Measurement of Cell Membrane Stability Evaluated by Electrolyte Leakage as a Drought and Heat Tolerance Test in Rice (Oryza sativa L.)

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Measurement of Cell Membrane Stability Evaluated by Electrolyte Leakage as a Drought and Heat Tolerance Test in Rice (*Oryza sativa* L.)

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To explore possibilities of adaptation of cell membrane stability measurement as a evaluation of drought and heat tolerance (or injury) in rice (*Oryza sativa* L.), we have measured electrolyte leakage (EL) from leaf discs exposed to desiccation and temperature stresses. In desiccation test with polyethylene glycol (PEG), EL increased from 10% to 80% with increasing from 20% to 60% of PEG concentration [PEG]. We have used 30% of [PEG] at which 25% of EL was occured. EL was increased with the time of PEG treatment; accordingly, 34 hours as a period of submerged leaf were selected. We have investigated an optimal temperature and treatment period, and around 42°C is likely to be suitable for testing membrane thermostability. EL was increased almost linearly with the time of leaf tissues submerged in test temperature *(42°C)* solution; we selected 24 hours for the following experiments. EL of PEG test was greater in senescing leaf (the 4th leaf from the youngest fully expanded leaf) than leaf in which senescence is less advanced (the youngest, fully expanded leaf). EL of leaf tissue was also increased with water deficit, indicating physiological status of plant itself can be reflected by the technique. Therefore, this technique is considered to be useful to assess injury, presumably at the membrane level, resulting from an environmental stress in rice.

INTRODUCTION

Plant survival and production under environmental stress are conditioned by complex mechanisms. Many studies point to cell membrane as an initial site of stress injury, the function and structure of plant cell membranes is drastically damaged by environmental stress (Liebermann et al., 1958; Mckersie and Tomes 1980; Mckersie *et al.,* 1982; Siminovich *et al.,* 1964). Thus, evaluation of cellular membrane integrity as a measure of environmental stress tolerance appears to be relevant criterion (Sullivan, 1972).

Most commonly, changes in the electrical impedence and electrolyte leakage have been measured to detect stress injury of cell membrane. Leakage will vary in relation to the membranes' abilities to take up and retain solutes and, therefore, will reflect stress-induced changes in both membrane potentials and membrane permeability. This technique is a modification of a method developed by Dexter *et al.* (1932) and Dexter (1956) for measuring freezing resistance. It is based on desiccation or heat shock in vitro of leaf discs by a solution including osmotica or adjusted temperature, and subsequence measurement of electrolyte leakage expressed as specific conductance of the aqueous bathing solution in which the discs were immersed, indicating degree of damage of cell membrane resulting from these treatments.

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Sullivan and Ross (1979) have conducted many experiments concerned with the relationship between electrolyte leakage following a desiccation treatment and the general ability of the plants to tolerate stress, and they found that membrane integrity and stability to the stress as evaluated by electrolyte leakage correlate well with tolerance of other plant process to the stress. To date, this method has been successfully used to measure membrane integrity in plants subjected to a variety of environmental stress (Liebermann *et al.*,1958; Mckersie and Tomes, 1980; Mckersie *et al.*,1 *982;* Siminovich *et al.*,1964). This method was found to be efficient in estimating stress tolerance of several crop plants, including wheat *(Triticum aestivum* L.) (Blum and Ebercon, 1981; Premachandra and Shimada,1987a), orchardgrass (*Doctylis glomerata* L.) (Premachandra and Shimada,1987b), and maize (*Zea mays* L.) (Premachandra *et al.*, *1989).* However, no attempt has been made to be established in rice (*Oryza sativa* L.).

Electrolyte leakage measured was markedly influenced by age, sampling part and season, degree of stress hardening, and plant species. Therefore, these factors should be taken into consideration at the measurement. In the present study, our objective was to explore possibilities of adaptation or cell membrane stability measurement as a measure of drought and heat tolerance in rice with a few alterations to Sullivan's methods.

MATERIAL AND METHODS

Plant material

Rice cultivar, Koshihikari was water-cultured using Yoshida's standard nutrient solution (Yoshida *et al.*,1976) containing 100ppm of SiO_2 in a green house. The culture solution was renewed once a week, and pH of the solution was adjusted to 5.0 to 5.5 with 1N NaOH and 1N HCl. Three samples were subjected to drought stress which consisted of exposing to 0.2 M mannitol (-0.68MPa of water potential) until leaf water potentials reached approximately 70% of control plant on the average. To conduct Si-deficient treatment, four plants were cultured in Yoshida's standard nutrient solution without SiO_2.

Measurement of electrolyte leakage

Polyethylene glycol *test*

Leaf samples were taken from the uppermost fully expanded leaves and were cut into 1 cm pieces. Thirty or twenty leaf pieces were put into a 100ml flask and washed slowly with three changes of deionized distilled water to remove surface adhered electrolytes. Follwing the washing, the leaf pieces were submerged in 30ml of polyethylene glycol (PEG) solution for a given period at 10°C in the dark 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 in the dark. Then the flask was warmed to 25℃, shaken well and the electrical conductivity was measured. Following the conductivity measurement, the leaf tissues were killed by autoclaving for 15 minutes to release all ions from the tissue, cooled to 25°C and then the electrical conductivity was measured. Degree of electrical leakage (EL) is evaluated using following equation:

$$EL=(ECi/ECt-Ci/Ct)\times 100$$

where, ECi is the initial electrical conductivity, ECt is the total electrical conductivity, Ci is the initial electrical conductivity of non-desiccated control. Ct is the total electrical conductivity of the control.

Heat shock treatment

Procedures and calculations were as above. Instead of incubating samples with an osmotic medium, ten leaf pieces were heated in 30ml of deionized water at 30, 35, 40, 45°C for 24 hours in the dark. After the treatment period, the electrical conductivity was measured according to PEG treatment.

RESULTS AND DISCUSSION

PEG test for estimating cell membrane stability is long-standing method for assessing drought tolerance. This method is based on the measurement of the electroconductivity of aqueous media containing leaf discs that were previously water stressed in vitro by exposure to a solution of PEG. Therefore, the values obtained with PEG test have been considered to be influenced by factors in terms of membrane permeability such as leaf surface wax content, thickness of cuticular layer and cell wall, changes in cytoplasmic lipids, and osmotic adjustment (Premachandra *et al.*, 1989, 1992).

In this study, first, we have investigated the relationship between PEG concentration ([PEG]) and electrolyte leakage (EL) of the leaf tissues (Fig. 1). EL increased from 10% to 80% with increasing from 20% to 60% of [PEG]. For testing drought tolerance in

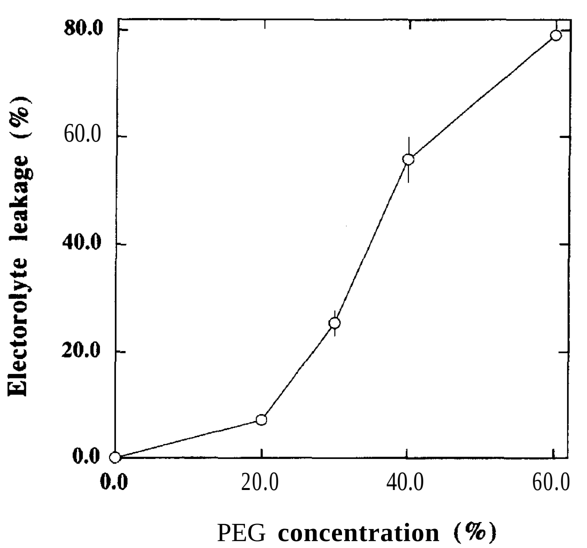

Fig. 1. Relationship between PEG concentration and elctrolyte leakage.

orchardgrass, Premachandra and Shimada (1987b) used 50% of [PEG] at which the difference between cultivars was maximam. In sorghum, 30% of [PEG] was used in the same reason (Premachandra *et al.*, 1992). The similar degree of leakage was occurred at 30% of [PEG] in rice leaves, therefore, this [PEG] was used as a standard osmotic medium for further work in rice.

With the time of submerging leaves in PEG solution, EL was increased (Fig. 2). Accordingly, 24 hours were selected for the following experiments. An experiment was also conducted to find out the suitable number of the leaf pieces. The differences of injury among the two samples (10 and 20 leaf pieces) were small and not statiscally significant (data not shown).

Although the mechanisms probably differ, drought resistance are often positively corelated with heat resistance in plants. Sullivan (1971, 1972) has described a technique employing leaf discs for evaluating tolerance to high temperature in terms of membrane thermostability. This method is based on heat shock in vitro of leaf discs by a solution adjusted temperature and subsequent measurement of EL from plant tissue into an aqueous medium.

Since the amount of the EL is a function of membrane permeability, the membrane thermostability (the degree of damage resulting from high temperature) can be assessed in terms of electrolytic conductance. The results obtained with this technique correlate with degree of the heat stability of photosynthetic activity in isolated chloroplasts (Sullivan *et al.*, 1977) and with degree of heat tolerance of whole plants (Sullivan, 1972). Therefore, this technique considered to be useful in investigating the membrane competence as affected by temperature stress.

In this study, we have investigated a optimal temperature and treatment period to applicate this technique on rice. Martineau et *al.* (1979) have tested membrane

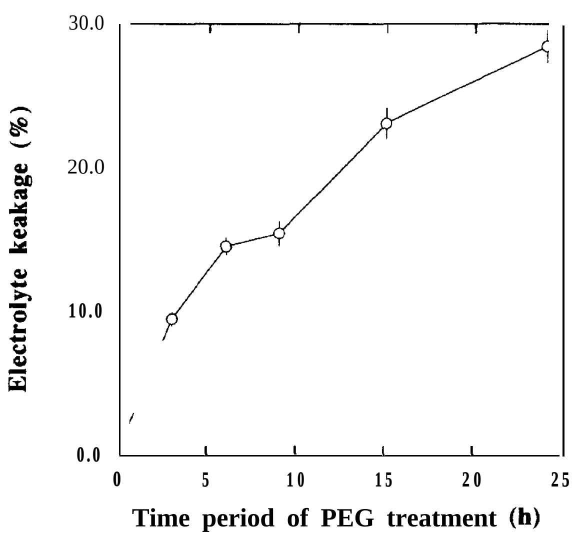

Fig.2. Effect of time period of 30% PEG treatment on electrolyte leakage.

thermostability of soybean in the range of 30°C to 60℃, and they have rated around 48°C at which 50% of EL was occurred a suitable temperature in soybean leaves. We have tested the leakage in the range of temperature 30°C to 45°C to test the thermostability (Fig. 3). EL increased from 8.1% to 71.6% with temperature increase from 40°C to 45°C. Threfore, around 42°C is likely to be suitable for testing membrane thermostability in rice leaves.

With the time of leaf tissues submerged in test temperature (42°C) solution, EL was

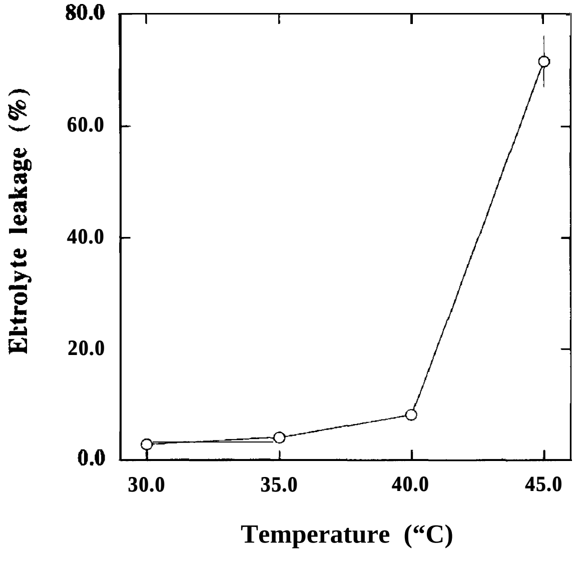

Fig. 3. Relationship between temperature of test solution and elctrolyte leakage.

increased almost linearly (Fig. 4), we selected 24 hours for the following experiments.

To test the ability of this technique to correctly describe membrane leakage expressed physiological status of plant itself, we selected model systems representing examples of physiological processes with well-known effects on membrane permeability (Table 1). These phenomena were water stress and senescence that have been shown to make membrane more leaky. In addition, we compared the leakage of silicated leaves with leaves grown under silica deficient condition, because silica is considered to increase the tolerance to environmental stress of plants, such as salt (Ahadam *et al.,* 1992), drought (Emadian and Newton, 1989), and temperature (Larcher *et al.,* 1991).

EL of PEG test was greater in senescing leaf (the 4th leaf from the youngest fully expanded leaf)than leaf in which senescence is less advanced (the youngest fully expanded leaf). Blum and Ebercon (1976, 1981) and Sullivan (1972) have reported that the younger leaf tissues are more tolerant to drought than older tissues in wheat and sorghum, respectively. Our results were well comparable with their results. The concept that membrane failure may be involved in leaf senescence was first suggested by Sacher

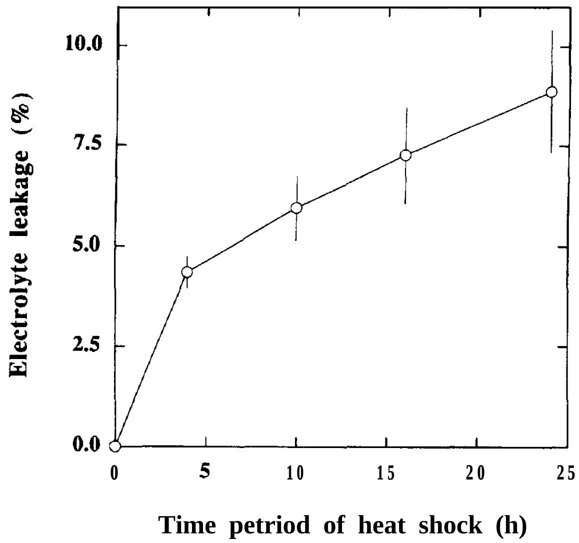

Fig. 4. Effect of time period of heat shock with 42°C test solution on electrolyte leakage.

Table 1. Effects of senescence, water stress and silicon on electrolyte leakage.

Leaf	Electrolyte leakage (%)
control	29.1±2.9
senescing	33.7±1.8
stress	38.4±1.8
-Si	38.6±2.1

Control, the youngest fully expanded leaf; senescing, the fourth leaf from the control leaf; stress, water-stressed leaf of the control leaf; -Si, the youngest fully expanded leaf grown in the nutrient solution without SiO_2.

(1957). Electron microscopy revealed a decrease in plastid volume and disruption of the internal mambrane system from senescing leaves (Butler and Simon, 1970). Biochemical studies showed that along with these morphological changes, there is change in the lipid composition of the membrane during senescence (Barton, 1966). The senescence-induced injury is likely to be assessed by the measurement of EL.

EL of leaf tissue was also increased with water deficit of leaf. Water deficits profoundly alter both the structure and the functions of membranes, leading to destructive events such as transition from liquid-crystalline to gel phase, fusion and increased permeability (Crowe *et al.*,1987; Ferrari-Ilion *et al.*,1984; Maroti *et al.*,1984;

Senaratna *et al.,* 1984). This results indicate that EL reflect stress-induced changes in the condition of cellular membranes.

It has been well documented that dry matter production and grain yield of rice is significantly increased by silica application to rice paddies. The growth-promoting effects by silicon application was significant when the plants were grown under stress-inducing conditions, especially drought. This indicate that silica involved in the stress tolerance, assumably with increasing cell membrane stability to water stress condition, In this study, EL was increased in Si deficit leaf to the same levels as water-stressed leaf, indicating silicon has an effect on increasing cell rigidity related to a building drought tolerance.

Collectively, these experiments indicate that the EL from tissues can evaluate the damage to membrane incurred by stress experiences, and thus, this technique would provide useful information to describe the behavior of plant tissues against environmental stresses in rice.

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