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Drought tolerance selection of soybean lines generated from somatic embryogenesis using osmotic stress simulation of poly-ethylene glycol (PEG)

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Abstract. *Sunaryo W, Widoretno W, Nurhasanah, Sudarsono. 2016. Drought tolerance selection of soybean lines generated from somatic embryogenesis using osmotic stress simulation of poly-ethylene glycol (PEG). Nusantara Bioscience 8: 45-54.* Somaclonal variation is an alternative source to create genetic variability including generating a novel character like drought tolerance. The objective of this research was to select the drought tolerant lines derived from somatic embryogenesis (somaclones) of 4 soybean genotypes using drought stress simulated by Polyethylene Glycol (PEG). Soybean seeds (R_1 generation) generated from 37 somaclones (R_0 generation) were grown on semi hydroponic system using Greenleaf as supporting medium. The seedlings were watered with half strength of liquid Murishage and Skoog nutrient. The PEG solution treatment of either 0% or 15% (PEG: w/v = -0.41 Mpa osmotic potential) was applied in the MS solution, from the seedlings of 14 days old until harvesting period. The vegetative growth, intensity of leaf firing, plant biomass, and drought sensitivity index variables were observed during the PEG application. The same procedure was applied to the seeds, propagated conventionally, from the same genotypes as controls. The results showed that somatic embryogenesis had altered the drought sensitivity of soybean genotypes under drought stress simulated by PEG. Interestingly, the different drought tolerance level was showed by the R_1 plants. Some lines were increased and the other lines were decreased compared to the control genotypes. From a total of 185 R_1 plants, 4 plants increased their tolerance against drought stress and grouped as tolerant genotype.

Keywords: Drought, Drought Sensitivity Index, PEG, somatic embryogenesis, somaclonal variation

INTRODUCTION

Drought stress is one of the main obstacles in soybean production in many countries including Indonesia. Drought stress caused the decrease 50% of the total yield of soybean production (Frederick et al. 2001). The significant decrease of about 24-50 % was reported from the greenhouse and field studies at different locations and times (Sadeghipour and Abbasi 2012). In Indonesia, particularly in the areas that have limited water supply from both natural and/or technical drought stress led to a noticeable yield loss in soybean production. These areas are widespread throughout Sumatra, Java, Sulawesi and Kalimantan islands. In addition, the climate change that has altered the rainfall and other climate factors causes the deficiency and reduction of ground water and water supply, which significantly influence the soybean production. Among various biotic and abiotic stresses exposing its growth, water supply shortage is the biggest cause of the decline in the soybean production (Frederick and Hesketth 1993). Therefore, the development of soybean varieties tolerant to drought stress is one of the most important breeding programs (Tuinstra et al. 1998).

Various attempts have been conducted to create drought tolerant lines such as using conventional breeding programs by crossing the commercial soybean variety with an exotic soybean germ plasm to increase genetic diversity. However, these efforts often run into problems due to the limitation of drought tolerant soybean germ plasm and the unavailability of effective selection methods for drought tolerance. Most selection methods for drought tolerance are performed in the field/soil with or without irrigation (Sakardiva and Yadav 1994), and/or used the pots to simulate drought conditions (Laohasiriwong 1986; Harnowo 1992; Hamim 1995; Cellier et al. 1998). Using these methods, the homogeneity of drought stress pressure can not be controlled, and the stress level is difficult to measure. Therefore the possibility to get the false result is very high.

The selection method for drought tolerance character with a better level of homogeneity can be performed by using an osmotic solution of poly-ethylene glycol (PEG) to simulate drought stress condition. PEG has an ability to control the water potential reduction homogeneously linear to soil water potential (Michel and Kauffmann 1973). The use of PEG solution with various concentrations of 5, 10, 15 and 20% for soybean drought tolerance selection at vegetative growth has been studied by Sunaryo et al. (2005). The PEG concentrations of 5, 10 or 15 % can effectively differentiate the drought tolerance of soybean genotypes similarly with the field experiment result. On the other hand, the PEG concentration at 20 % caused early

plant death because of dramatic leaf firing led to the failure of leaf photosynthesis. PEG was also effective as a selective agent of soybean drought tolerance at germination stage showed by the ability of PEG to classify genotypes based on drought tolerance/sensitivity (Widoretno et al. 2002).

Somaclonal variation, genetic variation resulted from tissue or cell culture, is an alternative source of new genetic diversity that is very useful for plant breeding programs including generating a novel character like drought tolerance. The initial work of somaclonal variation was reported by Larkin and Scowcroft (1981). After that, many researchers reported the successful efforts to screen and select new useful characters from somaclonal variationin several crops (Kumar 1985; Koornef 1991; Griga et al. 1995; Ignachimuthu et al. 1997; Kuksova et al. 1997). The breeding programs using somaclonal variation have been extensively reported to create new varieties resistant to herbicide and plant diseases, resistant to extremely abiotic stresses, and to create new varieties with improved plant quality and yield (Ignachimuthu et al. 1997).

The benefit of somaclonal variation for soybean improvement program has been reported in several studies. Gray et al. (1986) obtained resistant callus against stem brown spot disease. Song et al. (1994) was successful to generate plants resistant to leaf brown spot disease. Wrather and Freytag (1991) generated 10 soybean plants resistant to herbicide *Atrazine*. Plants resistant to kanamycin and hygromycin antibiotics were also successfully generated by Hinchee et al. (1988) and Finer and McMullen (1991), respectively. Recently, the field experiment to evaluate the invitro selected lines of soybean using PEG had been performed (Widoretno et al. 2012) and 10 variants had the potential to be developed as drought resistant genotypes.

In this paper, the study of drought tolerance selection of somaclonal regenerants at first generation (R_1) using PEG selection method was reported.

MATERIALS AND METHODS

Plant materials

Three elite soybean genotypes, B 3731, MLG 2999, MSC 8606 and one superior variety, Tidar, were used in this study. From previous study B 3731 and MLG 2999 were known as drought tolerant genotypes, on the other hand, MSC 8606 was classified as a sensitive genotype (Soepandie et al. 1996). As many as 185 R_1 generation seeds of the four genotypes were used for drought tolerance selection, consisted of 44, 50, 63 and 28 seeds of B 3731, MLG 2999, Tidar and MSC 8606, respectively. Those R_1 generation seeds were the selfed seeds produced from somatic embryogenesis plants (Somaclones/R_0 generation) of 11 somaclones B 3731, 11 somaclones MLG 2999, 9 somaclones Tidar and 6 somaclones MSC 8606. As a control, seeds derived from conventionally propagation (selfed seeds) of the four soybean genotypes were used.

The somaclones (R_0 generation) development and R_1 generation seeds production

The somaclones were generated by secondary somatic embryogenesis. Immature cotyledons were used as explant and inoculated insolid MS (Murishage and Skoog 1962) medium modified with B5 vitamin and amino acid addition and supplemented with 40 mg/l 2, 4 Dichlorophenox acetic acid (2, 4 D) to initiate primary somatic embryos. The primary somatic embryos were subsequently transferred to secondary somatic embryo initiation medium composed of the modified MS solid medium containing 10 mg/l 2, 4 D and 10 mg/l NaphtalenaAcetic Acid (NAA) 30 g/l sucrose. To initiate germination, the secondary somatic embryos were transferred to MS solid medium containing 30 g/l sucrose and 2 g/l charcoal. The survive seedlings indicated by green color, root axis and cotyledon (not a horn shapecotyledon) emergence were transferred to elongation and growth medium composed of ½ MS solid medium containing 2 mg/l GA3, 4 mg/l BAP, B5 vitamins and 20 g/l sucrose. To trigger the shoot and root emergence the seedlings were retransferred to the solid MS medium containing charcoal. The seedlings having shoot and root were transferred to acclimatization medium consisted of coconut and rice husk for three days in invitro culture chamber and one week in green house. After around ten daysin the acclimatization stage, the plants were grown in soil in green house until the R_1 generation seeds were harvested.

Experimental condition and PEG application

Experiments were carried out in semi-hydroponic growing media containing the mixture of coconut and rice husk with a ratio of 3: 1. Seeds were grown in wrapped plastic pots (30 cm height and 10 cm diameter), to prevent sunlight exposing the root. Each pot was sown with 2 seeds and after one week the plants were selected and left for one plant per pot. The pots were placed in green house with full sunlight.

The plants were watered using liquid ½ MS nutrient (Murishage and Skoog 1962) solution every 2 days (50 ml per pot). The solutions were combined with PEG solution treatments and the volumes were increased to 100 ml after the plants have three trifoliar leaves (± 14 days after planting). The treatments were liquid ½ MS nutrient solution without PEG as control, and the same liquid containing 15% PEG 6000 (equivalent to -0.41 MPa) for drought tolerance selection. The application of PEG was terminated after the plants were 28 days old. Plants were controlled from pests and diseases, and maintained until the end of observation (36 days after sowing).

Data collection and analysis

Plant variables such as plant height, number of nodes, number of trifoliar leaves, and the intensity of leaf firing were observed at 18, 24, 30, and 36 days after sowing. At the 36 days after sowing, the entire plants were harvested.The measurement of root length, root dry weight, shoot dry weight, total plant weight (biomass), and the ratio of root dry weight/shoot dry weight were carried out. The intensity of leaf firing was calculated based on leaf

symptoms (Figure 1). The formula used to determine the intensity of leaf firing was adapted from the formula proposed by Natawigena (1985):

$$P = \frac{\sum(nxV)}{ZxN} x100\%$$

Where:
P = Intensity of leaf firing (%)
n = Number of trifoliar leaves for each category of symptoms.
V = Category score of symptom (0, 1, 2, 3, 4, 5) (Figure 1)
N = Total number of trifoliar leaves observed in a plant.
Z = The highest score of symptoms (5)

To determine the sensitivity of a genotype against drought stress the formula proposed by Fischer and Maurer (1978) was used:

$$\mathbf{S} = \frac{(1 - Yp/Y)}{(1 - Xp/X)}$$

Note:
S = Drought sensitivity index
Yp = Mean value of a genotype grown under stress.
Y = Mean value of a genotype grown in normal condition (control).
Xp = Mean value of all genotypes were grown under stress.
X = Mean value of all genotypes in normal condition (control).

The genotype response was grouped based on the value of drought sensitivity index which categorized as a tolerant if $S \leq 0.5$, Mildly tolerant if $0.5 < S \leq 1.0$ and sensitive if $S > 1.0$.

RESULTS AND DISCUSSION

General growth response

Several negative effects of drought stress were observed on the vegetative growth of soybean from both somatic embryogenesis (R_1 generation) and from conventional propagation (Figure 2-6). The vegetative growth of plants derived from tissue culture (R_1 generation plants) was not better than the conventional propagation (control) plants against drought stress in genotype B 3731, observed from plant height, number of leaves and number of nodes, although the intensity of leaf firing in the control plant was

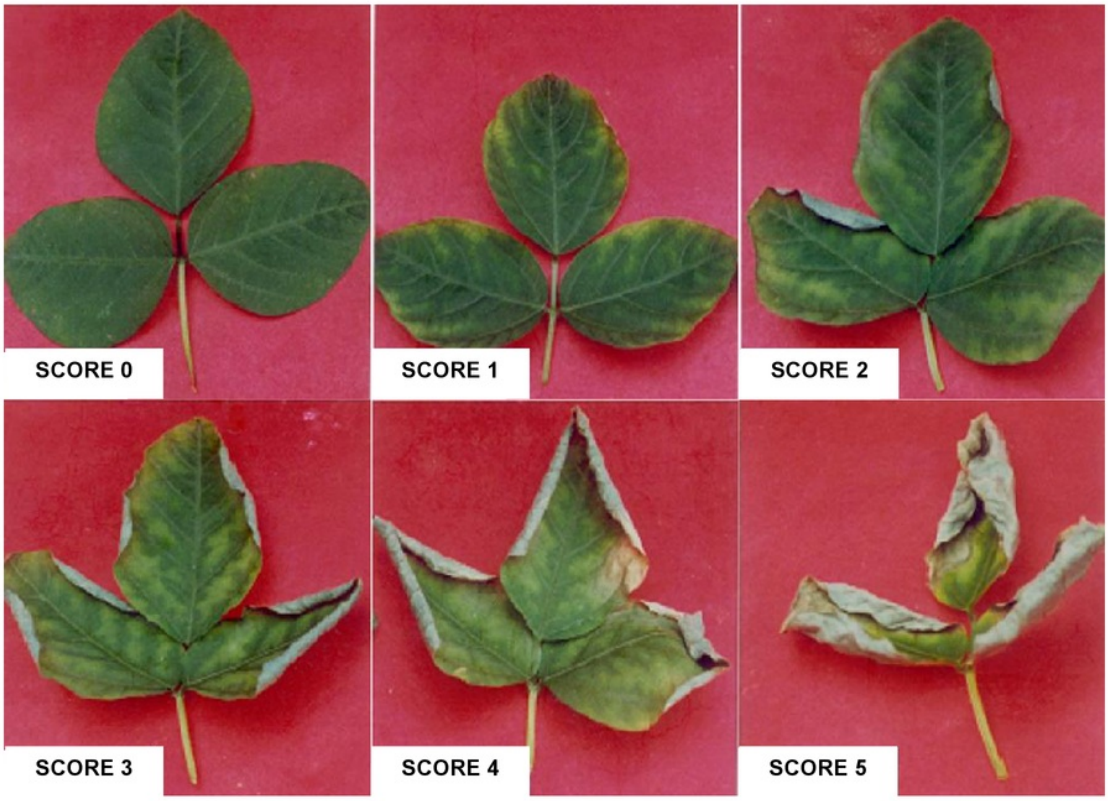

Figure 1. Drought tolerance scoring based on intensity of leaf firing (Sunaryo et al. 2005)

higher (±80%) than in the R_1 generation plants (±70%) (Figure 2). In addition, the reduction of the root length, root dry weight, shoot dry weight, plant biomass and root/shoot dry weight ratio was more drastic in the R_1 generation plants than those of the control plants (Figure 6). A different result was showed by MLG 2999 genotype to respond the drought stress. The plants derived from tissue culture (R_1 generation plants) grew faster and more tolerant against drought stress than control plants as showed by all variables observed (Figure 3 and 6). On the other hand, response of the R_1 generation plants against drought stress were almost the same with the control plant in 'Tidar' genotype. It was observed from plant height, number of leaves and number of nodes, and the leaf firing intensity of the R_1 generation plants was higher (100%) than that of the control plant (±90%) (Figure 4). The same results could be observed from the root length, root dry weight, shoot dry weight, total dry weight and root/shoot dry weight ratio characters, in which the plants derived from tissue culture showed significant reduction compared to the control plants (Figure 6). This indicates that in genotype 'Tidar', the plants derived from tissue culture (R_1 generation) was more sensitive against drought stress than those of conventionally propagated plants (control). In MSC 8606 genotype, the vegetative growth of the R_1 generation plants was a bit better than the control plants, noticed from the plant height, number of leaves, and number of nodes, although the leaf firing intensity of those plants were the same (100%) (Figure 5). The root dry weight, shoot dry weight, and the total dry weight (plant biomass) of the R_1 generation plants were more decreased than the control plants (Figure 6), showing that plants derived from tissue culture tend to be more sensitive against drought stress rather than those of from conventional propagation.

Drought sensitivity index of the tissue culture vs conventionally generated plants

Drought sensitivity index value (S) (Fischer and Maurer et al. 1978) was used to determine and group the drought sensitivity of the plants. Based on the mean of S value from different plant variables, genotypes B3731 and MLG 2999 (conventionally propagated/control plants) were grouped in the Mildly tolerant genotypes which had 0.86 and 0.90 S value, respectively (Table 1). On the other hand, Tidar and MSC 8606 were grouped to the sensitive genotypes with the S value of 1.12 and 1.01, respectively. The plants generated from somatic embryogenesis (R_1 generation) showed a different response. The B 3731 plants generated from somatic embryogenesis (SC. B3731) were grouped as sensitive genotype. The drought sensitivity of SC. MLG 2999 and SC. MSC 8606 were not altered, the same from the conventionally propagated plants. Interestingly, the drought sensitivity of SC. Tidar was increased to a Mildly tolerant (S value = 0.99) (Table 1).

Drought sensitivity of the R_1 generation plants based on the somaclones genotype (R_0 generation)

Since the drought sensitivity index as showed in Table 1 was calculated from the mean value of the R_1 generation plants, the S value of the R_1 generation was calculated based on its R_0 genotype or the somaclones source, to represent the drought sensitivity index value individually (Tables 2 and 3). These were to investigate whether the genetic variations in tissue culture could be genetically inherited to the progeny. The only S values of the Intensity of Leaf Firing (ILF) and Plant Biomass (PB) variables were used in this research since the indication of drought sensitivity index showed by these characters was similar to the mean of S values presented in the Table 1. In addition, the intensity of leaf firing and plant biomass variables were suggested to show the strongest indication for the drought sensitivity index calculation (Sunaryo et al. 2005).

The mean of S value among the R_1 generation genotypes was varied, showing a different drought sensitivity of the R_1 plants derived from different somaclones (R_0 genotypes) (Table 2). From 11 somaclones of B 3731 genotype, 8 somaclones were grouped into Mildly tolerant and 3 somaclones represented a sensitive genotype. In MLG 2999, 5 somaclones were Mildly tolerant and 6 somaclones were grouped as sensitive genotypes. There were 5 from a total of 9 somaclones of Tidar genotype showed a strong indication as Mildly tolerant genotype. All somaclones derived from MSC 8606 genotype were classified into sensitive genotypes.

Drought sensitivity of individual R_1 generation plants

The observation of S value of individual R_1 plants showed an interesting phenomenon of somaclonal variation in soybean (*data not shown*). Tolerant genotypes that were not found in the control plants, were appeared in the individual R_1 generation plants. One R_1 generation plant from somaclone A.5.8.- line (SC. B 3711 genotype) and three from SC. MLG 2999 genotype, each from somaclone D.2.2.-, D.4.4.-, and D.4.8.- were grouped as tolerant genotypes (Table 3), whereas none of the somaclones/R_0 generation genotype showed by the average S value of R_1 generation plants (Table 2) exhibited a tolerant genotype. Sixteen individual R_1 generation plants were Mildly tolerant genotypes against drought stress in "Tidar", although their somaclones/R_0 generation genotypes (B.4.1.1., B.4.2.-, B.4.3.-, B.5.5.1) showed by the average S

Table 1. The S (Drought Sensitive Index) value based on vegetative growth variables of R_1 generation plants (SC) compared to control plants (C) against drought stress simulated by PEG

Genotype	PH	NN	LN	ILF	RDW	SDW	PB	Mean Value
B 3731 (C)	0.81	0.80	0.79	0.77	0.95	0.93	0.94	0.86
SC. B 3731	0.94	1.06	1.03	0.85	1.12	0.97	1.00	1.00
MLG 2999 (C)	1.11	0.73	1.00	0.52	0.88	1.06	0.98	0.90
SC. MLG 2999	0.83	0.91	0.88	0.98	1.07	0.94	0.95	0.94
Tidar (C)	1.08	1.12	1.12	1.46	0.95	1.05	1.04	1.12
SC. Tidar	1.16	1.03	1.03	0.97	0.80	0.97	0.96	0.99
MSC 8606 (C)	1.11	1.00	1.00	1.03	0.86	1.04	1.02	1.01
SC. MSC 8606	0.82	1.12	1.12	1.41	0.85	1.12	1.09	1.08

Note: PH (Plant Height), NN (Number of Nodes), LN (Leaf Number), ILF (Intensity of Leaf Firing), RDW (Root Dry Weight), SDW (Shoot Dry Weight), and PB (Plant Biomass).

Table 2. The mean of S (Drought Sensitive Index) value based on Intensity of Leaf Firing (ILF) and Plant Biomass (PB) variables of the R_1genotypes grouped by the somaclonessource (R_0 generation)

Genotype	ILF	PB	Mean value	Phenotype
B 3731 (C)	0.77	0.94	0.86	Mildly tolerant
SC. B 3731				
A.5.3.1	0.93	1.11	1.02	Sensitive
A.5.7.-	0.40	1.21	0.81	Mildly tolerant
A.5.8.-	0.74	0.85	0.80	Mildly tolerant
A.5.9.-	0.91	0.97	0.94	Mildly tolerant
A.5.11.-	0.67	0.96	0.82	Mildly tolerant
A.6.15.-	0.86	0.97	0.92	Mildly tolerant
A.6.20.3	0.96	0.87	0.92	Mildly tolerant
A.6.29.-	1.21	1.06	1.14	Sensitive
A.6.31.1	1.08	1.00	1.04	Sensitive
A.6.32.-	1.02	0.94	0.98	Mildly tolerant
A.6.34.-	0.57	1.05	0.81	Mildly tolerant
MLG 2999 (C)	0.52	0.98	0.75	Mildly tolerant
SC. MLG 2999				
D.2.2.1	0.63	1.10	0.87	Mildly tolerant
D.2.2.2	0.59	0.75	0.67	Mildly tolerant
D.2.2.3	0.33	0.71	0.52	Mildly tolerant
D.4.4.-	0.25	0.95	0.60	Mildly tolerant
D.4.6.-	1.30	1.06	1.18	Sensitive
D.4.7.-	0.67	1.12	0.90	Mildly tolerant
D.4.8.-	1.73	0.78	1.26	Sensitive
D.4.9.-	1.67	0.97	1.32	Sensitive
D.4.13.1	1.12	1.00	1.06	Sensitive
D.4.14.-	0.88	1.13	1.01	Sensitive
D.4.18.-	1.06	0.94	1.00	Sensitive
Tidar (C)	1,46	0,98	1,22	Sensitive
SC. Tidar				
B.4.1.1	0.58	0.99	0.79	Mildly tolerant
B.4.1.2	0.70	0.99	0.85	Mildly tolerant
B.4.1.1	1.06	0.96	1.01	Sensitive
B.4.2.-	1.62	0.99	1.31	Sensitive
B.4.3.-	1.00	0.99	1.00	Sensitive
B.5.5.1	1.34	0.97	1.16	Sensitive
B.5.5.2	0.86	0.97	0.92	Mildly tolerant
B.5.6.-	0.85	0.83	0.84	Mildly tolerant
B.5.7.-	0.76	0.97	0.87	Mildly tolerant
MSC 8606 (C)	1.03	1.02	1.03	Sensitive
SC. MSC 8606				
C.4.1.-	1.78	1.10	1.44	Sensitive
C.4.2.-	2.01	1.12	1.57	Sensitive
C.4.4.-	1.75	1.12	1.44	Sensitive
C.5.5.-	0.96	1.11	1.04	Sensitive
C.5.6.-	0.85	1.16	1.01	Sensitive
C.5.7.-	1.37	0.92	1.15	Sensitive

Note: ILF (Intensity of Leaf Firing) and PB (Plant Biomass)

Table 3. Drought Sensitivity of individual R_1 plants based on the Intensity of Leaf Firing (ILF) and Plant Biomass (PB) variables.

Genotype	Number of plants	Drought sensitivity of individual R1 plants		
		Tolerant	Mildly tolerant	Sensitive
SC. B 3731				
A.5.3.1	4	0	0	4
A.5.7.-	2	0	0	2
A.5.8.-	3	1	1	1
A.5.9.-	4	0	2	2
A.5.11.-	4	0	3	1
A.6.15.-	4	0	2	2
A.6.20.3	4	0	2	2
A.6.29.-	4	0	2	2
A.6.31.1	4	0	2	2
A.6.32.-	4	0	2	2
A.6.34.-	7	0	2	5
Subtotal	44	1	18	25
SC. MLG 2999				
D.2.2.1	4	0	1	3
D.2.2.2	4	0	3	1
D.2.2.3	4	1	3	0
D.4.4.-	4	1	1	2
D.4.6.-	6	0	1	5
D.4.7.-	4	0	0	4
D.4.8.-	6	1	4	1
D.4.9.-	4	0	2	2
D.4.13.1	4	0	2	2
D.4.14.-	4	0	0	4
D.4.18.-	6	0	5	1
Subtotal	50	3	22	25
SC. Tidar				
B.4.1.1	6	0	3	3
B.4.1.2	8	0	6	2
B.4.1.1	8	0	4	4
B.4.2.-	6	0	3	3
B.4.3.-	8	0	4	4
B.5.5.1	8	0	5	3
B.5.5.2	8	0	5	3
B.5.6.-	8	0	7	1
B.5.7.-	3	0	2	1
Subtotal	63	0	39	24
SC. MSC 8606				
C.4.1.-	3	0	1	2
C.4.2.-	4	0	0	4
C.4.4.-	6	0	0	6
C.5.5.-	7	0	1	6
C.5.6.-	4	0	1	3
C.5.7.-	4	0	2	2
Subtotal	28	0	5	23
Total	185	4	84	97

value of R_1 generation plants were sensitive genotypes (Table 2-3). Other interesting data were showed by individual R_1 generation plants derived from MSC 8606 genotype. The control plants as well as somaclone genotype plants were grouped as sensitive genotype. Nevertheless, around 17 % (5 from a total of 28) of the R_1 individual plants were Mildly tolerant genotypes.

Discussion

Drought stress simulated by PEG (15%) had a negative effect on the vegetative growth of all soybean genotypes tested. The vegetative growth of genotypes derived from both somatic embryogenesis (R_1 generation) and fromconventional propagation were inhibited. Drought stress caused stunted phenotype, reduced the number of

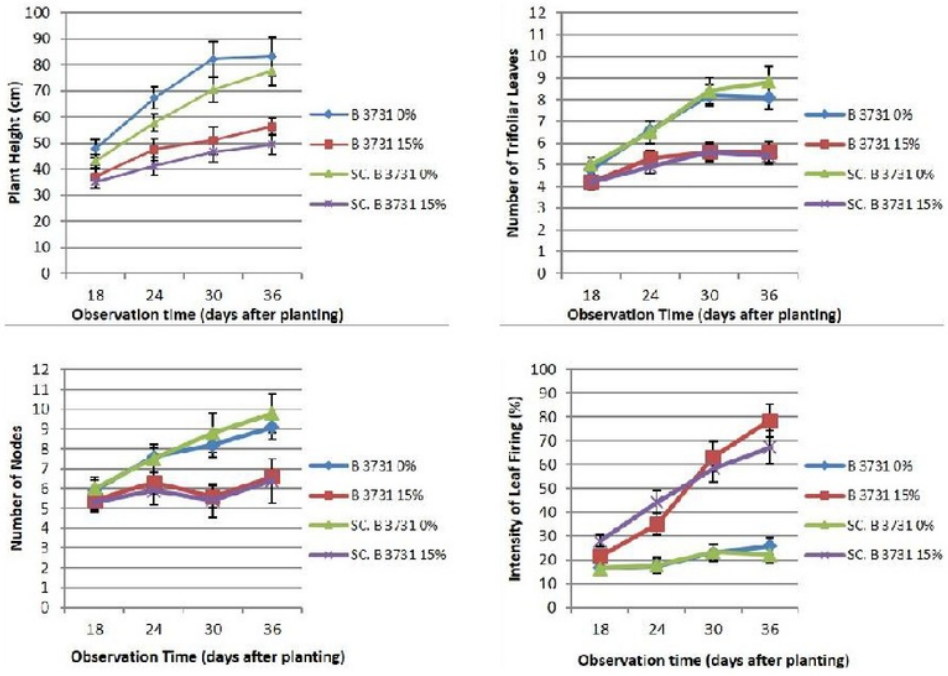

Figure 2. Vegetative growth response of soybean genotype"B 3731" derived from somatic embryogenesis and conventional propagation under drought stress simulated by PEG at different time of observation

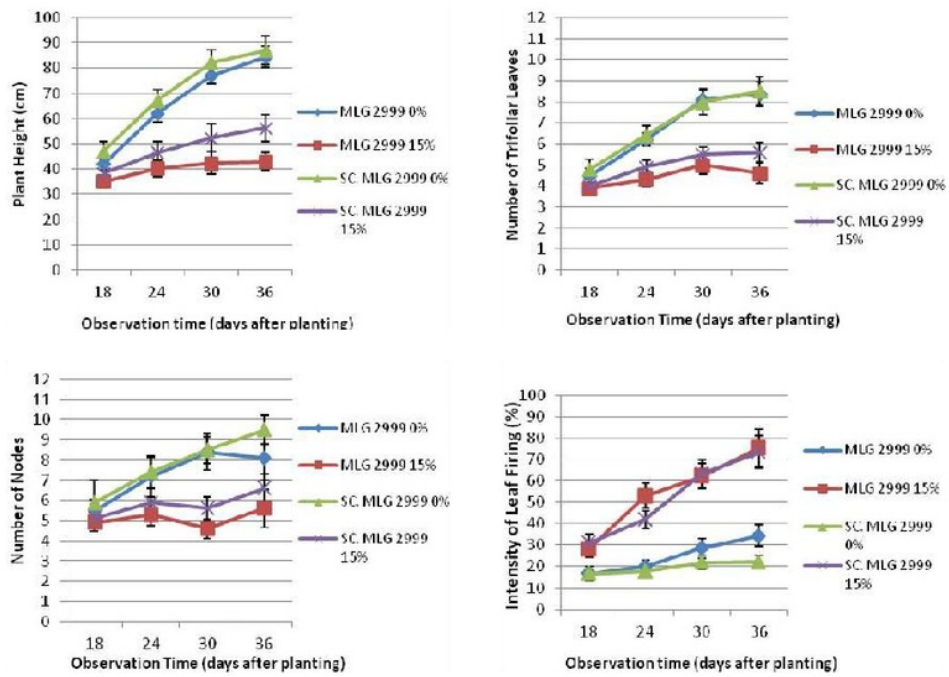

Figure 3. Vegetative growth response of soybean genotype "MLG 2999" derived from somatic embryogenesis and conventional propagation under drought stress simulated by PEG at different time of observation.

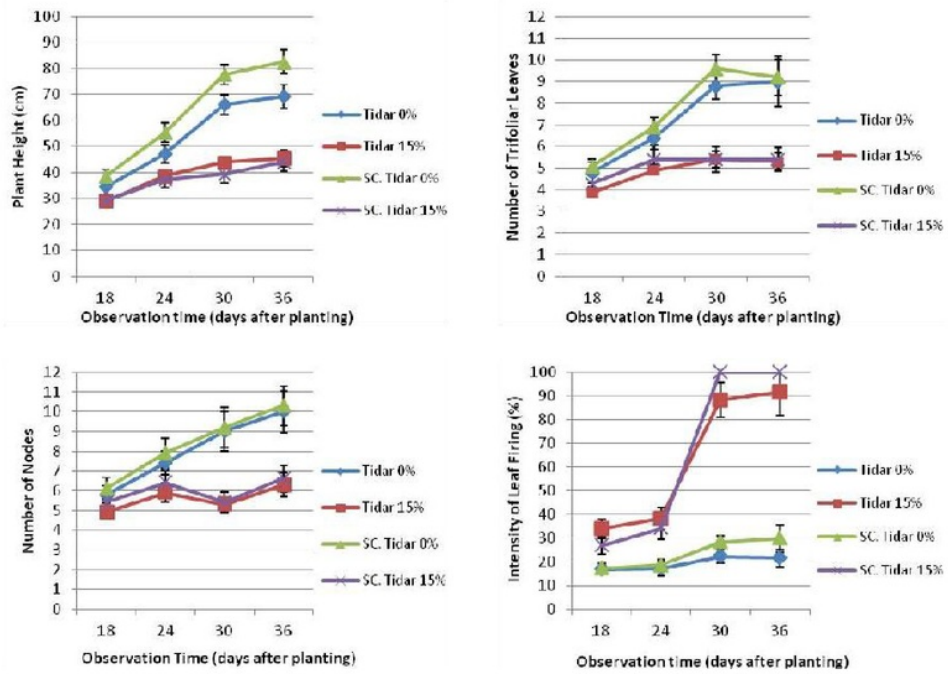

Figure 4. Vegetative growth response of soybean genotype "Tidar" derived from somatic embryogenesis and conventional propagation under drought stress simulated by PEG at different time of observation.

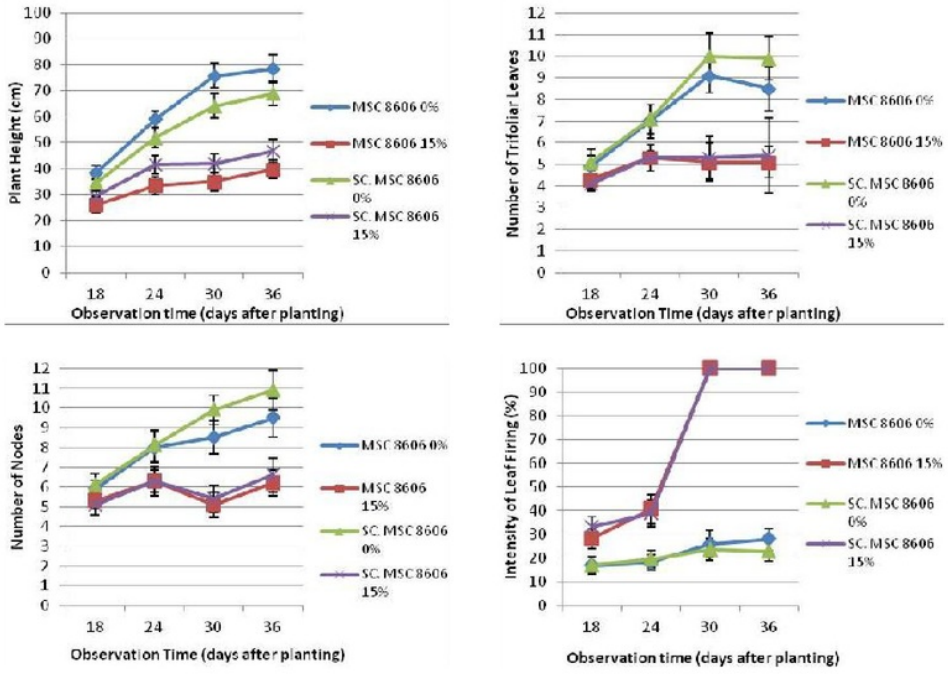

Figure 5. Vegetative growth response of soybean genotype "MSC 8606" derived from somatic embryogenesis and conventional propagation under drought stress simulated by PEG at different time of observation.

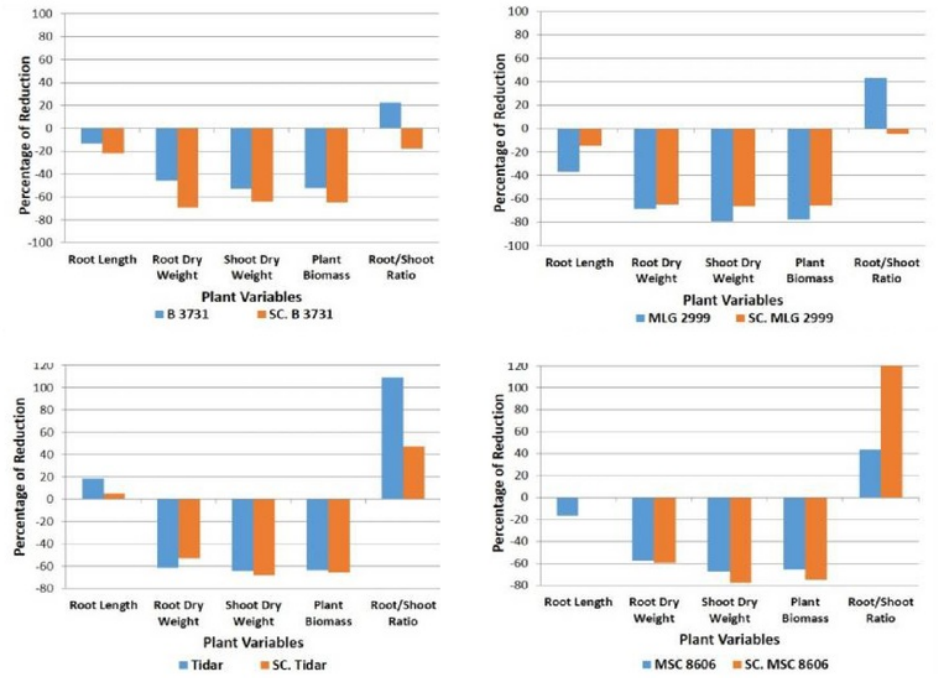

Figure 6. Different responses of genotypes derived from somatic embryogenesis (somaclones) compared to selfing seeds against drought stress simulated by PEG indicated by percentage of reduction root length, root dry weight, shoot dry weight, plant dry weight and root/shoot ratio.

trifoliar leaves and nodes, and increased the intensity of leaf firing (Figure 2, 3, 4 and 5). The drought stress condition also reduced root length, root dry weight, shoot dry weight, plant biomass, but it increased the ratio root/shoot dry weight (Figure 6). Plant exposed by drought stress shows an increase of its root/shoot dry weight as a response to reduce water loss and maintain water uptake through an extensive root system (Turner et al. 2001).

The same indication was also found in the previous studies showing that drought stress simulated by PEG inhibited the vegetative growth (Widoretno et al. 2002; Widoretno et al. 2003a; Sunaryo et al. 2005). However, in this study PEG application at 15 % caused decreased root length, whereas in the previous studies the opposite effect was observed. Negative effects caused by drought stress indicate that drought stress simulated by PEG has the same effect from the real drought stress in field/soil condition. Several studies conducted in the field were reported to have the same effect on soybean growth and yields (Harnowo 1992; Hamim 1995). Jones (1992) reported that drought stress exposed at the vegetative growth phase resulted in the inhibited plant growth, decreased cell division and elongation. Therefore plants were stunted and increased their root growth as well as root/shoot ratio. The increased root biomass, length, density and depth was a morphological plant characater as a drought avoidance mechanism (Subbarao et al. 1995). Plants also improve their root structure such as deep and thick root system to extract water from considerable depths (Kavar et al. 2007). The different responses of plants due to the drought stress simulated by PEG were observed in tobacco, grass, rice, chili, and (Krishnasany and Irulappan 1993; Mullahey et al. 1996; Perez-Molphe-Balch et al. 1996; Komori et al. 2000). The negative effect of PEG is tightly associated with its ability to dissolve in and bind water molecule resulting in the decrease of water potential and amount required for plant growth (Steuer et al. 1981). Water has an important function as basic material of photosynthesis reaction resulting carbohydrates needed for plant cell division and elongation (Salisbury and Ross 1985). According to drought sensitivity index, to determine the sensitivity of a genotype against drought stress, response of the plants derived from tissue culture against drought stress might be different from the conventionally propagated plants. B 3731was grouped into moderate tolerance genotype, but its embryo somatic derived plants were sensitive. In contrary to that, Tidar which was included into sensitive genotype, its embryo somatic derived plants were grouped into moderate tolerance genotype against drought stress (Table 1). These findings indicate that tissue culture via somatic embryogenesis could create genetic alteration especially in the response against drought stress. The alteration might be an increase or decrease of drought sensitivity.

In addition, the drought sensitivity among the R_1 generation genotypes which were grouped based on their original somaclones or R_0 generation genotypes varied (Table 2), meaning that the R_1 plants derived from different somaclones have different genetic variability. Each somaclone has different genetic change potencies resulted in somaclonal variation. Somaclonal variation has been reported affected by various factors such as genotype, culture condition and environment, plant growth regulator, explant sources, callus period, and length of culture in vitro culture duration (Evans et al. 1986; Koornef 1991; Ignacimuthu 1997). Genotype plays an important role in inducing genetic variation during tissue culture, as it influences the frequency plant regeneration and somaclonal variation.

Somaclonal variation in soybean was reported for various qualitative and quantitative traits (Ranch et al. 1985; Barwale and Widholm 1987 and 1990; Hildebrand et al. 1989; Stephens et al. 1991; Shoemaker et al. 1991). The tissue culture might alter the genetic constitution of plants via chromosome doubling and alteration, gene mutation and cytoplasm changes (Kumar 1985). The other possible mechanism of genetic change is the chromosome mutation, transposon element activation, DNA methylation, gene amplification and deletion and chromosome cross over during mitosis (Koornef 1991).

In this experiment, the genetic variation among individual R_1 generation plants that were not appear in the R_0 generation genotype were found (Table 3). The genetic variation occurring during tissue culture process would exist in the R_0regenerant plants and might be inherited in the next generation (R_1 generation). The genetic changes in R_1 generation seeds are a result of segregation process during meiosis and assorted independently in the self-fertilization and could potentially undergo phenotypic changes. The inherited traits could be selected for the specific characters useful for plant breeding program like drought tolerance. Each R_1 generation plant will have different genetic potential (Koornef 1991). If the genetic change is a dominant trait, it will immediately appear in the regenerants. Conversely, if the altered gene is recessive then the changes will appear in the next generation.

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