Japan. Jour. Crop Sci. 56(1) : 92—98 (1987)

The Measurement of Cell Membrane Stability Using Polyethylene Glycol as a Drought Tolerance Test in Wheat

Gnanasiri S. PREMACHANDRA and Tohru SHIMADA
(Department of Grassland Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080)
Received May 12, 1986

Genetic improvement of drought resistance in crop plants depends on the identification of drought resistance mechanisms and the development of suitable methods for measuring drought resistance. The two major mechanisms of drought resistance are drought avoidance and drought tolerance. Drought avoidance consists of mechanisms to reduce water loss from the plant and of mechanisms to maintain water uptake to maintain high tissue water potential. Drought tolerance refers to the ability of the plant to withstand low tissue water potentials.

BEWLEY[1] reported the critical role of cell membrane stability under conditions of moisture stress as a major component of drought tolerance. The rate of injury to cell membranes by drought may be estimated through measurement of electrolyte leakage from the cells.

SULLIVAN[8,9] has described a technique, using leaf discs, for evaluating heat tolerance in terms of membrane stability. BLUM et al[2,3]., and KRISHNAMANI et al[5]., later developed this technique to measure cell membrane stability as a measure of drought tolerance of sorghum, wheat and soybean genotypes respectively. They applied a desiccation treatment using polyethylene glycol (PEG) solution to induce stress on leaf tissues.

High molecular weight, solid form of polyethylene glycol 6000 was used for desiccation in most of the experiments. The liquid form of PEG 600 is very convenient to work with and results obtained from using it with sorghum are comparable to those obtained with PEG 6000 (SULLIVAN et al[10].,). Twenty percent solution of PEG 600, vol/vol, or a 43% solution of PEG 6000, wt/vol, gives an osmotic stress of about-18 bars.

When the leaf tissue is exposed to high concentration PEG solution, cellular membrane permeability is increased and electrolytes diffuse out of the cells. If the tissue is subsequently bathed in deionized water for a specified period, the amount of electrolyte leakage can be evaluated by electrical conductance measurement. Since the amount of electrolyte leakage is a function of membrane permeability (the degree of injury induced by the PEG solution), desiccation tolerance of different genotypes can be assessed in terms of electrical conductance. The results obtained with this technique correlate with observations on genotypes known to be drought tolerant from field experience (MARTRNEAU et al[6,7].,).

Drought hardening (conditioning) or adaptation to drought has been receiving increasing attention. Due to the nature of the rainfall pattern, several major cropping areas of the world experience drought stress more frequently and severely during the grain filling period (DENNIS et al[4].,). Under limited irrigation, drought stress may be allowed to develop early in the growth cycle to induce a conditioning response which may lessen the adverse effects of severe stress during the later stages. The major facet of such conditioning is identified as "osmotic adjustment". In this study a phenomenon of cell membrane adjustment to drought stress is studied.

This investigation was carried out to explore possibilities of adaptation of cell membrane stability measurement as a measure of drought tolerance in wheat, with a few alterations to Sullivan's method.

Materials and Methods

Cultivars of spring wheat and winter wheat were grown in pots inside the green house of Obihiro University during May to November 1985 to obtain experimental materials for this study.

Leaf samples were taken from the uppermost fully expanded leaves and were cut into 1 cm pieces. One gram of leaf pieces was put into a 100 ml flask and washed slowly with three changes of deionized distilled water for about 90 minutes to remove surface adhered electrolytes. Following the washing, the leaf pieces were submerged in 30 ml of PEG solution and were allowed to stand in the solution for 24 hours at a cool temperature of about 10°C to minimize secondary effects. PEG 600 was used in this study. The concentration of the solution and the duration were adjusted to the severity of desiccation desired. After the treatment period, the leaf pieces were washed quickly for three times with deionized distilled water. Thirty ml of deionized distilled water were then added and kept for 24 hours at 10° C. Then the flask was warmed to 25°C, shaken well and the electrical conductivity was measured. Following the conductivity measurement, the leaf tissues were killed by autoclaving for 15 minutes, warmed again to 25°C and then the electrical conductivity was measured for the second time. Desiccation treatment and non-desiccated controls were consisted of three replications.

The PEG treatment affects membrane integrity, which permits electrolyte leakage. Degree of membrane stability to stress is evaluated by ion leakage. The percentage of injury occurred to leaf tissues by desiccation was calculated using the first and the second electrical conductivity measurements.

Percent injury

$$=[1-(1-T1/T2)/(1-C1/C2)]\times 100$$

T1 = first conductivity measurement.
T2 = second conductivity measurement.
C1 = first conductivity measurement of control.
C2 = second conductivity measurement of control.

A preliminary test was conducted to find the appropriate concentration of PEG solution to be used in the experiments. Influence of age of the leaf and the sampling position of the leaf on drought tolerance were investigated. Uppermost fully expanded leaves of the plants of different ages were sampled and tested in order to study the influence of age of the plants on drought tolerance.

A drought hardening experiment was conducted using three spring wheat cultivars. At the twenty three days after seeding, drought hardening was initiated. Water was withheld for six to eight days until leaf rolling was observed at predawn. Hardened plants were subjected to three cycles of such stress while the control plants were watered daily. Leaf samples from hardened and control plants were tested for drought tolerance when the plants were fifty days old.

An experiment was conducted to study the influence of number of hardening cycles on drought tolerance. Seeding was done on 24 June, 1985 and the plants in three groups of pots were subjected to one, two and three cycles of stress commencing at 39, 30 and 21 days after seeding respectively. Plants were tested for drought tolerance at fifty days after seeding.

An experiment was conducted to determine the influence of age of the plant on hardening treatment. Seeding was done at four stages commencing on Sept. 4, 1985 with one week intervals. Plants of three, four, five and six weeks were subjected to a drought stress of ten days on Oct. 9, 1985 until leaf rolling was observed at predawn. After the stress period the plants were watered for three days and the leaf samples were taken for testing.

Genetic variability associated with drought tolerance of eleven spring wheat cultivars and fourteen winter wheat cultivars were measured with a hardening treatment of one stress cycle, during August and November 1985.

Results and Discussion

The relationship between the concentration of PEG solution and the percent injury of the leaf tissues of spring wheat cultivar Kitamiharu 47 is shown in Fig. 1. Twenty five percent of PEG solution was selected as most suitable for testing spring wheat cultivars because it represents nearly 50% of injury of tissues.

Effect of the age of the leaves of two spring wheat cultivars on drought tolerance is shown in Fig. 2. Percent injury increased with the increase of the age of the leaves. Differences between cultivars were not found. BLUM et al[2,3]., and SULLIVAN[9] also concluded that the younger leaf tissues are more tolerant to drought than older tissues in wheat and sorghum respectively. Therefore sampling proce-

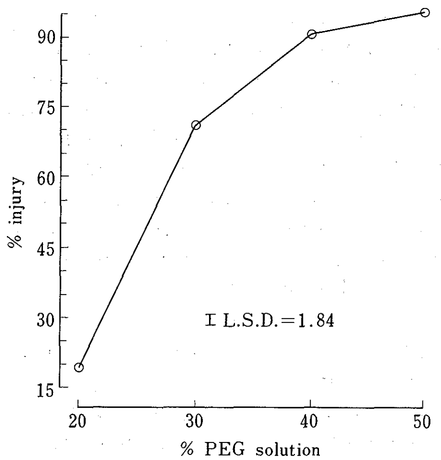

Fig. 1. The relationship of PEG concentration and percent injury of spring wheat cultivar Kitamiharu 47.

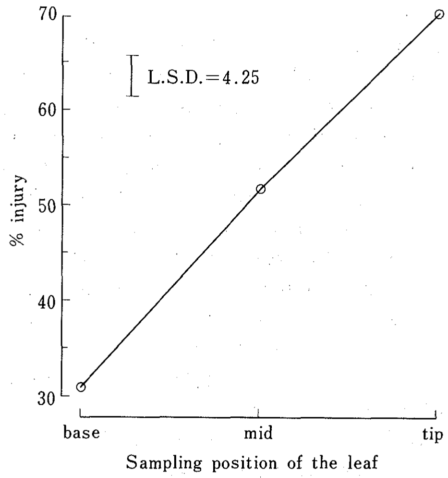

Fig. 3. Influence of sampling position of the leaf on percent injury of spring wheat cultivar Pitic 62.

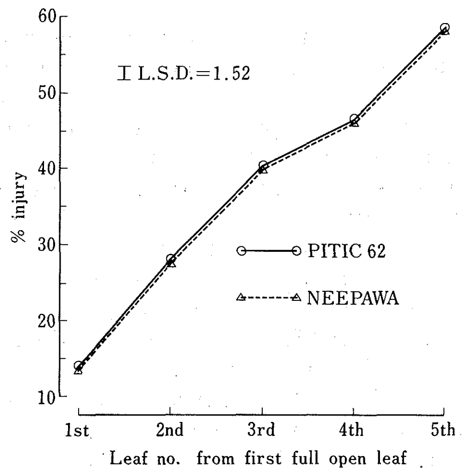

Fig. 2. The influence of age of the leaf on percent injury of two spring wheat cultivars.

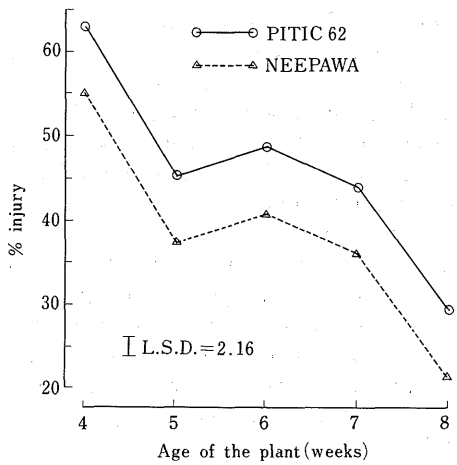

Fig. 4. Influence of the age of the plant on percent injury of two spring wheat cultivars (tested during Aug. Sep. & Oct. 1985).

dures for genotypic comparisons should take careful account on age of the leaves.

The results of the influence of sampling position of the leaf on percent injury is shown in Fig. 3. The difference of percent injury in base, middle and tip parts of the leaf is considerably large and statistically significant. Special care should be taken in sampling procedures to obtain samples always from the same position of the leaf in each treatment. The results were free from the error caused by this factor since whole leaf blade was used in this study.

The influence of age of the plant on drought tolerance of two spring wheat cultivars is shown in Fig. 4. A decrease in percent injury with the increasing of plant age was observed. Different results were obtained in two experiments conducted by Blum et al[2,3]., where they found decrease in drought tolerance with the plant age of sorghum and wheat. This experiment was conducted during August, September and October. Increase in drought tolerance with the age of the plant in this experiment can be attributed to the cold hardening of leaf tissues due to the decrease in environmental temperature towards October.

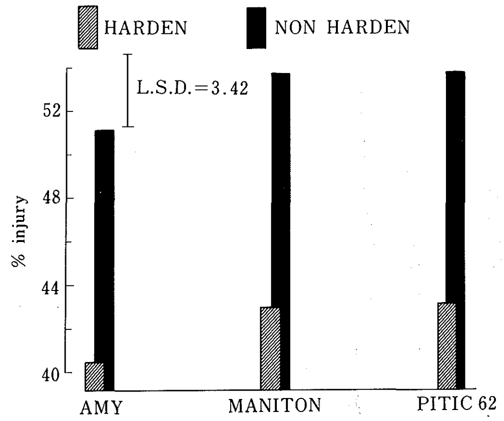

Fig. 5. The influence of drought hardening on percent injury of three spring wheat cultivars.

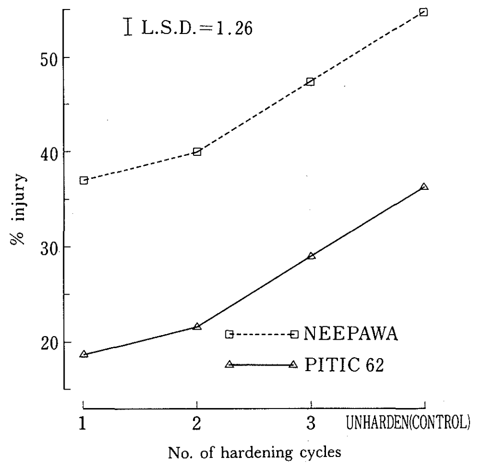

Fig. 6. The influence of no. of hardening cycles on percent injury of two spring wheat cultivars.

Fig. 5 illustrates the influence of drought hardening on drought tolerance of three spring wheat cultivars. Percent injury was found to decrease in plants subjected to periods of drought stress. BLUM et al[3]., observed significant differences in percent injury in water stressed plants, in a similar test conducted with eight wheat cultivars. In an experiment conducted by KRISHNAMANI et al[5]., using soybean leaf discs, leakage of electrolytes was 40% less in prestressed plants than control. These results supports the phenomenon of cell membrane adjustment to drought stress.

Influence of number of hardening cycles on drought tolerance of two spring wheat cultivars is shown in Fig. 6. Percent injury

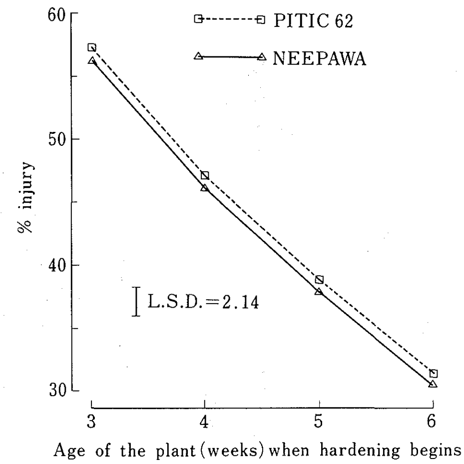

Fig. 7. The influence of age of the plant on drought hardening of two spring wheat cultivars.

increased when the number of hardening cycles were increased. Difference between cultivars were always the same. These results indicate that one hardening cycle is most effective for drought conditioning of spring wheat. Increase in the natural injury with the increase of number of hardening cycles was found in the drought sensitive cultivar Neepawa. The natural injury happened to leaf tissues under water stress conditions during hardening cycles. The magnitude of natural injury can be estimated from the item (C1/C2) in the calculation fomula of percent injury. These values for one, two and three hardening cycles were 19.1, 65.4 and 84.2 for cultivar Neepawa and 10.8, 9.9 and 9.2 for cultivar Pitic 62 respectively.

Fig. 7 illustrates the effect of age of the plant on drought hardening of two spring wheat cultivars. Percent injury decreased with the age of the plant at which hardening begins, and the differences between cultivars were always the same. Increase in effectiveness of drought hardening with the age of the plant was influenced by aging of the plant, but the result seems to be confounded by the seasonal effect which was discussed in Fig. 4.

Cultivar differences of eleven spring wheat cultivars and one winter wheat cultivar on drought tolerance are shown in Fig. 8 in the increasing order of percent injury. Influence of drought hardening is also shown together. Percent injury of the winter wheat cultivar

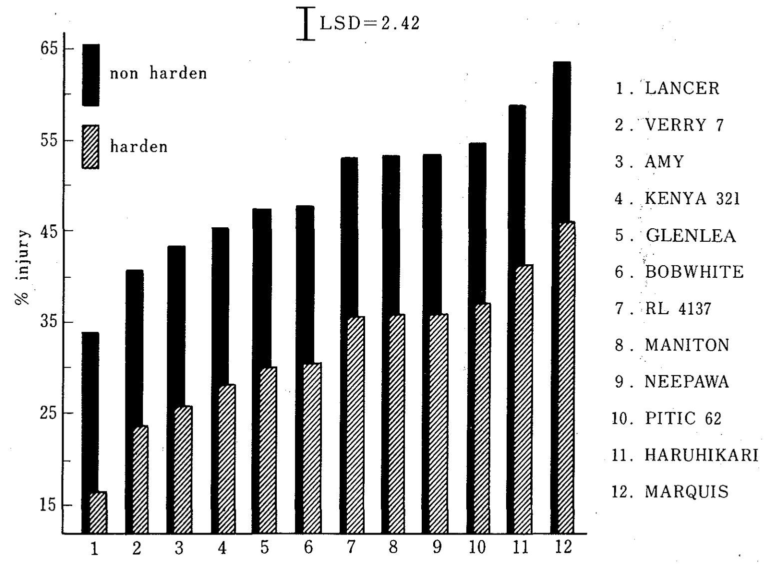

Fig. 8. The cultivar differences of percent injury of eleven spring wheat cultivars and one winter wheat cultivar (Lancer).

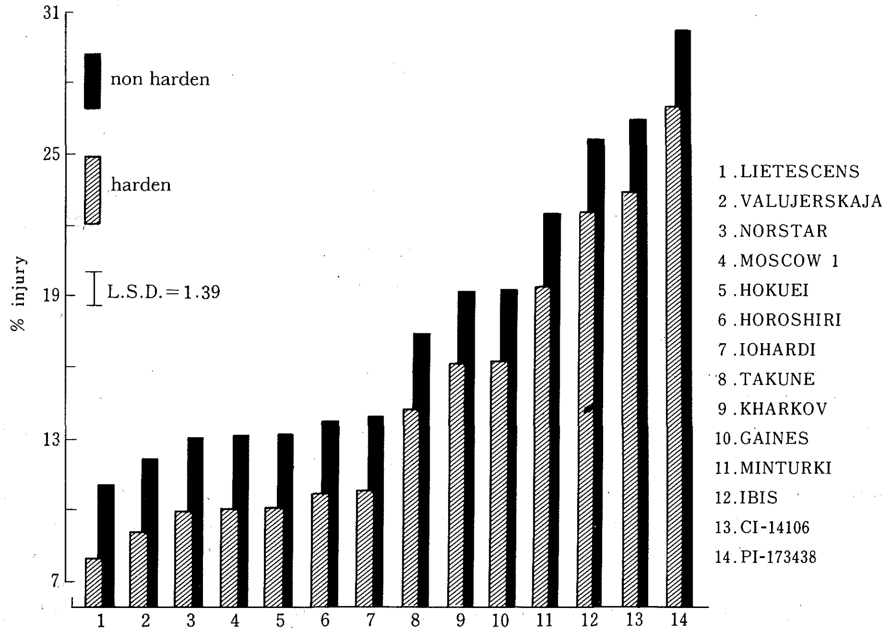

Fig. 9. The cultivar differences of percent injury of 14 winter wheat cultivars.

Lancer, was the minimum and the spring wheat cultivars showed differences in drought tolerance clearly. Highest ability of drought tolerance of winter wheat cultivar indicates that there should be a relationship between the drought tolerance and cold tolerance. It was found in a similar experiment done for the assessment of drought tolerance of several grass species, that the order of cold tolerance was the same as that of drought tolerance (unpublished data). Percent injury was less in hardening treatment than in unharden in all of the cultivars and the pattern (cultivar differences) was the same as unharden treatment. This indicates that the response of each wheat cultivar on drought hardening was almost the same. These results agree with the results of a field experiment conducted by BLUM et al[3]., to find the drought tolerance of eight spring wheat cultivars under irrigated and water stressed conditions. They obtained extremely low percent injuries in water stressed plots than irrigated plots.

Fig. 9 illustrates the drought tolerance of fourteen winter wheat cultivars in the increasing order of percent injury. Influence of drought hardening is also shown here. It was understood in a preliminary test that 60% concentration of PEG solution was the most suitable for testing winter wheat and it was used in this experiment. Differences of percent injury between cultivars are clearly indicated and are statistically significant. Influence of hardening treatment was the same as in spring wheat cultivars and the pattern was the same as unharden. This concludes that screening for drought tolerance of crop species or cultivars could be done under normal conditions or under water stressed conditions, but the conditions should be the same for all the species or cultivars being tested. Different results were obtained by BLUM et al[3]., where maximum cultivar separation in percent injury of wheat occured under favourable moisture conditions compared to stressed conditions. He concluded that initial screening should be done under favourable moisture conditions.

Desiccation tolerance tests using PEG were found to be comparatively efficient in the evaluation of drought tolerance between cultivars. Special care should be taken on age of the plants, age of the leaves and the sampling position of the leaf when obtaining samples for testing, for accurate assessment. Further studies on physiological meanings and genetical basis of the drought tolerance measured by this technique are needed for field studies and breeding programmes.

Summary

A technique of measuring cell membrane stability described by SULLIVAN[8,9] was used as a measure of drought tolerance to evaluate cultivars of spring wheat and winter wheat, with a few alterations. This involves the electrical measurement of injury of leaf pieces stressed by exposure to high concentration solution of polyethylene glycol. Cell membrane adjustment to drought stress (drought hardening) is also discussed here.

The results indicated that this technique was efficient in evaluating drought tolerance of wheat cultivars. However drought tolerance was markedly influenced by age of the leaf, sampling position of the leaf, sampling time of seasons, degree of drought hardening, and age of the plant at drought hardening. So it is very important to take careful attention on these factors at the time of measurement.

Acknowledgments

We wish to thank Dr. S. SHIBATA for his valuable suggestions and to Dr. Y. AMANO for supplying experimental seed material.

References

1. BEWLEY, J.D. 1979. Physiological aspects of desiccation tolerance. Ann. Rev. Plant Physiol. **30** : 195—238.
2. BLUM, A., and A. EBERCON 1976. Genotypic responses in sorghum to drought stress III. Free proline accumulation and drought resistance. Crop Sci. **16** : 428—431.
3. ——— and ———— 1981. Cell membrane stability as a measure of drought and heat tolerance in wheat. Crop Sci. **21** : 43—47.
4. DENNIS, P.G., C.Y. SULLIVAN and D.G. WATTS. 1983. Moisture deficits and grain sorghum performance : Drought stress conditioning. Agron. J. **75** : 997—100.
5. KRISHNAMANI, M.R.S., J.H. YOPP and O. MYERS, Jr. 1984. Leaf solute leakage as a drought tolerance indicater in soybean. Oyton. **44** : 43—49.
6. MARTINEAU, J.R., J.E. SPECHT, J.H. WILLIAMS and C.Y. SULLIVAN 1979a. Temperature tolerance in soybeans. I. Evaluation of a technique for assessing cellular membrane thermostability. Crop Sci. **19** : 75—78.
7. ———, J.H. WILLIAMS and J.E. SPECHT 1979b. Temperature tolerance in soybeans. II. Evaluation of segregating populations for membrane thermostability. Crop Sci. **19** : 79—81.
8. SULLIVAN, C.Y. 1971. Techniques for measuring plant drought stress. In Drought Injury and Resistance in Crops. (Eds.) K.L. Larson and J.D. Eastin. Crop Sci. Soc. Am., Madison Wis.
9. ——— 1972. Mechanisms of heat and drought resistance in grain sorghum and methods of measurement. In Sorghum in the Seventies.(Eds.) N. G. P. Rao and L.R. House. Oxford & IBH Publishing Co., New Delhi, India.
10. ——— and W.M. ROSS 1979. Selection for drought and heat resistance in grain sorghum. In Stress Physiology in Crop Plants. (Eds.)H. Hussel and R. Soaples, John Willy and Sons, N.Y. 263—281.

〔和　文　摘　要〕

ポリエチレングリコールを用いて細胞膜安定性を測るコムギの干ばつ耐性の測定法

ニヤーナシリ S. プレマチャンドラ・嶋田　徹
（帯広畜産大学）

コムギの干ばつ耐性の測定法として SULLIVAN[8,9] がソルガムに用いた方法を取りあげ，コムギに対する本法の有効性ならびに適用の際の留意点を検討した．予備試験の結果から，分げつ最上位の展開葉を 1 cm の長さに切断し，この 1 g サンプルをポリエチレングリコール 600 (PEG) の高張水溶液（春播きコムギでは PEG 20%，秋播きコムギでは PEG 60%）に 24 時間浸漬する方法を用いた．浸漬処理によって生じた細胞膜の損傷程度は，処理後サンプルを脱イオン水中に浸漬し，浸出した電解質の量を電気伝導度計で測定することにより評価した．

春播きコムギ 11 品種および秋播きコムギ 14 品種にこの方法を適用したところ，損傷程度に大きな品種間差異が認められ，干ばつ耐性の測定法として本法が有効であることが示された．しかし，損傷程度は，葉位，葉齢，葉身上の部位，季節，生育中に与えられた水分ストレスの程度やその時期などにより大きな影響を受けたので，測定に際してはこれらの要因について十分留意する必要があることが認められた。また本法の有効性をより明らかにするために，本法により評価される干ばつ耐性の生理的，遺伝的基礎および栽培的意義についてさらに検討が望まれた．