オーチャードグラス(Dactylis glomerata L.)の耐旱性測定法 (2):

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Measurement of Drought Tolerance in Orchardgrass (*Dactylis gromerata* L.)

II. Evaluation of polyethylene glycol test

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Synopsis

PREMACHANDRA, G.S. and T. SHIMADA (1987) : Measurement of drought tolerance in orchardgrass (*Dactylis gromerata* L.). II. Evaluation of polyethylene glycol test. J. Japan. Grassl. Sci. 33, 149-156.

Effectiveness of the polyethylene glycol (PEG) test of measuring cell membrane stability (CMS) for selecting drought tolerant genotypes in orchardgrass (*Dactylis gromerata* L.) was investigated. The PEG test was compared with two other tests. Genetic variability of CMS was also investigated.

Drought was induced artificially in pot-grown plants and in excised-leaves, and % injury in leaf tissues by drought stress was measured to compare with that in PEG test. Percent injury in leaf tissues of pot-grown plants in 12 orchardgrass cultivars was not correlated significantly with that in the PEG test ($r = 0.59$). However % injury in excised-leaves was correlated well with that in the PEG test ($r = 0.67^*$).

Variances in % injury in the PEG test were significant between cultivars, plants or ramets. The magnitude of variance component estimated from mean square was the smallest in ramets which represented the environmental variation. Heritability of % injury in the PEG test was 73.0 %.

CMS measured by the PEG test was therefore concluded to be a promising criterion in breeding for drought tolerance.

Key words: Cell membrane stability, Drought tolerance, Genetical variability.

Introduction

Effectiveness of measuring cell membrane stability (CMS) as an indicator of drought tolerance has been studied by many researchers.[1,2,3,4,6,7] All of them concluded that the degree of CMS was correlated well with tolerance of genotypes. Using this method PREMACHANDRA and SHIMADA[5] revealed that there were significant differences among cultivars of ordhardgrass (*Dactylis gromerata* L.) and environmental variations in terms of CMS.

Informations on genetical variability and heritability of selective characters are essential for effective screening. However, little is known about those of CMS. MARTINEAU et *al.*[4] estimated genetical variability and heritability of CMS to heat stress in soybean populations. They concluded that substaintial genetic variability existed and selection of heat tolerant

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genotypes was feasible.

This study was done to evaluate the effectiveness of PEG test as a measure of drought tolerance in orchardgrass. Genetical variability of CMS was also investigated.

Materials and methods

Exp. 1. CMS measurement using PEG test ; Eleven orchardgrass cultivars were grown in the experimental fields of Obihiro University. When they were about 6 months old uppermost fully opened leaves were sampled in early October 1986. CMS measurement was done using PEG 600 by the method reported earlier.[5)]

Exp. 2. CMS measurement in pot-grown plants under drought condition ; Four orchardgrass cultivars (Hayking, Kay, Obihiro-1 and kitamidori) were grown in pots in a glass house during May to August 1986. Six pots (42 cm × 30 cm × 12 cm) were used for each cultivar. Three weeks after seeding, seedlings were thinned out to 72 plants per pot. Ninety days old plants were treated without watering, for drought induction. The half of the plants were watered regularly for control. Uppermost fully expanded leaves were sampled 8,12, and 16 days after treatment. Middle portion of the leaves was cut into 1 cm pieces immediatly after sampling. One gram of leaf pieces was put into a 100 m*l* flask and washed slowly with three changes of deionized distilled water for about 90 minutes. The leaf samples were replicated three times. The washed leaf pieces were submerged into 30 m*l* of the water and were kept for 24 hours at 10°C. The flasks were then warmed to 25°C and shaken well, and the electrical conductivity of the water was measured. The leaf tissues were then killed by autoclaving for 15 minutes, and warmed to 25°C for measuring electrical conductivity again.

CMS of leaf tissues was evaluated by electrolyte leakage as % injury from the following equation.

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

T1, T2 = first and second conductivity measurements of treatment, respectively.

C1, C2 = first and second conductivity measurements of control, respectively.

Twelve orchardgrass cultivars were grown in the pots in the glass house. Plants were treated for drought for 12 days wiht 3 replications as before.

Exp. 3. CMS measurement in dehydrated excised-leaves ; Four orchardgrass cultivars (Hayking, Kay, Obihiro-1 and Kitamidori) were grown in the pots in the glass house during May to August 1986. Three weeks after seeding, seedlings were thinned out to 72 plants per pot. When the plants were about 90 days old, uppermost fully expanded leaves were sampled. They were separated into 7 groups of 50 leaves for drying. Thirty leaves were used for non-dehydrating control test. The seven groups were spread on the laboratory tables and dried for 4, 8, 12, 16, 20, 24 and 28 hours respectively. Temperature and relative humidity were about 23°C and 60%, respectively. Middle portion of the leaves were cut into 1 cm pieces. One gram of leaf pieces was put into a 100 m*l* flask. Both dehydrated and control leaf samples were replicated three times. CMS measurement was done by the method described previously.

Leaves of 14 field grown orhardgrass cultivars were sampled during August 1986 and dried for 16 hours. Cultivar differences in % injury were investigated.

Four orchardgrass cultivars (Hayking, Kay, Obihiro-1 and Kitamidori) were grown in the

pots in the glass house during May to August 1986. Each cultivar consisted of 6 pots. Ninety days old plants were treated for drought hardening by withholding of watering for 12 days. Control plants were watered regularly. After the hardening period, plants were watered for two weeks and the uppermost fully expanded leaves were sampled and dried for 16 hours under the same conditions as described above. The half of the leaves were not dried as control. CMS was then measured by the same method.

Exp. 4. Estimation of genetic variability and heritability ; Seedlings of 11 orchardgrass cultivars were grown in the field. Five enough grown plants of equal size were sampled in May 1986. Each plant was divided into 2 ramets. Ten ramets of each cultivar were planted in a single row with a spacing of 50 cm. Spacing between rows was also 50 cm.

Leaf samples were collected from the field in October 1986. Uppermost fully expanded leaves from each ramet were sampled. CMS was measured using 40% PEG 600 solution with 3 replications. Heritability estimates were calculated as the ratio of genetic variance to total variance.

Results and discussion

Percent injury in 11 orchardgrass cultivars is shown in Fig. 1. Table 1 shows % injury in leaf tissues at 3 stages of 4 pot-grown orchardgrass cultivars under drought condition. Percent injury increased with the number of days and the ranking of the cultivars was the same at 8 and 12 days. At 16 days the leaf samples were too dry and % injury was also too

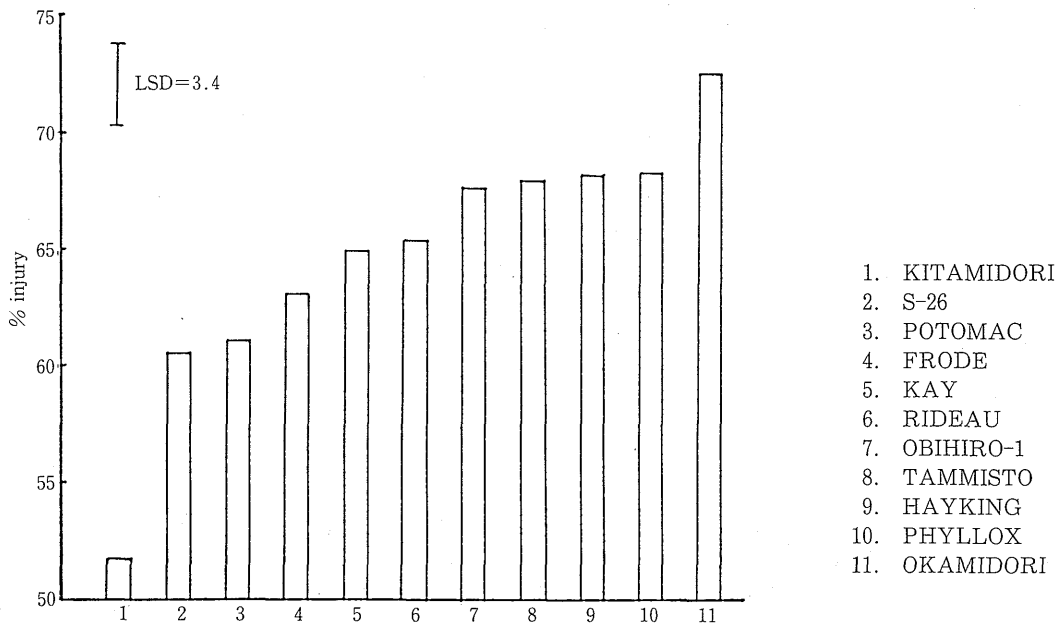

Fig. 1. Cultivar differences of % injury in PEG test of measuring cell membrane stability in 11 orchardgrass cultivars.

Table 1. Percent injury of Leaf tissues in 4 orchardgrass cultivars at 8, 12 and 16 days without watering.

Cultivar	Percent injury		
	8 days	12 days	16 days
Hayking	12.3	53.2	87.7
Kay	19.0	65.6	84.1
Obihiro 1	17.4	55.0	87.4
Kitamidori	10.7	50.8	85.1
LSD (0.05)	1.1	5.8	8.2
Mean	14.8	56.2	86.1
CV (%)	26.7	11.6	2.0

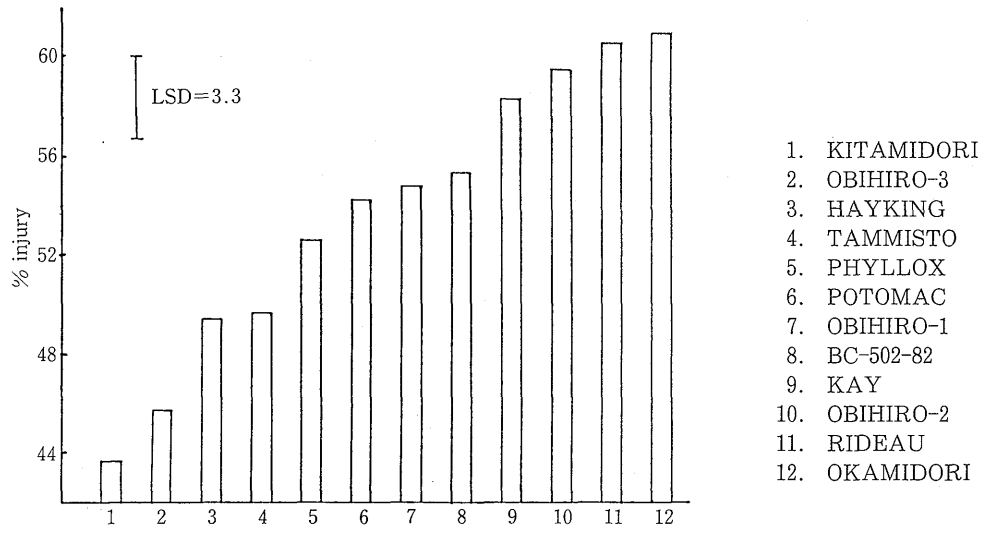

Fig. 2. Cultivar differences of % injury of leaf tissues at 12 days without watering in 12 orchardgrass cultivars.

high. Therefore, drought inducement for 12 days is considered the most suitable.

Cultivar differences in % injury of the leaf tissues of 12 orchardgrass cultivars after 12 days of drought inducement are shown in Fig. 2. Great differences were seen among cultivars. Ranking of the cultivars were different from the results of PEG test.

Table 2 illustrates % injury of dehydrated excised-leaves at different drying periods. Cultivar differences were also seen well in 12, 16 and 20 hours, but the ranking of cultivars was the same only in 12 and 16 hours. Dehydration of excised-leaves for 16 hours is therefore considered as the most suitable (c.v.% = 38.8). Krishnamani *et al.*[3] used a similar method as a drought tolerance test for two soybean cultivars. They dried the soybean leaf discs under a

Table 2. Percent injury of dehydrated leaf tissues in 4 orchardgrass cultivars for 4, 8, 12, 16, 20, 24 and 28 h after excision.

Cultivar	Percent injury after dehydration of						
	4 h	8 h	12 h	16 h	20 h	24 h	28 h
Hayking	1.2	2.2	3.8	6.6	10.9	30.5	30.2
Kay	0.6	2.1	7.0	16.3	16.8	30.8	44.0
Obihiro 1	1.2	4.1	4.8	9.2	13.5	31.2	42.4
Kitamidori	2.0	2.3	5.5	10.2	11.8	25.0	42.6
LSD (0.05)	0.3	0.4	0.8	1.5	2.8	2.0	4.2
Mean	1.3	2.7	5.3	10.6	13.3	29.4	39.8
CV (%)	0.5	0.4	25.5	38.8	19.6	10.0	16.2

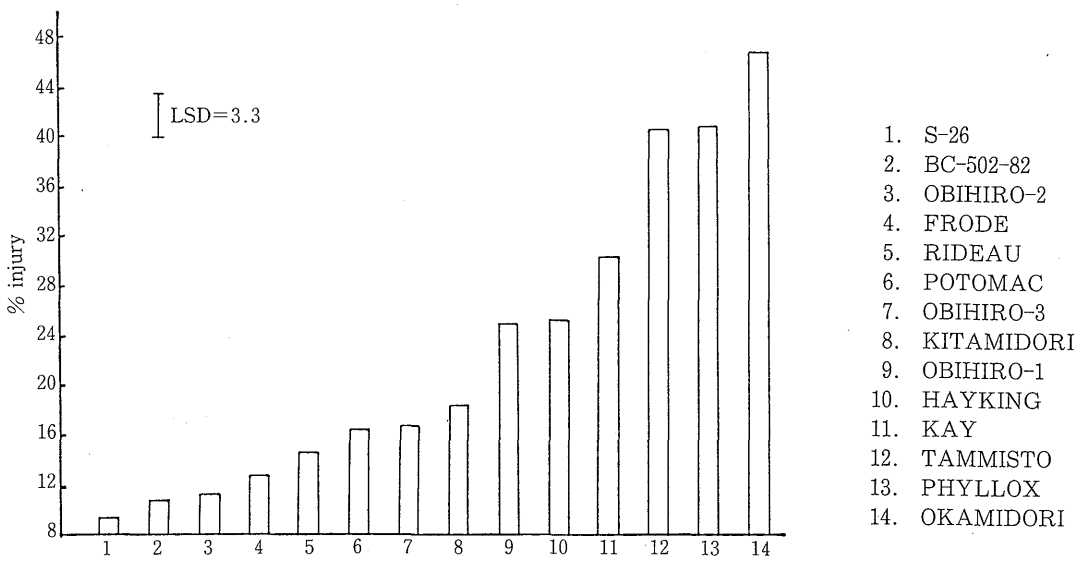

Fig. 3. Cultivar differences of % injury of leaf tissues dehydrated for 16 hours after excision in 14 orchardgrass cultivars.

fluorescent light and found cultivar differences in leaf solute leakage.

Differences of % injury of dehydrated excised-leaves in 14 orchardgrass cultivars are shown in Fig. 3. Differences were seen well among cultivars. Ranking of the cultivars was also well comparable to the results in PEG test, but was not related to CMS in pot-grown plants under drought condition.

Fig. 4 illustrates the influence of drought hardening on % injury in excised-leaves of 4 orchardgrass cultivars. Percent injury decreased in hardened plants. The same materials were tested using PEG method with the results that % injury decreased in hardened plants. Correlation coefficient between two % injury values was 0.79*. Krishnamani *et al.*[3] reported that the leakage of electrolytes from a soybean cultivar was decreased by drought hardening.

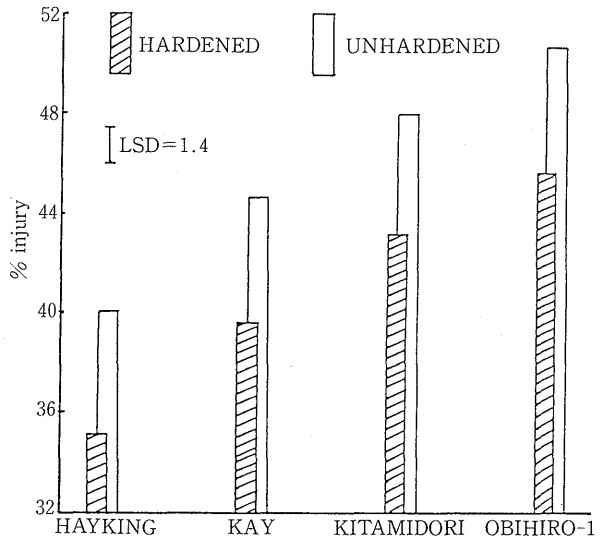

Fig. 4. Percent injury of leaf tissues dehydrated for 16 hours after excision with the influence of drought hardening in 4 orchardgrass cultivars.

Table 3. Analysis of variance, variance components and heritability of cell membrane stability in 11 orchardgrass cultivars indicated as % injury (PEG test).

Source	Degree of freedom	Mean square	F value	Statistical significance	Expectation of mean square
Between cultivars	10	924.9	3.9	<0.01	$\sigma^2+3\sigma_R^2+6\sigma_P^2+30\sigma_C^2$
Between plants	44	238.6	4.0	<0.01	$\sigma^2+3\sigma_R^2+6\sigma_P^2$
Between ramets	55	59.8	42.7	<0.01	$\sigma^2+3\sigma_R^2$
Error	220	1.4			σ^2

(1) Variance components estimated from expectation of mean squares
$\sigma_R^2=19.5$ (=Variance between ramets)
$\sigma_P^2=29.8$ (=Variance between plants)
$\sigma_C^2=22.9$ (=Variance between cultivars)

(2) Heritability $=\frac{\sigma_P^2+\sigma_C^2}{\sigma_R^2+\sigma_P^2+\sigma_C^2}=\frac{52.7}{72.2}\times 100=73.0\,(\%)$

Adjustment of cell membranes to drought stress is supported by the above results.

A significant correlation ($r = 0.67^*$) was obtained between PEG value and dehydrated excised-leaves, while not between pot-grown plants under drought conditions and PEG value ($r = 0.59$) or dehydrated excised-leaves ($r = 0.08$). This discrepancy is considered due mainly to water absorption by roots. Anyway the present results indicate the effectiveness of PEG method for measuring drought tolerance in orchardgrass.

Table 3 illustrates the analysis of variance, variance components estimated from the

expectation of mean squares and heritability of CMS. Significant differences were obtained between cultivars, between plants within a cultivar and between ramets within a plant. Variance components of ramets, plants and cultivars were 19.5, 29.8 and 22.9, respectively. Variance component of ramets within a plant which represents the environmental variance was the smallest. Heritability estimated from variance components was 73.0%. High heritability value indicates that CMS could be used effectively for selecting drought tolerant plants in plant breeding programmes.

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オーチャードグラス（*Dactylis glomerata* L.）の耐旱性測定法

II, ポリエチレングリコール法の評価

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要　約

オーチャードグラス耐干性個体を選抜するための検定法として，細胞膜安定性（CMS）を測るポリエチレングリコール法（PEG 法）が有効か否か検討した。そのため他の 2 つの耐干性検定法の結果と PEG 法による結果を比較した。またあわせて，PEG 法により測定した CMS の遺伝的変異性を推定した。

ポットに給水を停止する方法ならびに切り取った葉を実験台上に置く方法により人為的に乾燥ストレスを与え，その結果生じた葉の組織の損傷程度を CMC として測定した。給水停止処理による 12 品種の被害度と PEG 法による被害度との間には有意な相関係数が得られなかったが（r=0.59），切り取り処理による被害度と PEG 法による被害との間には有意な相関係数が得られた（r=0.67*）。

11 品種から 5 個体を選び，さらに各個体を 2 ラメートに株分けする方法で個体群を養成し，PEG 法により CMS を測定した。被害度は品種間，品種内個体間，個体内ラメート間でいずれも有意であった。また分散成分から推定された遺伝率は 73.0% と大きかった。

これらの結果から，PEG 法は，耐干性育種のための検定法として有望な方法であることが認められた。

キーワード：細胞膜安定性，耐干性，遺伝的変異性.

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