Seed Priming with PEG 8000 for Improving Drought Stress Tolerance of Soybean (*Glycine max*)

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Abstract: The experiment was carried out to evaluate the effects of seed priming with osmoticum Polyethylene glycol (PEG) 8000 in improving tolerance of soybean to drought stress. Green house factorial experiment in a completely randomized design with three replications was conducted. Treatments consisted of five levels of seed priming (dry seeds – untreated, PEG concentration: 0, 100, 200, 300 g L^{-1} water, respectively) and three levels of drought stress treatments (100 % field capacity, 75% field capacity and 50% field capacity). Results showed that seedling growing from primed seeds differed significantly with respect to plant height increment, shoot/root ratio, chlorophyll content and protein content. However, seed priming with PEG had no effect on relative growth rate (RGR), number of stomata, 100 grains weight and grain yield. Among the various concentration of PEG used, priming with 300 g PEG L^{-1} water significantly increased chlorophyll content and protein content. Drought stress treatment applied significantly affected plant height, shoot-root ratio, chlorophyll and protein content. Seed priming and drought stress treatments proved to be significant with respect to shoot-root ratio, 100 grains weight, protein and chlorophyll content. Seeds treated with 300 g PEG L^{-1} water demonstrated to be superior to the non-primed and all other primed seeds when water stress increased (50% field capacity). The results indicate that seed priming with PEG can improve plant growth in soybean by conferring more resistant seedlings to drought stress.

Keywords: Seed priming; drought tolerance; soybean

1. Introduction

Soybean is one of the most important oil seed crops, of Indonesia. There are about 133.7 mil ha of upland spread across the main islands outside of Java, Sumatra, Kalimantan, Sulawesi and Papua where soybean is cultivated. In addition, soybean is planted in rotation with rice in the dryland/ upland. However, there are many limiting factors in growing soybean uplands. The prominent factor is the limited of available water for plant growth. Water deficit is reported to inhibit soybean growth and yield (Ahmadvand *et al*., 2012).

Several methods have been used to precondition the seeds to improve germination and seedling establisment in many vegetables and field crops including seed priming. Seed priming is a pre-germination treatment in which seeds are held at a certain level of water potential that allows imbibitions, but prevents radical extension. Priming the seeds has been successfully demonstrated to improve germination and emergence in seed of many crops. Priming also allows for controlling the rate of seed water imbibition so that seed could become resistant to stress and apart from stimulating growth (Ghassemi *et al.,* 2011).

It enhances seed and seedling vigor leading to better stand establishment and yield, so that plants can grow in unfavorable environmental conditions (Khalil *et al.,* 2010). Improvement through priming is affected by factors such as plant species, water potential during priming, priming duration, temperature, and storage condition of priming seeds.

Seed priming treatments can be performed with osmoconditioning or matri-conditioning. Osmoconditioning can be done by priming seeds in a osmoticum solution. NaCl, KNO_3 and KH_2PO_4 and high-molecular-weight compounds such as mannitol and Polyethylene Glycol (PEG) are commonly used osmoticums. Of these osmoticums, PEG is commonly used for osmopriming. Osmoticum concentration can regulate the amount and speed of water such radicle emergence can be delayed. This condition allows the activation phase last longer and reduces the half-life (T50) by 40 per cent. This means that 40% of the initial phase of growth can be protected from environmental stress (Widoretno *et al.,* 2002).

Seed priming by soaking seeds in PEG 300 g L^{-1} water for 6 h was found to increase Actual Growth Rate (AGR), Crop Growth Rate (CGR) and grain yield and Relative Growth Rate (RGR) was higher at 18 h seed priming duration in soybean (Arif *et al*., 2010). Advantages of seed priming in plants such as wheat, sweet corn, beans, barley, cucumber has been reported by Ghassemi *et al*. (2011) while the use of PEG 6000 for seed pretreatment in soybean was reported by Sadeghi *et al.* (2011). It was found that soybean primed for 12 h with PEG equal to - 1.2 MPa generally showed better condition than non primed treatment.

Considering the advantage of PEG for seed priming, research was carried out to investigate its effects on growth and grain yield of soybean under various drought stress level in the field and to find the optimum dosage of PEG.

2. Materials and Methods

2.1. Methods

The experiment was conducted from July 2012 to November 2012 at Glasshouse facility of Agricultural Science of Hasanuddin University, Makassar, Indonesia. The experiment was arranged factorial in completely randomized design with three replications. Treatments included five levels of seed priming: dry seed (non primed), PEG 0 g L^{-1}, 100 g L^{-1}, PEG 200 g L^{-1} and PEG 300 g L^{-1} water and three levels of drought stress treatments (100% field capacity, 75% field capacity and 50% field capacity). Data were analyzed using ANOVA and means compared using DMRT. Seed of soybean (cv. Anjosmoro) were obtained from Central Research Institute for Food Crops – South Sulawesi, Indonesia.

2.2. Seed Priming

Seeds were primed for 6 h in solutions containing polyethylene glycol (PEG 8000) in the concentration of 0, 100, 200, 300 g L^{-1} water, respectively. Dry seed (non primed) were used as control treatment. The treated seeds were washed with running tap water after priming and dried back to its original moisture content (12 % seed moisture) at room temperature. Priming was done 12 hours before planting.

2.3. Green House Experiment

An aggregate sample of top soil from 30 cm depth was analyzed for soil moisture content at field capacity and permanent wilting point using three replicates and six pots per treatment per replicate. Moisture content of air dried soil was measured by gravimetric method to determine the quantity of water needed to attain 100 %, 75 % and 50 % field capacity. There were 18 pots for each treatment. Five seeds were planted at a depth of 3 cm in pots of 24 cm diameter and watered daily up to fourteen days from sowing. Plants were thinned on the fourteenth day so as to maintain one plant per pot. Drought stress was imposed from fourteenth days after sowing and stopped at R7 growth stage (beginning maturity). Each pot was irrigated with water of desired treatment.

2.4. Parameters

Plant height increment between 14th to 50th days after sowing was recorded. Shoot and root dry weight (g) were measured after plant harvested. Shoot/root ratio was calculated from the determined shoot and root dry weight (g). Determination of chlrophyll content (mg g^{-1}) was carried out in fully expanded of the 2nd leaves from the top, according to the method of Arnon (1945) that modified by Yoshida dan Parao (1976). Number of stomata (mm^2) were calculated from lower surface leaves. At maturity, plants were harvested and grains weigtht (g) per plant were calculated and than converted to grain yield ha^{-1}(kg), 100 grains weight (g) and protein content (%) according to Kjedhal Method were recorded.

RGR (mg g^{-1} of plant dry weight day^{-1}) was calculated from the determined dry matter using the following formula (Gardner *et al.,*1985):

$$RGR = \frac{\ln W_2 - \ln W_1}{T_2 - T_1}$$

W_1 = Initial weight

W_2 = Final weight

T_1 and T_2 are the time intervals (14^{th} and 50^{th} after sowing)

3. Results and Discussion

Analysis of variance (Table 1) indicated that plant height, shoot/root ratio, chlorophyll content and protein content were significantly affected by seed priming. However, priming the seeds did not significantly affect, RGR, number of stomata, 100 grains weight and grain yield. Imposition of drought stress significantly affected plant height, RGR, number of stomata, chlorophyll content, protein content and grain yield. The interaction between priming and drought stress proved significant with respect to shoot root ratio, chlorophyll content, protein content and 100 grain weight. Ahmadvan *et al*. (2012), showed that priming seed with PEG can increase germination capacity, root length, length of plumula, seedling dry weight and plant height than the dry seed.

Among the concentration of osmoticum (PEG) used for priming (Table 2), seed priming with PEG 300g L^{-1} water proved to be superior over non-primed and other primed seeds with respect to chlorophyll and protein content. Seed priming with PEG 300g L^{-1} water proved to be on par with priming at PEG 200g L^{-1} water and control (dry seed) with respect to plant height. Low shoot root ratio in seedlings from PEG 300g L^{-1} water priming indicated better root growth. Sadeghi *et al*. (2011) reported that seed priming with PEG increased growth rate of sorghum and melon under drought stress, saline stress, and low temperature stress. The research conducted by Arif *et al*. (2010) proved that RGR of seedlings from primed seeds (PEG 300 g L^{-1} water) were higher than those from untreated seed unlike the findings of the present study.

According to Arif *et al*., (2010), rapid plant growth on priming in early stages from primed seeds as evident in the study may be triggered by enhancement of pre-germination metabolism after the preplanting reimbibition on seed that readies the seeds for radicle emergence immediately and the direct effects of PEG on seed is fast and high uniformity of seedling emergence, vigorous plant, more tolerant to drought stress, early flowering and harvesting and high yield.

Significant effect of priming on protein content as evident in the study is in accordance with the findings of Bray (1995). According to the previous study, priming of leek seed increased protein biosynthesis. After the priming was completed, protein content in high vigor seed was higher than low vigor seed. When the primed seed was reimbibed, an increase in total protein content was observed, but there was not significant difference between the high and the low vigor seeds. This pointed out that protein biosynthesis might not occur in great amount during priming, it was sugested RNA synthesis occured during priming

Absence of significant effect of seed priming on grain yield in the present study may be due to short period of priming, varietal response, priming conditions and osmoticum concentration. Mubshar *et al*. (2008) reported that improvement through priming is affected by factors such as plant species, water potential of priming factor,

Table 1. Analysis of variance of seed priming and irrigation treatments on field performance of soybean

Source of Variance	df	Mean Square							
		Plant height	RGR	Shoot/root ratio	Chlorophylls contents	Number of stomata	100 grain weight	Grain yield	Protein content
Replication	2	2.022 ns	1.882 ns	575129.888 **	0.149 ns	2.022 ns	1.882 ns	575129.888 **	0.149 ns
Priming (P)	4	135.422 ns	3.814 ns	103168.671 ns	21.110 **	135.422 ns	3.814 ns	103168.671 ns	21.110 **
Irrigation (I)	2	251.622 *	1.187 ns	373674.996 *	12.350 **	251.622 *	1.187 ns	373674.996 *	12.350 **
P x S	8	31.789 ns	12.387 **	33629.047 ns	15.669 **	31.789 ns	12.387 **	33629.047 ns	15.669 **
Error	16	46.747	2.498	87.089.927	0.686	46.747	2.498	87.089.927	0.686

ns, * and ** : no significant and significant at the 0.05 and 0.01 , respectively

Table 2. Response of soybean seedlings to different concentrations of PEG (8000) priming solution

Treatments (Priming)	Plant height increment (cm)	RGR	Shoot/root Ratio	Chlorophyll content (mg g^{-1})	number of stomata	100 grain weight (g)	grain yield (kg ha^{-1})	protein content (%)
dry seed	34.79 x	0.137	6.480 x	0.324 y	26.33	12.39	1170.69	30.62 z
0 g PEG L^{-1} water	23.61 y	0.159	5.258 xy	0.300 y	26.33	12.78	991.65	31.16 yz
100 g PEG L^{-1} water	25.17 xy	0.142	6.008 x	0.327 y	22.33	12.48	1054.15	31.16 yz
200 g PEG L^{-1} water	33.44 x	0.143	4.074 y	0.335 y	24.67	14.00	1226.60	31.90 y
300 g PEG L^{-1} water	28.86 xy	0.159	4.233 y	0.533 x	32.78	13.15	1230.42	33.95 x

Different letters in each columm indicate significant difference at $p \leq 0.05$

Table 3. The effects of seed priming at different levels of irrigation on field performance of soybean

Treatments (Priming)	Irrigation (% F C)	Plant height Increment (cm)	RGR	Shoot/root ratio	Chlorophyll content (mg g^{-1})	Number of stomata	100 grain weight (g)	grain yield (kg ha-1)	protein content (%)
dry seed	100	34.17	0.134	7.240 a	0.314 b	25.00	12.7 3 bcd	1321,50	31.19 de
	75	31.70	0.141	5.440 abc	0.338 b	29.67	13.99 abcd	1101,04	29.32 ef
	50	38.50	0.135	6.760 ab	0.320 b	24.33	10.46 e	1089,54	31.35 de
0 g PEG L-1 water	100	20.67	0.162	5.833 ab	0.300 b	31.00	12.89 bcd	1036,21	32.68 cd
	75	25.67	0.178	5.430 abc	0.310 b	28.00	14.21 abc	1034,83	32.14 cd
	50	24.50	0.138	4.510 bc	0.291 b	20.00	11.25 de	903,92	28.67 f
100 g PEG L^{-1} water	100	17.50	0.136	6.053 ab	0.332 b	21.33	11.13 de	1167,33	32.05 d
	75	37.33	0.169	4.580 bc	0.312 b	28.33	14.88 abc	1110,67	35.00 ab
	50	20.67	0.123	7.390 a	0.336 b	17.33	11.44 de	884,46	34.49 bc
200 g PEG L^{-1} water	100	40.83	0.163	2.387 c	0.337 b	30.00	14.39 abc	1563,29	30.10 ef
	75	28.67	0.138	4.870 bc	0.323 b	25.00	12.33 cd	1116,67	30.39 e
	50	30.83	0.128	4.967 abc	0.344 b	19.00	15.27 ab	999,83	35.22 ab
300 g PEG L^{-1} water	100	33.00	0.161	5.257 abc	0.309 b	31.00	13.00 bcd	1448,25	30.66 e
	75	29.57	0.161	3.057 c	0.326 b	38.33	11.02 de	1132,00	35.23 ab
	50	24.00	0.154	4.387 bc	0.963 a	29.00	15.45 a	1111,00	35.95 a

Different letters in each columm indicate significant difference at $p \leq 0.05$

priming duration, temperature, vigor and seed storage condition. Rouhi *et al*. (2011) reported that high germination index were observed when seeds of corn were primed with PEG equal to 1.2 Mpa osmotic about 12 h.

Interaction between seed priming with PEG 300 g L^{-1} and drought stress (irrigation - 50% field capacity) is enumerated in Table 3. The results showed that seed priming with PEG 300g L^{-1} water produced plants more tolerant to drought stress (50% field capacity). Priming seeds with PEG 300g L^{-1} and providing irrigation upto 50 % field capacity produced plants with higher chlorophyll content, higher 100 grains weight and higher protein content. The interactive effect of seed priming at PEG 300g L^{-1} water and higher drought stress had resulted in low shoot root ratio. This points to the presence of better root growth in seedlings which may prove useful to the crop in combating moisture stress.

Rouhi *et al*. (2011) reported that there were many physiological mechanisms that can cause seed priming to improve plant resistance to stress conditions. The physiological mechanisms such us repairing dry seed cells damage especially during re-imbibition before planting, delay of second phase imbibition on germination is a good avoidance mechanism to stress.

Higher tolerance and performance of primed seeds may be due to slow membrane disintegration and effectiveness in mobilization of compounds. It seems that during storage membrane disintegration occur in dry seed. Seed priming with PEG can preserve plasma membrane better than unprimed seeds. Priming also proves effective in mobilization of compound such as proteins, amino acids and soluble sugar from storage organs to embryonic tissue during stress. Priming was reported to stimulate growth by modifying the enzymes of sucrose metabolism (Kaur *et al*., 2005).

Many researchers report that a part of, or even the entire process related to germination can be accelerated by priming treatment. Seed when primed before planting, are re-imbibed enabling, the treated seeds to absorb water rapidly immediately stimulating the germination metabolism. This is brought about by biochemical reaction required for seed germination viz., increased activation germination enzymes. Seed priming is also reported to increase antioxidant production (glutathione and ascorbate) that encourage germination by decreasing the peroxidation lipid activity (Sadeghi *et al.,* 2011).

4. Conclusion

Considering the above, it can be inferred that seed priming with PEG can improve plant growth in soybean and also impart tolerance to drought stress. Seed priming with PEG 300 g L^{-1} water before planting proved most efficient in increasing the adaptability of plants to severe drought (50% field capacity).

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