}_{v}$	$9,40^{b}_{v}$	$9,40^{b}_{v}$	$9,40^{bc}_{v}$	
g_2	$11,60^{a}_{v}$	$11,00^{ab}_{vw}$	$10,00^{b}_{vw}$	$8,60^{c}_{w}$	
g_3	$10,60^{abc}_{v}$	$10,40^{ab}_{v}$	$11,00^{ab}_{v}$	$11,00^{ab}_{v}$	
g_4	$9,40^{c}_{v}$	$11,60^{ab}_{v}$	$10,60^{ab}_{v}$	$9,40^{bc}_{v}$	1,69
g_5	$11,60^{a}_{v}$	$10,60^{a}_{v}$	$10,60^{ab}_{v}$	$9,60^{bc}_{v}$	
g_6	$11,20^{ab}_{v}$	$9,40^{b}_{v}$	$11,80^{a}_{v}$	$10,40^{ab}_{v}$	
g_7	$11,40^{a}_{v}$	$10,80^{ab}_{v}$	$10,80^{ab}_{v}$	$6,80^{d}_{v}$	
g_8	$9,60^{b}_{v}$	$10,40^{ab}_{v}$	$10,00^{b}_{v}$	$12,00^{a}_{w}$	
LSD*k*	2,44				

Note: numbers followed by the same letter in the column (vwxyz) and the row (abcd) are not significantly different at the level of 5%

Table 3: Average of Canopy Fresh Weight (g) of Different Soybean Genotypes with Various Percentage of Soil Water Content

Genotype (g)	Soil Water Content (K)				LSDg
	k_0	k_1	k_2	k_3	
g_1	$30,00^{bc}_{v}$	$21,40^{c}_{vw}$	$21,00^{b}_{vw}$	$18,80^{b}_{w}$	
g_2	$42,60^{a}_{v}$	$22,00^{c}_{w}$	$21,80^{ab}_{w}$	$18,00^{b}_{w}$	
g_3	$29,60^{bc}_{v}$	$37,60^{a}_{v}$	$28,80^{a}_{v}$	$27,20^{a}_{v}$	
g_4	$28,20^{bc}_{v}$	$25,40^{bc}_{v}$	$23,40^{ab}_{v}$	$22,40^{ab}_{v}$	7,56
g_5	$24,40^{c}_{v}$	$28,20^{bc}_{v}$	$22,00^{ab}_{v}$	$20,60^{ab}_{v}$	
g_6	$26,00^{ab}_{vw}$	$32,40^{ab}_{v}$	$24,60^{ab}_{vw}$	$17,00^{b}_{w}$	
g_7	$35,20^{ab}_{v}$	$31,80^{ab}_{v}$	$26,80^{ab}_{v}$	$15,60^{b}_{w}$	
g_8	$26,20^{c}_{vw}$	$32,00^{ab}_{v}$	$19,80^{b}_{w}$	$19,60^{b}_{w}$	
LSDk	11,14				

Note: numbers followed by the same letter in the column (VWXYZ) and the row (abcd) are not significantly different at the level of 5%

Al Uptake in Root and Canopy

Increased in the average of roots and canopy Al uptake was observed with increased of drought stress from the percentage of soil water content of 80-100% fc to 40-60% fc which correlated with decreased grain weight of the soybean genotypes (Figures 1 and 2). This is in line with the opinion of Kasim, et al (2001) which stated that Al tolerant crops have the ability to suppress the bad influence of Al, by reducing the Al^{3+} ion uptake by the roots, and has a variety of ways to neutralize the toxic effects of Al absorbed into the tissue hence roots growth are not disturbed.

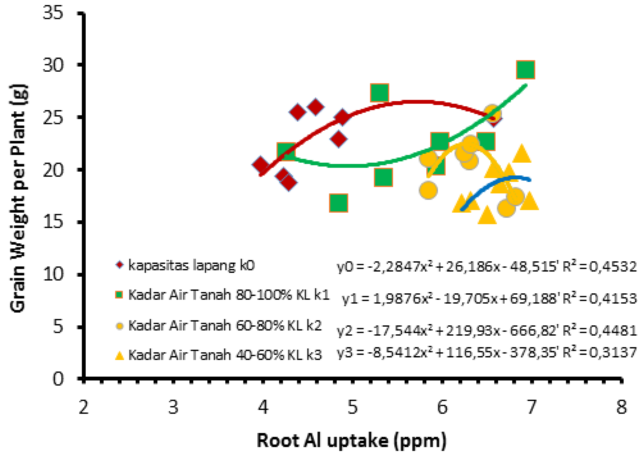

Figure 1: Relationship between Root Al Uptake on the percentage of soil water content of 40-60% fc (k_1), 60-80% fc(k_2), and 80-100% fc(k_3) and Grain weight per plant index of Soybean Genotypes

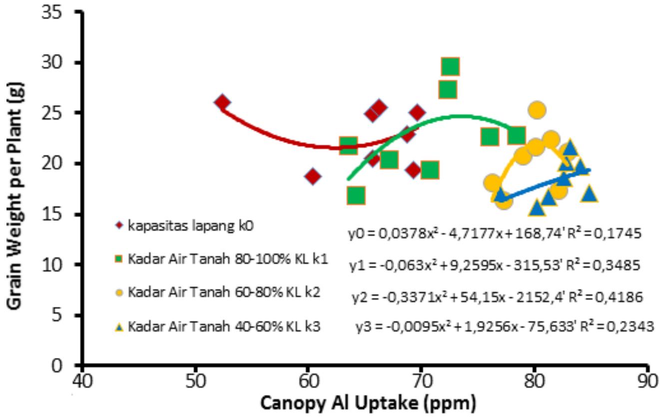

Figure 2: Relationship between Canopy Al Uptake on the percentage of soil water content of 40-60% fc (k_1), 60-80% fc(k_2), and 80-100% fc(k_3) and Grain weight per plant index of Soybean Genotypes

Leaf Relative Water Content

Table 4 shows that the genotype 1 shows the highest mean of leaf relative water content with a value of 60.91% and did not differ significantly with treatment g_2, g_3, g_4 and g_5 but different significantly from g_6, g_7 and g_8 on the percentage of soil moisture content of 40-60% fc. However, the percentage of soil water content treatment of 40-60% fc (k3) was not differed significantly from 60-80% fc (k2), and different significantly with 80-100% fc (k1) and field capacity (k0) (Table 4). This is consistent with Moaveni (2011) which stated that the relative water content of leaves is a measure of the status of water in plants as a physiological consequence of the soil water content, while the potential of water as a benchmark to estimate the status of water in plants serve for the continued transport of water from the soil. Makbul et al., (2011) reported that drought is the status of water in plants which can be determined by measuring the leaf water potential and relative water content as physiological indicators. Status of water on the leaves, is the interaction between leaf water potential and stomatal conductance, which will induce a drought signal from roots to the canopy to reduce the transpiration rate so that the stomata close when the water supply decreases.

Table 4: Average Relative Leaf Water Content (%) on the Different Soybean Genotypes with on Various Soil Water Content

Genotypes (g)	Soil Water Content (k)				LSD_g
	k_0	k_1	k_2	k_3	
g_1	$60,32^{b}_{vw}$	$67,99^{a}_{v}$	$51,45^{ab}_{w}$	$60,91^{a}_{vw}$	
g_2	$63,85^{b}_{v}$	$55,87^{b}_{vw}$	$48,65^{ab}_{w}$	$50,74^{ab}_{w}$	
g_3	$62,27^{b}_{v}$	$50,18^{bc}_{vw}$	$47,10^{ab}_{w}$	$52,64^{ab}_{vw}$	
g_4	$74,66^{a}_{v}$	$52,94^{bc}_{w}$	$45,58^{b}_{w}$	$53,51^{ab}_{v}$	10,43
g_5	$61,74^{b}_{v}$	$48,34^{bc}_{w}$	$44,91^{b}_{w}$	$43,88^{b}_{w}$	
g_6	$61,91^{b}_{v}$	$45,05^{c}_{w}$	$51,50^{ab}_{vw}$	$49,07^{b}_{w}$	
g_7	$65,67^{ab}_{v}$	$51,08^{bc}_{w}$	$56,07^{a}_{vw}$	$45,74^{b}_{w}$	
g_8	$64,85^{ab}_{v}$	$52,86^{bc}_{vw}$	$53,81^{ab}_{vw}$	$44,40^{b}_{w}$	
LSD_k	12,80				

Note: numbers followed by the same letter in the column (VWXYZ) and the row (abcd) are not significantly different at the level of 5%.

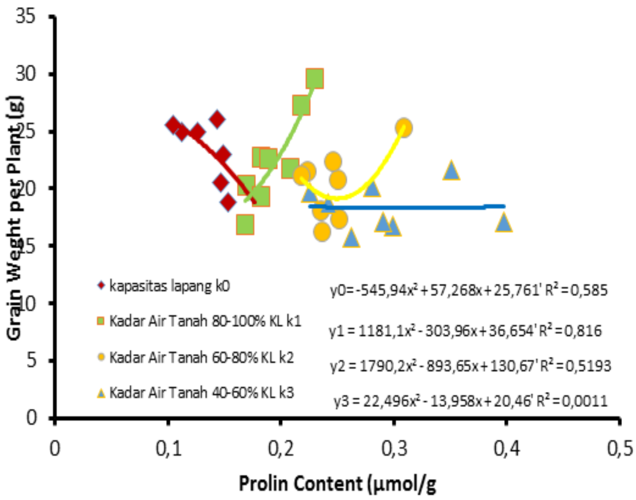

Figure 3: Relationship between Prolin Content on the Percentage of Soil Water Content of 40-60% fc (k1), 60-80% fc (k2), and 80-100% fc (k3) and the Grain Weight per Plant index of the Soybean Genotypes

Prolin Content

Increase in the average of proline accumulation was observed in this study in line with increased in drought stress when percentage of soil moisture content decline from 80-100% fc to 40-60% fc which correlated with decreased in Soybean genotypes grain weight. At the 40-60% fc of soil moisture content, the tolerant genotypes accumulated higher proline that correlated positively to the grain weight of the soybean genotypes (Figure 3). This is in accordance with Riadiz et al., (2008), which stated that the most important physiological response of plant adaptation to drought stress is to maintain turgor, which the mechanism can occur via decline in osmotic potential on the leaves and accumulation of solutes such as sugars, amino acids, organic acids, proline and glycine betaine. Mohammadkhan and Heidari (2008) showed increased proline accumulation on the primary roots of maize in the condition of drought stress.

Flowering Age

Table 5 shows that earlier flowering in soybean genotypes was observed along with increased in drought stress from soil moisture content percentage of 80-100% fc to 40-60% fc. Genotype 8 (genotype Orba) showed earlier flowering than g_2 (genotype Orba, 25 Gy) and g_6 (genotype Orba, 50 Gy). It is in line with Blum (2011) which stated that the most constitutive properties that shows resistance to drought, operationally primarily through avoidance in dehydration and effective use of water (EUW). Some examples are the depth of the root, leaf area of plants determined by the size of leaves or tillers, earlier flowering, leaf surface properties and morphology of the reproductive system that fertility is affected under conditions of stress. Decreased levels of soil water content from 50% to 25% fc significantly accelerate the age of plant flowering. Even faster than the flowering dates listed in the description of varieties (Soverda, et al., 2007).

Table 5: Average of Flowering Age (days) in Different Soybean Genotypes on Various Soil Water Content

Genotypes (g)	Soil Water Content (k)				Average	LSD_g
	k0	k1	k2	k3		
g1	39,67	39,67	35,00	35,00	$37,33^{ab}$	2,48
g2	42,00	42,00	35,00	35,00	$38,50^{bc}$	
g3	40,83	40,83	37,33	35,00	$38,50^{bc}$	
g4	42,00	37,33	35,00	35,00	$37,33^{ab}$	
g5	38,50	38,50	37,33	35,00	$37,33^{ab}$	
g6	42,00	39,67	42,00	38,50	$40,54^{c}$	
g7	40,83	37,33	35,00	35,00	$37,04^{ab}$	
g8	35,00	35,00	35,00	35,00	$35,00^{a}$	
Average	$40,10^{v}$	$38,79^{vw}$	$36,46^{wx}$	$35,44^{x}$		
LSD_k	2,38					

Note: numbers followed by the same letter in the column (VWXYZ) and the row (abcd) are not significantly different at the level of 5%

Number of pods and grains

Number of pods and grains per plant of soybean genotypes decreased with decreased in percentage of the soil water content from 80-100% fc to 40-60% fc (Table 6). This is consistent with the results of Widiatmoko et al., 2012, which stated that the availability of adequate water until the plant entered a phase of pod formation allegedly reduce pods loss. Drought prior to the time of flowering decreases total pods number per plant of the genotypes tested. The pods produced were only for the amount of 25 pods (Strain L / S: B6-G1). Blum (2011) stated that the mostly constitutive traits that

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cause the effect of resistance to drought, operationally through avoidance of the dehydration and water use efficiency (EUW). An example is the depth of the root, leaf area of plants determined by the size of leaves or tillers, earlier flowering, leaf surface properties and morphology of the reproductive system that fertility is affected by the stress conditions. Stomatal closure of the leaves will be hampering the exchange of CO_2 and O_2 from plant tissue with the atmosphere. It will also inhibit the nutrient absorption. This resulted in soybean plants slowing down its metabolism as a mechanism of plants to avoid drought stress and prepare for further growth when the water is available (Liu et al., 2003). According to Jaleel et al., (2009) water shortages reduce growth in some plants with affecting the physiological and biochemical processes, such as photosynthesis, respiration, translocation, ion absorption, carbohydrate, nutrient metabolism and hormone production.

Table 6: Average of Number of pods and Grains per Plant (pod.plant^{-1}) of Different Soybean Genotypes with Taraf Soil Water Content

Soil Water Content (k)	Average Number of Pods	Average Grain Number	LSD NP	LSD GN
k0	101,79^{v}	8,42^{v}	14,03	25,99
k1	100,50^{v}	83,59^{vw}		
k2	81,63^{w}	92,43^{wx}		
k3	73,21^{w}	78,53^{x}		

4. Conclusion

6 genotypes found to be tolerant were g_1 (genotype Menyapa, 50 Gy), g_2 (genotype Orba, 25 Gy); g_3 (genotype Tanggamus); g_4 (genotype Tanggamus, 25 Gy); g_5 (genotype Tanggamus, 50 Gy); g_7 (genotype Orba, 50 Gy) and 2 genotypes were found to be moderate, namely: g_7 (genotype Menyapa), g_8 (genotype Orba).

The percentage of soil water content of 40-60% field capacity can be used as an indicator for selection of soybean genotypes tolerant to drought stress.

Parameters of root length, fresh weight of canopy and root, canopy and root Al uptake, proline content and flowering age can be used as indicators for selection of soybean genotypes tolerant to drought.

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