トウモロコシ葉の細胞膜安定性のポリエチレングリコール法による検討

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Polyethylene Glycol Test for Evaluation of Cell Membrane Stability in Maize

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Abstract : The cell membrane stability of three maize cultivars was measured by the polyethylene glycol (PEG) test at different parts of leaf (base, middle and tip parts of the uppermost full open leaf). The % injury in PEG test was compared with other measurements such as stomatal and cuticular resistance and the physiological state of the leaf parts. Leaf water potential, osmotic potential and Mg contents in cell sap and leaf tissues were well correlated with % injury in PEG test. Osmotic potential of leaf tissues seems to influence the desiccation treatment in PEG test. Relative contribution of sugar to osmotic potential was higher than the other solutes but sugar contents in leaf tissues or cell sap did not suggest the existence of any relationship with % injury in PEG test.
Key words : Cell membrane stability, Drought tolerance, Polyethylene glycol.

トウモロコシ葉の細胞膜安定性のポリエチレングリコール法による検討：ニャーナシリ S. プレマチャンドラ・実岡寛文・尾形昭逸（広島大学生物生産学部）

要　旨：トウモロコシ葉の細胞膜安定性をポリエチレングリコール（PEG）試験法により評価した．さらにトウモロコシ3品種（K8388, P3424, P3358）の展開完了最上位葉の先端部，中央部，基部の3部位について浸透ポテンシャル，水ポテンシャル，気孔抵抗，クチクラ抵抗および各部位の糖，K, Ca, Mg 含量と PEG 処理によって生じた損傷程度（被害度）との関係から細胞膜安定性の支配要因を解析した．

水ポテンシャル，浸透ポテンシャル，葉組織と細胞液中の Mg 含量と被害度との間には高い相関が認められた．特に葉組織の浸透ポテンシャルが，PEG 処理によるストレス，すなわち被害度に直接影響を及ぼし，浸透ポテンシャルの低い品種ほど被害度の小さいことが推察された．浸透ポテンシャルの低下は，他の体内成分に比べアルコール可溶糖により高く依存していることが推察されたが，葉組織，細胞液中の糖含量と被害度との間には相関は認められなかった．
キーワード：細胞膜安定性，耐干性，ポリエチエングリコール．

The evaluation of Cell Membrane Stability (CMS) using Polyethylene glycol (PEG) as a measure of drought tolerance was introduced by Sullivan[13]. The technique is based on desiccation *in vitro* of leaf tissues by a solution of PEG and subsequent measurement of electrolyte leakage into an aqueous medium. PEG test was used for measuring drought tolerance by Sullivan[14] and Blum and Ebercon[1] for sorghum, Martineau et al.[7] and Krishnamani et al.[6] for soybean, Blum and Ebercon[2] and Premachandra and Shimada[9] for wheat and Premachandra and Shimada[10] for orchardgrass and it was concluded to be comparatively efficient in partitioning between cultivars. Premachandra and Shimada[11,12] compared the PEG test with two other tests and concluded that it can provide a measure of drought sensitivity in wheat and orchardgrass.

Premachandra and Shimada[9,10] observed differences in CMS at different parts of leaves in wheat and orchardgrass. In this study CMS was measured at different parts of the uppermost fully opened leaf in maize to assess the CMS measured by the PEG test and to ascertain whether it was correlated with other measures or the physiological state of the leaf parts.

Materials and Methods

Three cultivars of maize (K8388, P3424 and P3358) were grown in the experimental field of Hiroshima University during September and October 1987. Field plots consisted of 5 m long rows spaced 0.65 m apart with 0.25 m spacing between plants. Plants were grown under ordinary cultivation conditions with adequate irrigation. When the plants were about 55 days old, measurements were made on the base, mid and tip parts of the uppermost fully opened leaves with three replications.

PEG test

Leaf discs of 1.1 cm diameter were cut using a leaf puncture. Thirty leaf discs were put into a 100 m*l* flask and washed three times with

deionized distilled water. For the desiccation treatment, leaf discs were then submerged in 30 m*l* of 30% PEG 600 solution and were allowed to stand in the solution for 24 h at 10°C. The leaf discs were then washed quickly three times with deionized distilled water. For both desiccated and control samples, 30 m*l* of deionized distilled water were added and leaf discs kept again for 24 h at 10°C. The flask was warmed to 25°C, well-shaken and the electrical conductivity measured using a Conductivity Meter CM-6A, TOA Electronics Ltd. Following the conductivity measurement, the leaf tissues were killed by autoclaving for 15 min, warmed again to 25°C and the electrical conductivity measured for the second time. Three replicates were measured for the desiccation treatment (T) and non-desiccated control (C). Three additional replicates were made from the desiccation treatment for the analysis of cell leakage.

CMS of leaf tissues was evaluated as percentage injury from the following equation.

Percentage injury

$$= [1-(1-T_1/T_2)/(1-C_1/C_2)] \times 100,$$

where T_1 = first conductivity measurement, T_2 = second conductivity measurement, C_1 = first conductivity measurement of control and C_2 = second conductivity measurement of control.

Measurement of leaf water potential and osmotic potential

Leaf water potential was measured using a thermocouple psychrometer (Wescor microvoltmeter and C-52 sample chamber). Leaf discs of 0.5 cm diameter were taken and immediately placed in the sample chambers from 10.00 to 14.00 h. Leaf water potential was measured after an equilibration period of 1 h. Thereafter the leaf discs were frozen and again put into the sample chambers and the osmotic potential measured after 10 min was allowed for equilibration.

Stomatal resistance and cuticular resistance

Leaf diffusive resistance was measured from 10.00 to 13.00 h and the cuticular resistance was measured during night in the dark with an autoporometer (Lamda Ind. Co.).

Dry matter, cell sap and cell leakage analysis

Base, mid and tip parts of the uppermost fully opened leaves were sampled. Half of them were ovendried for dry matter analysis whilst the rest of the samples were frozen and then centrifuged at 4,000 r.p.m. to extract cell sap. Leaf dry matter, cell sap and cell leakage were analysed for sugar, Na, K, Ca, Mg and P contents. Sugar (80% hot ethanol soluble) was determined by anthrone method. Na and K were measured using a Flame photometer (Eiko Pla). Magnesium and Ca were measured using an atomic absorption spectrophotometer (Hitachi 208). Total P was determined by the molybdenum blue method.

Results and Discussion

Figure la illustrates the % injury in PEG test at base, mid and tip parts of leaves in 3 maize cultivars. CMS was highest (% injury was lowest) in K8388, lowest in P3358 and intermediate in P3424. Percentage injury was higher in the mid part than in the base and tip parts. The differences between cultivars and the leaf parts were statistically significant. Premachandra and Shimada[9,10] observed increasing % injury values from the base to the tip in wheat and orchardgrass. However, that of maize is different.

Figure 1b and c illustrate the leaf water potential and osmotic potential at the base, mid and tip parts of leaves in 3 maize cultivars. Both leaf water potential and osmotic potential were lowest in K8388 (which was high in CMS), highest in P3358 (which was low in CMS) and intermediate in P3424. Leaf water potential and osmotic potential were higher in the mid part than in the base and tip parts, exhibiting the same pattern as in % injury in PEG test.

Relationship between % injury in PEG test and leaf water potential and osmotic potential is shown in Fig. 2 . Both leaf water potential and osmotic potential were low at low % injury values. Significant correlations were obtained between % injury in PEG test and leaf water potential ($r=0.645^*$) and osmotic potential ($r=0.641^*$). This suggests that osmotic potential in leaf tissues may have influence on the degree of desiccation caused by treatment in PEG test.

Cuticular resistance measured at the base, mid and tip parts of leaves in 3 maize cultivars is shown in Fig. 1d. Cuticular resistance was highest in K8388 (which was high in CMS) and lowest in P3358 (which was low in CMS) only at the base and mid parts and did not show a good linear relationship with % injury

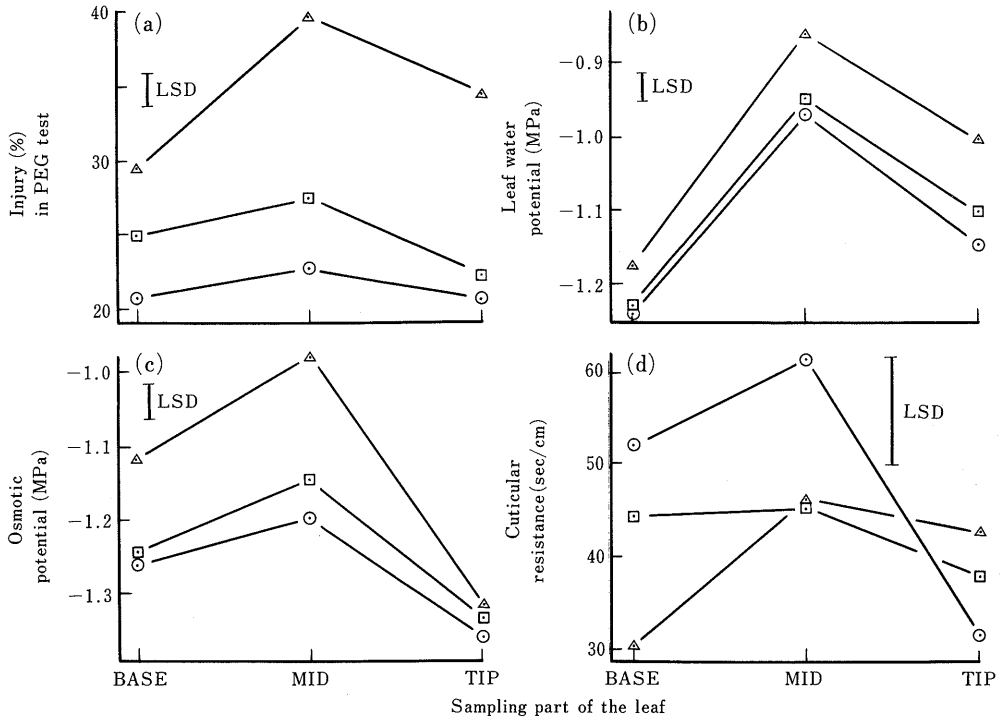

Fig. 1. Effect of part of leaf sampled on (a) Injury (%) in PEG test, (b) Leaf water potential, (c) Osmotic potential and (d) Cuticular resistance, in 3 maize cultivars (⊙ K8388, ⊡ P3424 and ◬ P3358).

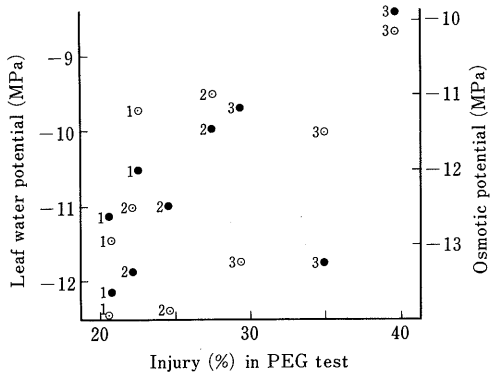

Fig. 2. Relationship between percentage injury in PEG test and ⊙ leaf water potential and ● osmotic potential measured at base, mid and tip parts of leaves in 3 maize cultivars (1, K8388; 2, P3424; 3, P3358).

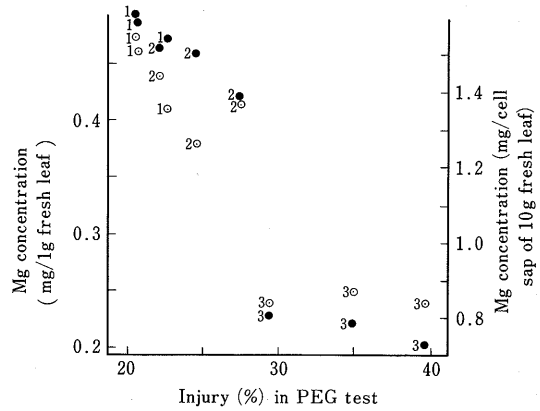

Fig. 3. Relationship between percentage injury in PEG test and Mg content (⊙, in leaf tissues and ●, in cell sap) measured at base, mid and tip parts of leaves in 3 maize cultivars (1, P8388; 2, P3424; 3, P3358).

in PEG test (r=0.063). Stomatal resistance also did not show a linear relationship with % injury in PEG test (data not presented).

Table 1 illustrates the correlation coefficients between % injury in PEG test and sugar (80% hot ethanol soluble), Na, K, Ca, Mg and P contents in leaf tissues, cell sap and cell leachate measured at the base, mid and tip parts of leaves in 3 maize cultivars. Magnesium contents in leaf tissues and cell sap and

Tabel 1. Correlation coefficients between percentage injury in PEG test and sugar, Na, K, Ca, Mg and P contents in leaf tissues, cell sap and cell leachate measured at base, mid and tip parts of leaves in 3 maize cultivars (K8388, P3424 and P3358).

Test materials	Percentage injury in PEG test					
	Sugar	Na	K	Ca	Mg	P
Leaf tissues	0.531	−0.411	−0.296	−0.473	−0.895**	−0.195
Cell sap	0.513	−0.015	−0.263	−0.044	−0.925**	−0.209
Cell leachate	0.502	0.710*	0.904**	0.187	0.300	0.833**

* : 5% ; ** : 1% level of significance.

Table 2. Relative solutes contribution to osmotic potential and percentage of solutes leached out from leaf tissues during PEG test measured at base, mid and tip parts of leaves in 3 maize cultivars (K8388, P3424 and P3358).

Cultivar and leaf part		Relative solutes contribution to osmotic potential (mg/cell sap of 10g fresh leaf)						Percentage of solutes leached out from leaf tissues					
		Sugar	Na	K	Ca	Mg	P	Sugar	Na	K	Ca	Mg	P
K8388	base	92.8	0.08	31.6	6.9	1.6	2.6	31.8	15.0	28.1	40.4	6.1	1.7
	mid	92.3	0.04	31.4	8.7	1.5	2.5	33.4	13.3	26.9	35.5	8.5	1.6
	tip	89.4	0.01	29.3	9.7	1.6	2.5	25.2	60.0	27.9	42.3	7.5	1.7
	mean	91.5	0.04	30.8	8.4	1.6	2.5	30.1	29.4	27.6	39.4	7.4	1.7
P3424	base	96.8	0.02	30.8	8.6	1.5	2.1	70.7	5.0	38.9	33.6	8.4	3.2
	mid	96.6	0.24	30.1	9.8	1.4	2.2	100.0	9.4	36.6	50.5	19.5	3.2
	tip	95.1	0.06	28.1	10.7	1.5	2.0	97.5	8.7	28.6	80.8	13.2	2.1
	mean	96.2	0.10	29.6	9.7	1.5	2.1	89.4	7.7	34.7	55.0	13.7	2.8
P3358	base	129.6	0.01	27.2	3.8	0.8	2.4	26.4	100.0	45.6	34.0	2.1	3.8
	mid	105.3	0.02	30.2	7.1	0.7	2.4	41.1	100.0	48.2	55.8	7.9	3.5
	tip	102.5	0.07	28.3	12.4	0.8	2.1	50.2	52.5	49.9	100.0	12.0	3.1
	mean	112.5	0.03	28.6	7.8	0.8	2.3	39.2	84.2	47.9	63.3	7.3	3.4
L.S.D.		1.7	0.03	0.3	2.0	0.02	0.06	8.2	14.4	5.7	18.1	4.0	0.4
Mean		100.0	0.06	29.7	8.6	1.3	2.3	52.9	40.4	36.8	52.5	9.5	2.6

Na, K and P contents in cell leachate correlated with % injury in PEG test. Magnesium contents in both leaf tissues and cell sap were high at low % injury values in PEG test (Fig. 3). This result suggests that high contents of Mg in leaf tissues might increase CMS.

Contribution of sugar K and Ca to osmotic potential was relatively higher than Na, Mg and P (Table 2). Cultivar K8388 which was highest in CMS contained a low sugar content but high contents of other solutes as compared with P3358 which was lowest in CMS. Since the major contributor to osmotc potential was sugar and the osmotic potential was lowest in K8388 (Fig. 1c), high sugar content was expected in K8388. Although the contents of K, Ca, Mg and P were relatively higher in K8388 than in P3358, their relative contribution to osmotic potential was considerably lower when compared to sugar. Therefore, the relationship of relative contribution of solutes, osmotic potential and the PEG test was not clearly defined. The contents of Mg in the three cultivars were well correlated with % injury in PEG test (Table 1) but did not correlate well with osmotic potential ($r = 0.550$). Therefore, while Mg may influence CMS in other ways it did not contribute to osmotic potential.

Ford and Wilson[3] noted that osmotic substances are different according to plant species. In sunflower inorganic ions are the major substances and sugar is minor[5]. Munns and Weir[8] concluded that sugar was the main osmotic substance in wheat. Itoh and Kumura[4] found variations of K and sugar concentrations at different leaf positions in soybean. It was understood from the present study that sugar plays a major role as an osmotic substance in maize.

Percentages of sugar, Na, K and Ca leached out from leaf tissues are relatively higher than Mg and P (Table 2). Since the relative contribution of Na to osmotic potential is low, K and Ca may be the major solutes which influence the electrical conductivity measurement in PEG test.

The results indicated that % injury in PEG test is influenced by the leaf water potential, osmotic potential and Mg content in leaf tissues and cell sap. Further studies on influence of factors related to cell membrane quality on CMS measured by PEG test will be helpful to investigate the mechanism of the PEG test. Relationship of the physiological factors and the cell membrane characteristics with the PEG test under drought conditions are essential to estimate the efficiency of the PEG test as a drought tolerance measurement.

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