A Simple Method of Seedling Screening for Drought Tolerance in Soybean

Young-Jin Kim*†, S. Shanmugasundaram**, Song-Joong Yun***,
Ho-Ki Park* and Moon-Soo Park*

*National Honam Agricultural Experiment Station, RDA, Iksan 570-080, Korea
**Asian Vegetable Research and Development Center, Shanhua, Tainan 74199, Taiwan, ROC
***Faculty of Biological Resources Science, and Institue of Agricultural Science and Technology, Chonbuk National University, Chonju 561-756, Korea

ABSTRACT : Water deficit is a serious constraint to soybean [*Glycine max* L. (Merr.)] production in rainfed regions of Asia, Africa, and America. This study was conducted to develop a simple and effective screening method for drought tolerance in soybean. Fifteen soybean cultivars, eight identified to be drought-tolerant and seven drought-sensitive in previous studies, were used for the evaluation of drought tolerance under the new screening conditions. The seedling screening method was consisted of a treatment in a PEG solution and drought treatment in parafilm-layered pots. 5-day-old seedlings were treated in a 18% PEG solution for 4 days and their wilting and hypocotyl browning were recorded. Three seedlings grown in a parafilm-layered pot containing peat moss were drought-stressed by withholding water from the third day after seedling emergence, and root and seedling growth were examined. Degree of drought tolerance were rated based on seedling vigor in the PEG solution and drought-stressed parafilm-layered pots, and also on the penetration ability of roots through parafilm layer. Most of seedlings of the drought-tolerant cultivars showed higher vigour and root penetration than those of the drought-sensitive cultivars under the new screening conditions. Our results indicate that the new method can be used as a simple and effective screening procedure for drought tolerance in soybean breeding programs.

Keywords : soybean, drought tolerance, seedling method, parafilm, PEG

Drought stress by water deficit is a serious yield-limiting constraint in a large soybean production area. Since the soybean crop in Korea is rainfed, it is prone to be drought-stressed. Therefore, cultivation of drought-tolerant variety is a primary means of minimizing yield loss from drought in case of under unpredictable rainfall and poor irrigation conditions. However, drought tolerant cultivars were not developed due to the research difficulties involved. A simple screening method for drought tolerance in soybean is not available now. Breeding for drought tolerance could be accomplished by selecting for seed yield under drought stress field conditions, but such a procedure requires full-season field data and is not always convenient or efficient. Futhermore, several screening methods are not always correlated with yield potential at field condition under drought stress.

Drought-resistance mechanisms fall into two categories: 1) drought tolerance, and 2) drought escape or avoidance. Drought tolerance might result singularly or in combination from a deep and profusely branched root system, good stomatal control of transpiration, osmotic adjustment and tolerance of dehydration (Morgan, 1984), and relate to water-use efficiency and transpiration efficiency (Specht, 2001). Early maturity and ability to resume growth after a drought can help escape drought.

A root system with longer root length and density at deeper layers is useful in extracting water in upland conditions. Vijayalakshmi and Nagarajan (1994) observed that drought-tolerant rice cultivars had better root penetration and distribution and greater root weight under water stress compared with drought-susceptible cultivars. Narayan (1991) also found that most cultivars with deep penetrating roots had higher yields under water stress. He further concluded that root-penetration depth was a better criterion than total root-length density in selecting suitable wheat cultivars under soil water stress. However, these methods for surveying the root pattern in the field requires too much labor and time.

Even though a number of drought-related traits and tests were identified, no single test and trait used in selection was sufficiently reliable in determining the overall drought response of a cultivar. Therefore, a combination of tests was used to categorize tolerant and susceptible group of cultivars. Several selection criteria were proposed to select genotypes based on their performance in stress and nonstress environment (Blum, 1988). Screening for drought resistance may require physical measurement of entire root system

†Corresponding author: (Phone) +82-63-840-2237 (E-mail) yjikim@rda.go.kr <Received July 25, 2001>

under actual or simulated stress conditions, determination of physiological processes such as characters related to photosynthesis, measurement of water status such as osmotic adjustment and tolerance to dehydration or assessment of ability to resume growth after drought. Yu *et al.* (1995) developed new method for screening root penetration. They developed and evaluated the use of a wax-petroleum layer system which consisted of 60% wax and 40% petrolatum white as a reference to measure genotypic variation in root-penetration ability of 11 rice cultivars in pots. Though many adaptive traits have been proposed for use in breeding program for drought-prone areas, unfortunately, few hard data are available for selection. However, these screening methods are not always correlated with yield potential under water stress and its application is limited since they require too much time and efforts. Therefore, rapid screening techniques are needed to select for tolerance stress.

The objective of this experiment was to develop a simple, mass-screening fast, and cost-effective method for drought-resistance screening in seedling stage of soybean.

MATERIALS AND METHODS

To develop a drought-tolerance screening model, we used 54 soybean cultivars which had different ecotype at the preliminary experiment in Taiwan (Kim, 1998). Fifteen soybean cultivars among them, eight identified to be drought-tolerant and seven drought-sensitive in previous studies, were used for the evaluation of drought tolerance under the new screening conditions. Of the fifteen cultivars, 10 had yellow, three green, one brown, and one black seed-coat, respectively. The hilum color was yellow (11), brown (1) and black (3). The 100-seed weight ranged from 6.6 g to 23.4 g. Five cultivars had a 100-seed weight of less than 10 g (Table 3).

Evaluation of soybean cultivars for drought tolerance at seedling stage

Fifty seeds per cultivar were sown in plastic trays, and the trays were allowed to soak water for four hours at room temperature. Thereafter, the water was drained. The trays were transferred to an environmental control room and maintained at 27°C in dark condition. Sufficient moisture was provided to the tray at 6-hr interval for four days. The germinated seedlings were then exposed to low light condition (light intensity of about 200 $\mu mol^{-2}\, sec^{-1}$) for hardening the seedlings. After one day exposure to hardening, 10 seedlings from each variety were transferred to a plastic seedling tray containing 18% polyethylene glycol (PEG, -0.15MPa) and remained for four days. On the fifth day, the seedlings were visually rated on a 0 to 9 scale for wilting, discoloration of the hypocotyl and death of the seedlings. The range of rating was 0 to no symptoms and 9 to seedling death (Table 1). Four days after germination, the percent germination was determined. After the hardening treatment, the length of the seedlings was measured.

Table 1. Judgment of drought tolerance by PEG treatment.

Symptoms	Visual rates for wilting
No wilting	0
A little	1
1/4 wilting	3
1/41/2	5
Above 2/3	7
All dead	9

Root penetration ability as an index of drought tolerance

The same fifteen cultivars used for seedling evaluation were used for this experiment. A plastic seedling tray with quadrilateral compartments was used. Each compartment is referred as a pot. A parafilm (American National Can, USA) strip was pressed into each pot so that the parafilm covered the whole pot. Peat moss was added over the parafilm in each pot, and three seeds of each cultivar were sown to each pot. In another tray, each pot was filled with moistened peat moss only. The tray with parafilm was placed over the tray with peat moss and kept at 27°C under a 14 hr photo period with light intensity of about 600 $\mu mol^{-2}\, sec^{-1}$. On three days after emergence of the seedlings, moisture was gradually withdrawn by withholding watering. The parafilm was impermeable to moisture. The experiment was laid out in a randomized complete block design with five replications.

The penetration ability of roots through the parafilm layer was determined by observing the number of days taken for the root to penetrate through the parafilm.

Field screening of soybeans for drought tolerance

Screening of genotype for drought tolerance was con-

Table 2. Degree of drought sensitivity by root penetration ability using parafilm.

Days to root penetration	Degree of drought sensitivity
1	0
2	1
3	2
No penetration	5

ducted at National Honam Agricultural Experiment Station (NHAES) in Iksan, Korea. Fifteen soybean cultivars were sown in the sandy loam soil on 20 May, 1999. Each variety was planted to a single 2 m-long rows 60 cm apart with a 15 cm-hill spacing. The crop was completely rain-fed. A randomized complete block design with four replications was used. At R2 to R5 growth stage, field was covered with vinyl plastic using 2.5 m-high metal frames to prevent plants from receiving rainwater and impose a drought stress. The sides were left open for ventilation during rain-free days. On rainy days, the sides were closed to prevent rain splash on the plants. Observations were made on plant height at maturity (R8 growth stage), yield per plant and visual rating of wilting of plants.

RESULTS AND DISCUSSION

The percent of germination varied from 40 in Ankur to 98 in G2314. The length of the seedlings in five days after germination varied from 131 to 174 mm. The mean seedling length for 15 cultivars was 149 ± 21.4 mm (Table 3).

Based on the results of drought-tolerance screening in field, varieties with a drought-tolerance visual rating, such as GC84040, IYT94040, and IAC100, are considered to be tolerant. Whereas, those with a drought-susceptible visual rating, such as G2739 and PI379618, are considered to be susceptible (Table 3).

Since the ultimate objective is to select genotypes which have minimum or no reduction in yield under drought condition, the genotypes selected through other screening procedures should be related to field performance. Therefore, results from the preliminary screening conducted at AVRDC, Taiwan were confirmed in Korea and related to field performance of these genotypes.

Morgan (1984) recognized that osmotic adjustment is an important mechanism of drought resistance (dehydration avoidance). Shiferaw (1996) observed that PEG was more suitable than mannitol and/or NaCl for drought -screening tests under osmotic stress in *Eragrostis*.

From the results of the preliminary experiment with 54 soybean cultivars in Taiwan, we developed a equation for drought-tolerance screening model. It has been used successfully to screen for drought tolerance from different cultivars at seedling stage based on root penetration ability methods.

$$Td = \alpha_{PEG} + \beta rp + \sum_{n=1}^{N} \gamma gn + Pd$$

$Td \leq 4$ is the drought tolerance

$Td > 13$ is the drought susceptible

Where : Td is drought-tolerance value

α_{PEG} is the drought-tolerance rating for wilting by PEG treatment

βrp is the degree of drought sensitivity by root penetration

N is seedling stage (V_2)

γgn is the genotype eigenvector value

Pd is the residuals.

Cultivars susceptible to drought may suffer from dehydra-

Table 3. Characteristics and field screening for drought tolerance of soybean varieties.

Varieties	Germination rate (%)	Sprout length (mm)	Seed coat color[†]	Hilum color[†]	100-seed weight (g)	Drought tolerance in field[‡]
G2866	81	138	y	y	12.5	MT
GC84040	52	137	y	y	17.8	T
GC86018	79	136	y	y	17.4	MT
IYT94040	75	131	y	y	16.4	T
IAC100	89	165	y	br	11.3	T
PI230970	97	165	g	bl	13.3	MS
PI459024	94	162	y	y	23.4	MT
TGX560-3D	95	152	g	y	9.7	MT
G2091	96	174	g	y	6.6	MS
G2314	98	153	bl	bl	8.5	MS
G2739	81	151	y	y	13.7	S
G3327	81	154	y	y	10.0	MS
G4782	94	154	br	y	6.6	MS
Ankur	40	138	y	y	16.1	MS
PI379618	73	132	y	bl	12.7	S

[†]y, yellow; g, green; br, brown; bl, black.
[‡]T, tolerance; MT, moderate tolerance; S, susceptible; MS, moderate susceptible.

Table 4. Screening of cultivars for drought-tolerance using seedlings and root penetration ability methods.

Varieties	Visual rates for wilting by PEG†	Degree of root penetration‡	Td(drought tolerance)§
G2866	2.7	1.0	3.97
GC84040	2.3	0	2.56
GC86018	1.4	2.2	3.84
IYT94040	2.3	0.6	3.27
IAC100	3.1	0.2	3.62
PI230970	2.7	1.0	3.85
PI459024	1.4	0.4	2.12
TGX560-3D	2.7	0.7	3.32
G2091	9.0	5.0	14.48
G2314	9.0	4.8	13.97
G2739	8.3	4.6	13.11
G3327	8.7	5.0	14.13
G4782	8.6	4.6	14.07
Ankur	8.3	5.0	13.13
PI379618	8.1	5.0	13.35

†Visual rates for wilting in seedlings treated with PEG 18% 0, no wilting; 9, all dead.
‡Drought sensitivity degree by root penetration ability using parafilm 0, one day to root penetration; 5, no penetration.
§$Td = \alpha_{PEG} + \beta rp + \sum_{n=1}^{N} \gamma gn + Pd$

tion due to 18% PEG treatment. In our experiment, such cultivars with discoloration (light-brown) symptom in the hypocotyl wilted and eventually died due to severe dehydration. The results of drought-tolerance screening using sprouts are shown in Table 4. Eight out of 15 cultivars could be rated as drought tolerant. Fig. 1 showed soybean seedlings treated with 18% PEG.

The ability of root to penetrate in search of moisture is a survival mechanism of plants in condition of an impermeable medium. In drought situations, moisture depletion occurs first at upper soil strata. However, depending upon the soil type, rainfall and drainage pattern as well as other factors, the moisture status at different depth will vary. Regardless, at times of drought the root system will desperately look for moisture. The penetration ability of root to deeper layers during drought is a distinct and effective component of drought resistance (Bohm, 1979).

Fig. 1. Behavior of soybean seedlings treated with 18% PEG.

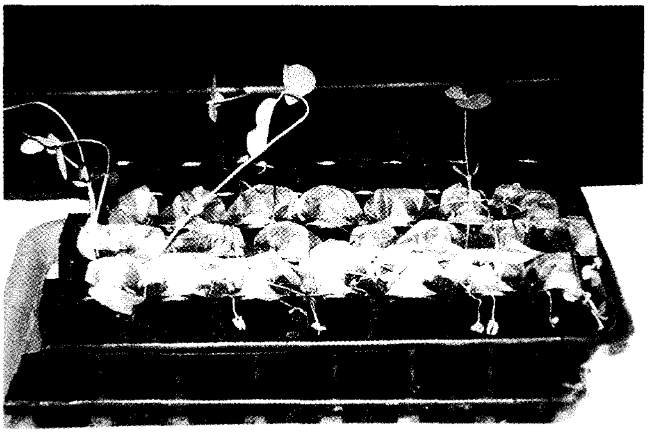

Fig. 2. Root penetration through parafilm. Drought-susceptible cultivars are dead, while drought tolerant cultivars survive.

The cultivars evaluated can be conveniently grouped into two major categories, namely 1) those whose roots penetrate through the parafilm and 2) those whose roots cannot penetrate through the parafilm, wilt and die. Eleven cultivars fall into category 1) and the others into 2). Among those whose roots penetrate through the parafilm, there are three groups (Table 2). Some cultivars penetrate readily within a day after withdrawal of water. It is likely that these cultivars either regardless of drought might penetrate or at the first sign of drought penetrate through the impermeable layer. They are GC84040, IYT94040, IAC100, PI459024, and TGX560-3D. On the other hand the cultivars which penetrate in 2 to 3 days probably can withstand drought for a day or two and their roots proceed to penetrate through the parafilm seeking moisture thereafter. They are G2866, GC86018, and PI230970 (Table 4).

Yu *et al.* (1995) developed a new method using a wax-petroleum layer system through which the roots of drought tolerant rice cultivars penetrate to seek water. Ray *et al.* (1996) used RFLP and found four quantitative trait loci (QTLs) associated with root penetration ability. The cDNAs corresponding to drought responsive genes have also been isolated and characterized (King and Nguyen, 1991). Fig. 3 showed that penetration ability of roots of soybean seedlings under drought condition. Roots of drought-tolerant soybean seedlings could penetrate through the parafilm layer.

Drought tolerance can result due to one or a combination

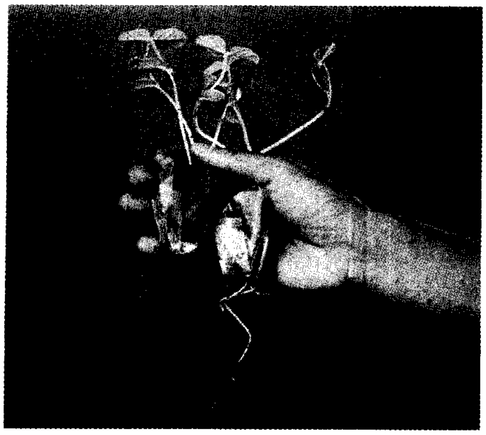

Fig. 3. Penetration of roots of the drought-tolerant soybean seedlings through the parafilm layer (right). Roots of the drought-susceptible soybean seedlings could not penetrate through the parafilm layer (left).

of different mechanisms. The above-ground- and below-ground-plant parts play a role in drought tolerance. Yield of a crop plant is a complex trait governed by a number of components. Presence or absence of pubescence on the leaves, stem and pods, thickness of the leaves, waxy layer on the leaves, size and number of stomata, transpiration rate, osmotic adjustment, tolerance to dehydration, and ability to produce deeper roots in search of water are a few of the mechanisms to overcome drought.

As described above, we developed a simple and efficient screening procedure of employing the PEG and root-penetration tests which allow evaluation of different drought-tolerance mechanisms operating in soybean plants. The roots of all drought-tolerant cultivars which were rated to be drought-tolerant in the seedling screening method using 18% PEG, were able to penetrate through the parafilm. Furthermore, screening results from the new method highly correlated with those from field tests. These results indicate usefulness of the new method in soybean breeding programs for drought tolerance.

REFERENCES

Blum, A. 1988. Plant breeding for stress environments. CRC Press, Florida, 223p.

Bohm, S. 1979. Methods of studying root systems. Ecological studies No. 33. Springer-Verlag, Berlin, 188p.

Carter, Jr. T. E. and T. W. Rufty. 1992. Soybean plant introductions exhibiting drought and aluminum tolerance. Proc. Inter. Symposium, AVRDC, pp. 335-346.

Hanson, W. D. 1991. Root characteristics associated with divergent selection for seedling aluminum tolerance in soybean. *Crop Sci.* 31(1) : 125-129.

Kim, Y. J. 1998. Screening for drought tolerance-simple and rapid methodologies. AVRDC Research report, pp. 1-46.

King, S. W. and H. T. Nguyen. 1991. Gene expression in sorghum during drought stress. *Sorghum Newsletter* 32 : 9-10.

Morgan, J. M. 1984. Osmoregulation and water stress in higher plants. *Ann. Rev. Plant Physiol.* 35 : 299.

Narayan, D. 1991. Root growth and productivity of wheat cultivars under different soil moisture conditions. Intern. *J. Ecology Environ. Sci.* 17(1): 19-26.

Quintero, H. F. M., D. d. S. M. Santos. 1997. Cell wall proteins of in vitro cultured chili pepper lines differing in water stress tolerance. *Plant Sci. Limerick* 128(2) : 217-223.

Rao, A. H., B. Karunasree, and A. R. Reddy. 1993. Water stress-responsive 23 KDa polypeptide from rice seedlings is boiling stable and is related to the RAB 16 family of proteins. *Plant Physiol.* 142(1) : 88-93.

Ray, J. D., L. Yu, S. R. McCouch, M. C. Champoux, G. Wang and H. T. Nguyen. 1996. Mapping quantitative trait loci associated with root penetration ability in rice (*Oryza sativa* L.) Theor. *And Appl. Gene.* 92(6) : 627-636.

Shiferaw, B. and D. A. Baker. 1996. An evaluation of drought screening techniques for *Eragrostic tef. Tropical Sci.* 36(2) : 74-85.

Specht, J. E., K. Chase, M. Macrander, G. L. Graef, J. Chung, J.P. Markwell, M. Germann, J. H. Orf and K. G. Lark. 2001. Soybean Response to Water : A QTL Analysis of Drought Tolerance. *Crop Sci.* 41(2) : 493-509.

Vijayalakshmi, C. and M. Nagarajan. 1994. Effect of rooting pattern on rice productivity under different water regimes. *J. Agron. Crop Sci.* 173(2) : 113-117.

Yu, L. X., J. D. Ray, J. C. OToole and H.T. Nguyen. 1995. Use of wax-petrolatum layers for screening rice root penetration. *Crop Sci.* 35(3) : 684-687.