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SENESCENCE OF RICE LEAVES

XV. Solute Leakage and Inorganic Phosphate Uptake

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Abstract

Solute leakage and inorganic phosphate uptake during senescence of rice leaves were investigated. In general, solute leakage increased during senescence. An interesting finding of this investigation was the pattern of the changes of K^+ release and conductivity during dark-induced senescence of detached rice leaves. Potassium ion was released to the external solution during the first 24 h of dark incubation, while in the following period K^+ was effectively taken up by leaf segments. The release of K^+ at earlier stage of dark incubation seems to play some important roles in dark-induced senescence.

Both abscisic acid (ABA) and cycloheximide (CHI) inhibited, but benzyladenine (BA) promoted uptake of inorganic phosphate. The uptake of inorganic phosphate promoted by BA and inhibited by ABA is likely mediated through coupling and uncoupling of oxidative phosphorylation, respectively. Uncoupling of oxidative phosphorylation is unlikely to be the primary action of CHI in inhibiting inorganic phosphate uptake.

Key words: Abscisic acid; leaf senescence; solute leakage; ***Oryza sativa***; phosphate uptake.

Introduction

In literature, there are a number of evidences demonstrating that the membranes lose some of their integrity during leaf senescence. For example, Das (1968) found that the rate of leaching of electrolytes from old bean leaves was 3 to 4 times that from young leaves and increased with age up to at least 6 weeks. Poovaiah and Leopold (1973) also showed that the rate of release of tritiated water from corn leaf discs after 4 days' senescence in the dark was 10 times the rate from the fresh leaves. Conventionally, leaf senescence is studied by incubating detached leaf segments or leaf discs with water or test solution under dark condition. It is likely that solutes released from leaf segments or leaf discs via cut edge may play importment roles in regulating dark-induced senescence. The main purpose of this investigation, therefore, was to examine whether solute leakage is the principal cause or the result of dark-induced senescence.

In a previous work, we reported that abscisic acid (ABA) stimulated but benzyladenine (BA) inhibited respiration rate during senescence of rice leaf segments and suggested that ABA acted as an uncoupler of oxidative phosphorylation (Kao, 1985). The uncoupling process is normally accompanied by an increase in oxygen uptake and a decrease in uptake of inorganic phosphate (Hemberg, 1978). For this reason, it was also of interest to study whether uptake of inorganic phosphate by rice leaf segments was inhibited by ABA and promoted by BA.

Materials and Methods

Plant Materials and Incubation Condition

Rice (*Oryza sativa* L. cv. Taichung 1) seedlings were cultured as previously described (Kao, 1980). The apical 3-cm segment excised from the third leaves of 12- to 15-day-old seedlings was used. A group of 10 segments was floated in a 50 ml flask containing 10 ml deionized water. For the experiments of inorganic phosphate uptake, a group of segments was vertically placed in a test tube, which contained 2 ml of 10^{-3} M phosphate solution (pH 6.0) with or without ABA, BA or cycloheximide (CHI). Incubation was carried out at 27°C in the dark for the desired period. All experiments were repeated at least three times. Similar results and identical trends were obtained each time. The data reported here were from a single experiment.

Chlorophyll Determination

Chlorophyll was extracted and determined as described before (Kao, 1980). Chlorophyll was expressed as A_{665} per 10 segments in 10 ml 80% ethanol.

Deiermination of Solute Leakage

Aliquots of incubation media were removed for analysis of K^+, conductivity, amino-nitrogen, inorganic phosphate and sugar. K^+ was measured by flame photometer, conductivity of the external solution was measured directly by conductivity meter. Sugar was estimated by the anthrone reagent method (Yoshida *et al.*, 1972). Phosphate was measured according to the method described by Yoshida *et al.* (1972). Amino-nitrogen was determined using ninhydrin absorbance at 570 nm (Kao, 1980).

In experments of the effect of plant age on the solute leakage of intact rice leaves, the method of Venkatarayappa *et al.* (1984) was followed to measure solute leakage. Ten apical 3-cm leaf segments taken from the third leaves of various ages were submerged in a test tube containing 10 ml deionized water and shaked continuously. After 5 h the conductivity of the solution was measured. The leaf segments were then boiled for 20 min, and the conductivity was again measured. The percentage leakage was calculated as followeds:

$$\text{Percentage leakage} = \frac{\text{Conductivity before boiling}}{\text{Conductivity after boiling}} \times 100$$

Determination of Inorganic Phosphate Uptake

Uptake of inorganic phosphate by rice leaf segments was determined according to the method reported by Hemberg (1978). Briefly, inorganic phosphate uptake was determined on the basis of the amount of phosphate remaining in the external solution after 24 h of incubation compared to the same external solution without leaf segments. Since inorganic phosphate may release from leaf segments during incubationtion. The values calculated as above were further correcetd by the amount of inorganic phosphate released from segments.

Results

Leakage of Solutes during Senescence

The senescence of rice leaves was followed by measuring the decrease of chlorophyll. Figure 1 shows the time course of chlorophyll content of leaf segments floating on water in the dark. The decrease of chlorophyll content was evident at 2 days after leaf detachment under dark condition.

The content of K^+ in the external solution increased during the first 24 h of dark incubation and declined subsequently, indicating that there was an efflux of K^+ during the first 24 h, while in the following period K^+ was effectively taken up

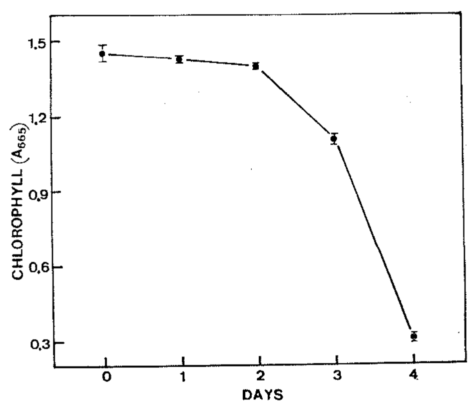

Fig. 1. Changes of chlorophyll content of detached rice leaves incubated in the dark.

(Table 1). Since leaf segments were rinsed with deionized water several times before the experiments, the release of K^+ is unlikely due to the release of K^+ present on the outside of leaves. The changes of conductivity of external solution were almost similar to those of K^+, i.e., conductivity increased during the first 24 h and declined subsequently (Table 1). These results seem to suggest that K^+ is responsible for part of conductivity. In contrast to our data, Trippi and Thimann (1983) showed that large increase of K^+ leakage and conductivity only occurred when senescence was far advanced.

The release of amino-nitrogen appeared in the external solution occurred only at the 4th day of dark incubation (Table 2), at that time leaf segments had the lowest level of chlorophyll (Fig. 1). This was because both proteolysis and membrane leakage are required for the release of amino-nitrogen. Generally, the levels of phosphate and sugar in the external solution increased slightly during the course of senescence (Table 2). The levels of phosphate and sugar found in rice leaf segments were rather high, which were about 0.3 μmol and 99 μg per segment, respectively. In view of those higher endogenous levels of phosphate and sugar, the amount released into external solution was negligible. Therefore, the release of phosphate and sugar seems unlikely to play the primary role in causing dark-induced senescence of rice leaf segments.

Table 1. *Changes of conductivity and release of K^+ from leaf segments senescing in the dark in 10 ml deionized water*

Days	K^+ (μg/segment)	Conductivity ($\mu\Omega$/cm)
1	0.42±0.02	0.28
2	0.12±0.08	0.12
3	0.04±0.02	0.19
4	0.04±0.03	0.13

Table 2. *Changes of leakage of amino-nitrogen, phosphate and sugar from leaf segments senescing in the dark in 10 ml deionized water*

Days	Amino-nitrogen (μmol/segment)	Phosphate (μmol/segment)	Sugar (μg/segment)
1	0	0.005±0.001	0.11±0.06
2	0	0.009±0.003	0.20±0.02
3	0	0.009	0.20±0.07
4	0.004±0.001	0.012±0.001	0.14±0.01

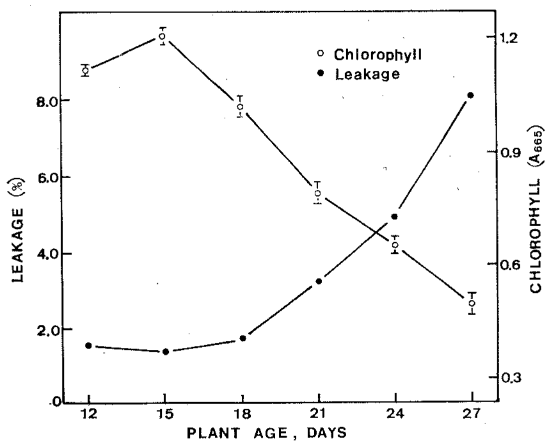

Fig. 2. Effect of plant age on the chlorophyll content and percentage leakage. Apical 3-cm leaf segments of the third leaves taken from various ages were used to determine chlorophyll content and percentage leakage.

The levels of chlorophyll and leakage of solutes in the third leaf from 12- to 27-day-old seedlings are shown in Fig. 2. Chlorophyll content in intact leaves decreased with increasing age, whereas the solute leakage increased with increasing age. However, chlorophyll degradation preceded solute leakage. Therefore, in intact seedling leaves, the change of membrane permeability, expressed by percentage leakage of solutes, was the result rather than the cause of senescence.

Uptake of Inorganic Phosphate by Rice Leaf Segments

Table 3 shows the effect of ABA on the uptake of inorganic phosphate by leaf segments detached from mature and senescing leaves. ABA inhibited the uptake of inorganic phosphate by both mature and senescing leaves. The uptake of inorganic

Table 3. *Effect of ABA on the uptake of inorganic phosphate by leaf segments incubated for 24 h in the dark*

Twenty apical 3-cm leaf segments were incubated in 2 ml phosphate solution (10^{-3}M, pH 6.0).

ABA	Amount taken up (*n*mol)	
	mature leaves (12-day-old)	Senescing leaves (17-day-old)
0	158	54
10^{-5}M	124	34
10^{-4}M	34	0

Table 4. *Effect of BA and CHI on the uptake of inorganic phosphate by leaf segments incubated for 24 h in the dark*

Twenty apical 3-cm leaf segments were incubated in 2 ml phosphate solution (10^{-3}M, pH 6.0).

Treatment	Amount take up (*n*mol)	
	Mature leaves (12-day-old)	Senescing leaves (17-day-old)
Water	140	74
BA, 10^{-5}M	179	130
CHI, 100 μg/ml	44	30

phosphate was also inhibited by CHI, an inhibitor of protein synthesis (Table 4). However BA, a synthetic cytokinin, promoted the uptake of inorganic phosphate by both mature and senescing leaves (Table 4). Data in Tables 3 and 4 all showed that senescing leaves had much lower ability to take up inorganic phosphate, suggesting that the ability of inorganic phosphate uptake was decreased during leaf senescence.

Discussion

From the data presented in this paper, it clearly showed that the loss of membrane permeability was certainly a normal accompaniment of senescence. The significant finding of this investigation was the pattern of the changes of K^+ release and conductivity shown in Table 1. Potassium ion was released to the external solution during the first 24 h of dark incubation, while in the following period K^+ was effectively taken up by leaf segments. These results were in contrast to those reported by Trippi and Thimann (1983), but similar to those found during germination of *Raphanus sativus* (Cocucci and Cocucci, 1977). During the first ten hours of germination, there was a general efflux of K^+, while in the following period K^+ was effectively taken up from the external medium.

Thimann and Satler (1979a, 1979b) demonstrated the parallelism between stomatal aperture and senescence. Senescence is strongly promoted by treatments that cause stomata to close. They, therefore, proposed that the stomatal aperture is the causal factor of leaf senescence. Recently, Gepstein (1982) demonstrated the existence of H^+-pump ATPase and close correlation between the cessation of H^+ pumping and acceleration of senescence. The transport of K^+ into and out of guard cells has been suggested to be an important factor in regulating the movement of guard cells (Zeiger, 1983). Recently, we found that stomatal aperture decreased prior to senescence, but increased in senescing rice leaves (unpublished data). These results would explain why K^+ was released prior to senescence and taken up by senescing leaves as shown in Table 1. It seems likely that the release of K^+ at earlier stage of incubation may play some important roles in dark-induced senescence of detached rice leaves.

Most, if not all, of the work concerning the effect of hormones on the uptake of inorganic phosphate is conducted by using sliced storage tissue. Relatively little work has been done by using leaf system. In potato pith discs, Hemberg (1978) found that ABA inhibited uptake of inorganic phosphate and stimulated oxygen uptake and suggested that it acted as an uncoupler of oxidative phosphorylation. By using rice leaves, we showed that both ABA and CHI inhibited, but BA promoted uptake of inorganic phosphate. CHI is generally used as an inhibitor of protein synthesis. But the use of CHI as specific protein synthesis inhibitor has been questioned by Ellis and MacDonald (1970), who suggested that CHI inhibited ion absorption as a consequence of its uncoupling of respiration. In the previous work, we found that ABA stimulated, but CHI inhibited respiration rate of rice leaves (Kao, 1985). Therefore, uncoupling of oxidative phosphorylation is unlikely to be the primary action of CHI-inhibited uptake of inorganic phosphate, but more likely to be the primary action of ABA. The primary action of CHI is probably on protein synthesis.

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水稻葉片老化之研究

(十五) 溶質之滲漏與無機磷之吸收

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本試驗主要探討臺中在來一號水稻葉片老化過程中溶質之滲漏與無機磷吸收之情形。一般而言，溶質之滲漏隨著葉片老化程度之增加而增加。研究結果顯示切離葉片在暗中老化時，早期 K^+ 由葉片流到培養液中，後期時葉片則吸收 K^+。早期 K^+ 之流出對於暗中切離葉片之老化扮演重要角色。

ABA 與 CHI 抑制葉片組織吸收無機磷，而 BA 促進無機磷之吸收。ABA 與 BA 對無機磷吸收之效應可能是經由氧化磷酸化反應之機制。而 CHI 之抑制無機磷吸收可能與其抑制蛋白質合成有關。