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Genotypic Variation in Leaf Water Status of Soybean

Yong-Moon Jin*, Hong-Suk Lee**, Suk-Ha Lee**,
Yong-Woong Kwon**, and Jeong-Nam Im***

ABSTRACT

Plant water status during growth is directly and indirectly associated with seed yield. The objective of the present study was to determine the genotypic differences in leaf water characteristics at an early growth stage of soybean [*Glycine max* (L.) Merrill] plants through the pressure-bomb technique. Measurements of water potential as well as relative water content (RWC) were made at the third leaf from the fully-expanded top leaf of eight different soybean genotypes grown for 31 to 35 days after field emergence. On the basis of the modified exponential model, pressure-volume (PV) curves were fitted well (R^2=0.92 to 0.99** for the curvilinear region and R^2=0.67** to 0.96** for the linear region), indicating that a segmented model using PROC NLIN of SAS could be used effectively to estimate the leaf water characteristics. The regression analysis for the pressure-volume (PV) curve revealed genotypic variation in the solute potential at saturation ($\Psi_{s,sat}$: −10.7 to −14.8 bar), solute potential at incipient plasmolysis ($\Psi_{s,ip}$: −14.3 to −18.3 bar), RWC at incipient plasmolysis (RWC_{ip} : 83.3 to 91.7%), high integrated turgor pressure from saturation to plasmolysis ($\int_1^b \Psi_P$: 0.39 to 0.81), and maximum volumetric modulus of elasticity (ε_{max} : 150 to 445 bar).**

Keywords : leaf water potential, pressure-volume curve, solute potential, plasmolysis, relative water content, maximum elastic module, turgor pressure

The occurrence of drought during crop growth is the most limiting environmental factor for yield. As irrigation for soybean production in the field is usually uneconomical, it is important to breed soybean genotypes showing a relatively high field drought tolerance (Jin et al, 1997; Sloane et al. 1990). Indirect selection for components of drought tolerance was suggested in a breeding program because drought tolerance was considered a highly complex trait. As yet, cultivars have not been developed specifically for drought tolerance. Changes in leaf water status in response to water deficient stress is one of the major physiological processes responsible for drought tolerance. Turgor in plant tissue might be maintained during drought by either dehydration postponement, dehydration tolerance, or both (Kramer, 1983). Genotypic variability for leaf water status had been demonstrated in soybean plants (Burch et al. 1978; Sloane et al. 1990), suggesting that leaf water status might be one of the criteria for selecting drought-tolerant soybean genotypes.

With the development of the pressure-volume technique by Scholander et al. (1964), the technique had been used extensively for quantifying leaf water potential (Ψ) and its components, solute potential (Ψ_s) and turgor (Ψ_p) under field conditions (Grimes & Yamada, 1982). The technique had also been extended to estimate the volumetric modulus of elasticity (ε) by Tyree & Hammel (1972) and Melkonians et al.(1982).

For the same set of PV data, PV curves might be drawn in two ways (Tyree & Richter, 1972), Ψ as a function of RWC^{-1} and Ψ^{-1} as a function of (1-RWC). Both types of PV curves were composed of a curved line from turgid cells and a straight line from flaccid cells and organs (Richter et al. 1979; Tyree & Hammel 1972). A variety of algorithms appeared for the analysis of PV curves to determine the water potential at the turgor loss point, which occurred at the intersection of the two lines (Schulte & Hinckley, 1985). Sinclair & Venables (1983) and Jane & Green (1983) have reported using non-linear least squares methods to fit the entire PV curve. Nowadays, turgor loss point could be estimated effectively with a segmented model using the procedure of PROC NLIN of SAS (1988).

PV curves provided a basic information about deriving values of parameters such as the osmotic potential at saturation and the turgor loss point which might allow us to determine genotypic variation in drought tolerance. The objectives of this study were to evaluate the performance of a segmented model using the procedure of PROC NLIN of SAS to fit the PV curve, and to determine whether there was a genotypic difference in the ability to maintain turgor of soybean leaves at an early growth stage when exposed to high pressure through the pressure-bomb technique. Also, the present study was performed to clarify whether leaf water status could explain seed yield in response to drought stress in our previous study or not.

MATERIALS AND METHODS

Eight soybean genotypes, consisting of five recommended varieties (Baegunkong, Hwangkeumkong, Jangbaegkong, Namcheonkong, and Paldalkong), two elite

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* National Institute of Agricultural Science and Technology, Suwon 441-707, Korea.
** Department of Agronomy, Seoul Nat'l Univ., Suwon 441-744, Korea.
*** National Crop Experiment Station, Suwon 441-100, Korea.
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lines from a pedigree breeding programme (Suwon 93 and Suwon 119), and one introduced variety from the USA (Hodgson 78) were evaluated for the leaf water status through the pressure-bomb technique. As was described in our previous paper (Jin et al., 1997), these eight soybean genotypes, diverse in seed size (12.3 to 26.2 g/100seeds) and maturity, showed significant variation of leaf area and total dry weight reduction in response to drought stress. Soybean plants were grown for 31 to 35 days after field emergence. Row spacing was 0.6m, and population was 15.3 plants/m within the row.

Leaf water potential was measured at the third leaf from the fully-expanded top leaf of each soybean plant. Three leaves per variety or line were sampled to overcome a limited number of observations. Then samples were sealed in 2 L bottles containing 200 mL distilled water, and were placed for 24 hours at 25℃ in the dark. Leaf was weighed to estimate the fully turgid leaf weight (W_s). The fully turgid leaf was inserted into the pressure chamber (Daiki, DIK-7000), and was successively overpressured with N_2 gas. Fresh weight was recorded before and after each water potential determination at 3 to 5 minute intervals to estimate the W_{fi} at each measurement. Determinations of fresh weight and water potential were repeated up to 15 times until leaf water potential reached −20 to −25 bars. At the end of a measurement series, the leaf sample was oven-dried at 80℃ for two days and dry matter (W_d) was measured.

The relative water content (RWC) of leaves was expressed as the amount of water in a leaf at each measurement relative to the maximum amount the leaf could hold when fully turgid. RWC was calculated as follows:

$$RWC = (W_{fi} - W_d) \times 100 / (W_s - W_d)$$

The PV curve was drawn by plotting leaf water potential as a function of RWC^{-1}. Typical PV curves were composed of a curvilinear and a straight line (Fig. 1). The curvilinear region of the PV curve was fitted on the basis of the modified exponential model by Schulte & Hinckley (1985). A segmented model was established for the computer analysis method as follows:

$$y_1 = e^{a(x-b)} - 1 + dx + e, \quad \text{if } x \leq b$$

$$y_2 = dx + e, \quad \text{if } x > b$$

The procedure PROC NLIN using DUD computational method of SAS (1988) was used to fit a segmented model and to estimate the joint point (b) in the model. Fig. 1 represents a typical PV curve from which estimates of $\Psi_{s,sat}$, $\Psi_{s,ip}$, RWC_{ip}, and $\int_1^b \Psi_p$ were made. Maximum elastic modulus (ε_{max}) was calculated from the values of Ψ_p by the equation, $\frac{d\psi_p}{dRWC} \times RWC$, at RWC=1.

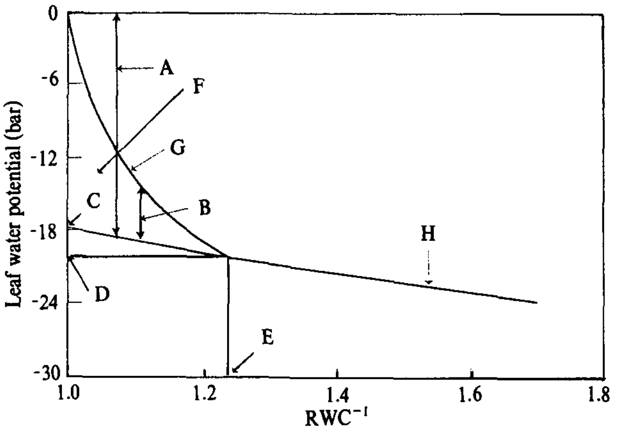

Fig. 1. Pressure-volume curve to search for leaf water characters related to the drought hardness.

A. Ψ_s : Solute Potential (Osmotic potential)

B. Ψ_p : Pressure potential (Turgor pressure)

C. $\Psi_{s,sat}$: Solute potential at full saturation

D. $\Psi_{s,ip}$: Solute potential at incipient plasmolysis

E. RWC_{ip} : Relative water content at incipient plasmolysis(x=b)

F. $\int_1^b \Psi_p$: Integrated turgor pressure from saturation to plasmolysis

G. $y_1 = e^{a(x-b)} - 1 + dx + e$, where $x \leq b$

H. $y_2 = dx + e$, where $x > b$

RESULTS AND DISCUSSION

The major problem in the analysis of the PV curve was to determine at what point the PV curve began to deviate from the curvilinear region. Regardless of soybean genotypes, modified exponential model by Schulte & Hinckley (1985) in connection with the procedure PROC NLIN of SAS (1988) provided a good fit to data of the PV curve in all soybean genotypes (Table 1). Comparison of R^2-values revealed that a segmented model was better predictive for the curvilinear region than the linear region of the PV graph. Estimates of the joint point varied from 1.09 (Namcheonkong) to 1.20 (Hwangkeumkong), indicating the possibility of genotypic variation in leaf water potential at the turgor loss point.

The regression analysis of Ψ on RWC^{-1} indicated that fairly large genotypic variations in $\Psi_{s,sat}$ from -10.7 (Hodgson 78) to −14.8 bar (Namcheonkong) was observed (Table 2). The range of these values was similar to that of Wenkert et al. (1978). Contrary to the genotypic variation in our study, Sloane et al. (1990) did not detect the genotypic variation in solute potential at the RWC=1 intercept between drought-tolerant PI 416937 and drought-sensitive Forrest, but in response of solute potential to drought stress. As the leaf tissue lost water, solute potential of the PI 416937 became progressively

Table 1. Estimated parameters in the regression equation of P-V curve.

Variety	a	b	d	e	R^2	
					$x \leq b$	$x \geq b$
Hodgson 78	−13.69	1.18	−25.56	14.91	0.92**	0.71**
Paldalkong	−14.69	1.17	−24.28	11.51	0.64**	0.67**
Jangbaegkong	−14.39	1.18	−23.04	9.30	0.98**	0.92**
Baegunkong	−16.85	1.17	−15.61	0.97	0.94**	0.77**
Suwon 93	−19.86	1.13	−25.35	12.77	0.96**	0.63**
Suwon 119	−25.51	1.10	−24.52	12.63	0.99**	0.84**
Namcheonkong	−29.27	1.09	−26.78	11.94	0.93**	0.93**
Hwangkeumkong	−13.63	1.20	−19.61	5.22	0.97**	0.96**

$y_1 = e^{a(x-b)} - 1 + dx + e$, where $x \leq b$
$y_2 = dx + e$, where $x > b$

Table 2. Estimated parametric values related to leaf water potential from the regression analysis of P-V curve.

Variety	$\Psi_{s,sat}$	$\Psi_{s,ip}$	ε_{max}	$\int_1^b \Psi_p$	RWC_{ip}
	·············· bar			··············	%
Hodgson 78	−10.7	−15.1	150	0.55	84.7
Paldalkong	−12.8	−16.9	176	0.58	85.5
Jangbaegkong	−13.7	−18.0	200	0.72	84.5
Baegunkong	−14.6	−17.2	272	0.73	85.5
Suwon 93	−12.5	−15.8	238	0.43	88.5
Suwon 119	−11.9	−14.3	311	0.34	90.9
Namcheonkong	−14.8	−17.3	445	0.39	91.7
Hwangkeumkong	−14.6	−18.3	200	0.81	83.3

$\Psi_{s,sat}$: Solute potential at saturation
$\Psi_{s,ip}$: Solute potential at incipient plasmolysis
RWC_{ip} : Relative water content at incipient plasmolysis
$\int_1^b \Psi_p$: Integrated turgor pressure from saturation to plasmolysis
ε_{max} : Maximum elastic modulus

lower than that of Forrest.

Genotypic variation was also observed in solute potential and RWC at incipient plasmolysis, which varied from −14.3 to −18.3 bar and 83.3% to 91.7%, respectively. Our data indicated that maximum elastic modulus (ε_{max}) was in the range of 150 to 445 bar, which was in a similar range in the data of Wenkert et al. (1978) and Schulte & Hinckley (1985). However, this was inconsistent with the estimates of ε_{max} of Kim (1983). His estimates of ε_{max} were $8.5 \times 10^2 \sim 1.6 \times 10^5$ bar which were greater than expected.

Maintenance of turgor potential (Kramer, 1983) as well as osmotic adjustment (Jones & Turner, 1978; Sloane et al., 1990), the biochemical accumulation of solutes in response to water stress, had been known to be associated with drought tolerance. Lower RWC_{ip}, $\Psi_{s,sat}$ and $\Psi_{s,ip}$ and higher $\int_1^b \Psi_p$, based on the PV graph analysis, were thought to be closely related to the resistance of crops to drought stress. Hwangkeumkong, Jangbaegkong, Baekunkong, Paldalkong, and Hodgson 78 showed a relatively lower values of RWC_{ip} (Table 2). Of five soybean genotypes showing low RWC_{ip}, Hwangkeumkong, Jangbaegkong, and Baekunkong were low in $\Psi_{s,sat}$ and $\Psi_{s,ip}$ and high in $\int_1^b \Psi_p$ suggesting that these three soybean genotypes seemed to be comparatively tolerant to leaf water deficiency.

In our previous study (Jin et al., 1997), those three soybean varities showed fairly high average seed yield across drought stress treatments. However, the yields of Hwangkeumkng and Baekunkong, except Jangbaegkong, decreased significantly when they were subjected to drought stress treatment (Jin et al., 1997). The lack of association of leaf water characteristics in the present study with yield reponse to drought stress in the previous study (Jin et al., 1997) might be due to the instantaneous measurement of leaf water characteristics at an early growth stage, which could not account for the whole physiological processes responsible for drought tolerance. In addition, this was thought to be a big timing interval between early leaf water characteristics and final harvest of soybean plants.

Crop yields in response to drought stress are obviously dependent upon leaf water characteristics since leaf water status is closely associated with drought tolerance. It would also seem reasonable to presume that there would be a close relation of crop growth and yield to leaf water status. However, this generally has not been the case with leaf water status, even though we found the genotypic variation in leaf water characteristics. This might be partially due to the fact that crop yield was determined by the integrated physiological processes rather than by a single environment. Also, this emphasized the further investigation of the seasonal changes in leaf water characteristics rather than instantaneous measurement of leaf water status to clarify the close association of crop yield with leaf water status in response to drough stress. In addition, further studies on genotypic variation in recovery rate after drought stress is needed to better explain the crop yield.

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