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SENESCENCE OF RICE LEAVES

XIV. Changes of Respiration during Senescence of Detached Leaves

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Abstract

Some natures about respiration of detached rice leaves during senescence under both light and dark conditions were investigated. The rise of respiration rate during senescence was preceeded by a steep fall. The maximum respiration rate, which occurred at 3rd day of incubation under both light and dark conditions, was about twice the minimum respiration rate. The sensitivity of respiration to cyanide decreased as senescence progressed. Furthermore, during senescence the decrease was also observed in the degree of inhibition of respiration by malonate, suggesting the role of the TCA cycle system in total respiration decreased during senescence. The respiration rate was lowered by cycloheximide, α,α'-dipyridyl, Ni^{2+}, and CO_2 treatments, which delayed dark senescence. However, light and low temperature, which also delayed dark senescence, promoted the respiration rate.

Under both light and dark conditions, benzyladenine (BA) inhibited but abscisic acid (ABA) promoted respiration. Evidence indicated that in senescent rice leaves respiration and ATP production were still coupled together. BA seems to keep respiration and ATP production coupled more tighter, whereas ABA makes these two become uncoupled. This conclusion was based on observations that (a) BA treated leaves was more sensitive to dinitrophenol than senescent leaves and (b) ABA treated leaves showed relatively little effect of DNP.

Key words: Leaf senescence; *Oryza sativa L.*; respiration; rice.

Introduction

The relation between respiration and leaf senescence is not a simple one. If senescence is viewed as a general decline in function (Thimann 1980), steady decline of respiration rate would be expected. On the other hand, synthesis of degradative enzymes and nutrient mobilization processes during senescence all seem to require higher respiratory activity (Stoddart and Thomas, 1982; Thimann 1980). Studies on respration changes (determined either by CO_2 evolved or O_2 uptake) during

senescence have been made on either attached or detached leaves of several plant species including *Acer*, barley, *Lolium temulentum*, oats, pea, *Perilla frutescens* and tobacco (Rhodes 1980). Recently, Satler and Thimann (1983) extensively studied the relation between respiration and senescence in oat leaves. In general, respiration rate increases during senescence in various plant materials.

Since no similar work has been made by using rice leaves and a number of other metabolic changes have already been followed in rice leaves during senescence (Kao, 1978; 1980; 1981; Kao and Yang, 1983; Wang and Kao, 1983; Wang *et al.*, 1982), a systematic study of respiration changes in our senescing rice leaves would be valuable. The present work describes some natures about the respiration of detached rice leaves during senescence. The results also showed that not all treatments, which retarded senescence, inhibited respiration rise.

Materials and Methods

Plant Material and Incubation Condition

Rice (*Oryza sativa* cv. Taichung Native 1) seedlings were cultured as described elsewhere (Kao and Yang, 1982). The apical 3 cm of the third leaves of 9-day-old seedlings was used for experiments. Ten rice leaf segments were floated on 10 ml of distilled water or test solution in a 50-ml flask. The flasks were then incubated at 30°C either under light (80 μmol quanta $m^{-2} s^{-1}$) provided by a mixture of cool-white and Grolux tubes, or in darkness. All experiments were repeated at least four times. Similar results and identical trends were obtained each time. The data reported here were from single experiment.

Chlorophyll Determination

Chlorophyll was extracted with boiling 80% ethanol and determination as described before (Kao, 1980). Chlorophyll was expressed as A_{665} per ten leaf segments.

Determination of Respiration Rate

Leaf segments were placed into 14-ml test tubes containing 1.5 ml of deionized water, and sealed with rubber serum caps after flushing with fresh air. Repiration rates were determined by measuring the CO_2 accumulated in darkness at 30°C during a 2-h period; rate was linear for 4 h. One-ml gas sample was withdrawn from the headspace of the test tube. CO_2 was assayed using a gas chromatograph (Model 800, Carle Instruments, Inc., USA) equipped with silicagel column and a thermoconductivity detector. Respiration rate was expressed as μl CO_2 evolved per g fresh weight per h.

Results

Typical time course curves for the respiration of detached rice leaves under light and dark conditions are shown in Fig. 1. Respiration rate decreased during early stage of incubation, but it started to increase after about 12 and 48 h incubation in the light and dark, respectively. Under both light and dark conditions, respiration peaked at a later stage (about 72 h after incubation), during which time leaf segments had low chlorophyll level (Fig. 2). The maximum respiration rate was about twice the minimum respiration under both light and dark conditions.

Table 1 and 2 present the responses to cyanide and malonate, respectively.

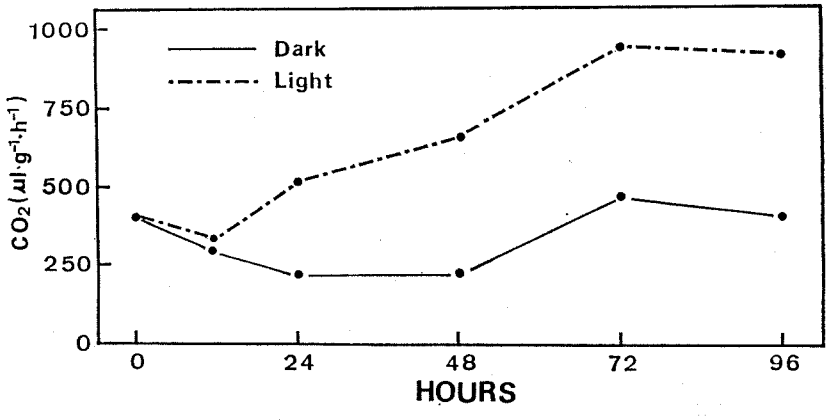

Fig. 1. Changes of respiration during senescence of rice leaf segments under light and dark conditions.

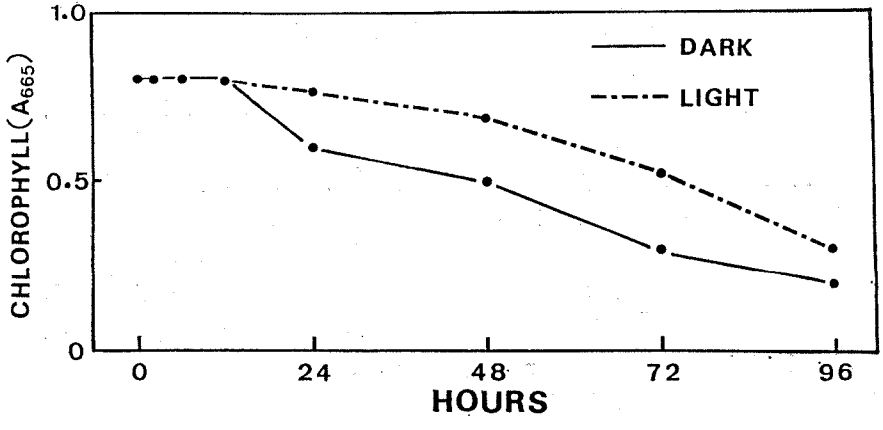

Fig. 2. Changes of chlorophyll level in rice leaf segments incubated under light and dark conditions.

Table 1. *Cyanide sensitivity of respiration during senescence of rice leaf segments*

Leaf segments were floated on water for 0, 2 or 4 days, then vacuum infiltrated with water or KCN (10 mM) for 5 min in darkness and CO_2 evolution was measured 30 min later.

Time (days)	CO_2 ($\mu l \cdot g^{-1} h^{-1}$)					
	Darkness			Light		
	−CN	+CN	% inhibition	−CN	+CN	% inhibition
0	460	90	80	460	90	80
2	301	135	55	550	189	66
4	413	150	64	543	178	67

Table 2. *Malonate sensitivity of respiration during senescence of rice leaf segments*

Leaf segments were floated on water for 0, 2 or 4 days, then vacuum infiltrated with water or malonate (20 mM) for 5 min in darkness and CO_2 evolution was measured 30 min later.

Time (days)	CO_2 ($\mu l \cdot g^{-1} h^{-1}$)					
	Darkness			Light		
	−malonate	+malonate	% inhibition	−malonate	+malonate	% inhibition
0	432	313	28	432	313	28
2	303	257	15	514	487	5
4	422	403	5	555	503	9

It clearly showed that the sensitivities of respiration to cyanide and malonate decreased during senescence under both light and dark conditions.

Light is known to retard chlorophyll loss (Thimann *et al.*, 1977). For rice leaf segments, light also strongly delayed chlorophyll loss as compared with the dark control (Fig. 2). However, light substantially promoted respiration rate throughtout the course of senescence (Fig. 1). Low temperature, which retarded chlorophyll loss under dark condition, also promoted respiration (Table 3). In contrast to the effect of light and low temperature, cycloheximide (CHI), α, α'-dipyridyl (DP), Ni^{2+} and CO_2 treatments, which significantly retarded chlorophyll loss under dark condition, all inhibited respiration rate (Table 3).

It has long been recognized that cytokinins are effective in retarding the senescence of most, if not all, leaves. The effect of cytokinins in retarding senescence is species- or variety-specific, for the rice variety used in this investigation,

Table 3. *Effects of CHI, DP, Ni^{2+} and low tew temperature or CO_2 treatment on respiration rate and chlorophyll content of rice leaf segments in darkness*

Chlorophyll and respiration rate were determined after 3.5 days. Control in the experiment 2 was CO_2-free which was achieved by hanging in the flask a center well containing a filter paper wetted with 0.2ml of 20% KOH; for CO_2 treatment the leaves were incubated in 3% CO_2.

Treatment	Chlorophyll (A_{665})	CO_2 ($\mu l \cdot g^{-1} h^{-1}$)
Experiment 1		
Control	0.408	378
CHI, 0.1 mM	0.967	142
DP, 0.1 mM	0.905	124
Ni^{2+}, 10 mM	0.969	75
Low temperature, 5°C	1.037	546
Experiment 2		
Control	—	383
CO_2	—	286

BA has been found to be the most active cytokinin in retarding senescence in the dark (Kao, 1988). Although BA effectively retarded senescence of leaf segments in the light, as well as in the dark (Kao and Yang, 1984), BA significantly inhibited respiration rate (Fig. 3). ABA has been shown to promote chlorophyll loss of rice leaf segments during senescence under both light and dark conditions (Kao and

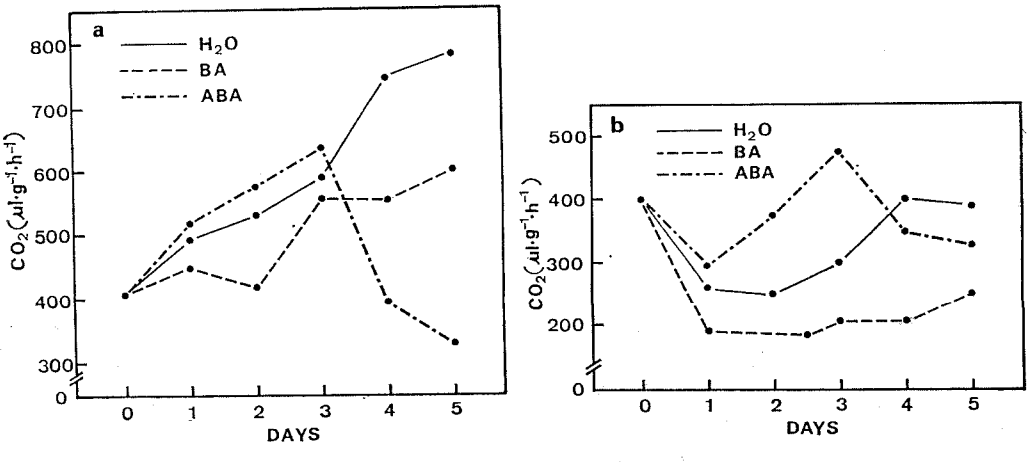

Fig. 3. Changes of respiration of rice leaf segments incubated in BA (10^{-5}M) or ABA (10^{-4}) under light (a) and dark (b) condition.

Yang, 1984). Respiration rate was also promoted by ABA (Fig. 3). The chlorophyll level in ABA treated leaf segments reached the lowest level at the third day under both light and dark conditions, indicating senescence was well advanced at the third day (data not shown). Death symptoms were shown after 3-day incubation. This would explain why respiration rate of ABA treated leaf segments started to decline after 3-day incubation in light and darkness.

The respiration rate in senescent and BA treated leaf segments was increased by DNP about 100% and 130% over the control rate, respectively, in darkness; and about 160% and 190%, respectively, in light (Table 4). On the other hand, ABA made the respiration rate highly DNP-insensitive (Table 4).

Table 4. *Effect of DNP on respiration of water, BA or ABA treated rice leaf segments*

Leaf segments were pretreated with water, BA or ABA for 3 days under light or dark condition, then vaccum infiltrated with or without DNP for 5 min in darkness and CO_2 determination was made 30 min later.

Pretreatment	DNP	Respiration	
		CO_2 $\mu l \cdot g^{-1} \cdot h^{-1}$	Increase over minus DNP (%)
dark + water	−	360	
	+	698	94
dark + BA	−	214	
	+	484	126
dark + ABA	−	431	
	+	577	34
light + water	−	613	
	+	1596	160
light + BA	−	516	
	+	1491	189
light + ABA	−	721	
	+	812	13

Discussion

A variety of patterns of respiratory behavior during leaf senescence has been observed. Beevers (1976) stated that respiratory activity was maintained at a fairly constant rate until the terminal phase of senescence when a rapid decline in respiration occurred. A steady decline in the respiration rate throughout the period of senescence has been observed by Richardson (1957) and Smilie (1962). In some leaves, on the other hand, the rise of respiration rate is preceded by a steep fall or a constant rate (Aharoni and Lieberman, 1979; McGlasson *et al.*, 1975; Tetley and Thimann, 1974; Woolhouse, 1967). In our system, respiration rate initially

decreased but increased at a later stage when leaf segments were senescent (Figs. 1 and 2). It seems that the rise of respiration in the present system is the result rather than the cause of leaf senescence.

The form and timing of the respiration rise during leaf senescence is suggestive like the climateric rise in the respiration of ripening fruits. In climateric fruits, the onset of respiration rise accompanied by the onset of ethylene production, whereas the ethylene production in rice leaves occurs considerably early than maximum respiration rate (Kao and Yang, 1984). Thus, it is unlikely that ethylene plays any causative role in inducing the rise of respiration rate during the senescence of rice leaf segments.

Compared with the dark controls, light was shown to promote respiration rate substantially throughout the course of senescence. Since respiration was determined under dark condition, the higher respiration rate is unlikely due to photorespiration, but is more likely due to the accumulation of respirable substrates from photosynthesis (Satler and Thimann, 1983).

Recently, Satler and Thimann (1983) showed that the respiration of oat leaves was insensitive to cyanide, but that of senescing leaves was sensitive to cyanide. In our rice system, however, the sensitivity to cyanide decreased as senescence progressed (Table 1). Our results are consistent with those found in potato tuber slices. The respiration of fresh potato slice is predominantly cyanide sensitive, but becomes cyanide insensitive with aging (Hackett *et al.*, 1960). Furthermore, during senescence of rice leaves the decrease was also observed in the degree of inhibition of respiration by malonate (Table 2). It seems that the respiration of the leaves developed a changed reliance on succinate oxidation by the mitochandria. The role of the TCA cycle system in total respiration, therefore, may decrease during senescence. The decrease of sensitivity of respiration to malonate has also been found in wheat and barley leaves during senescence (Lustinec and Pokorna, 1962; Udvardy *et al.*, 1964). The decrease of sensitivities to cyanide and malonate in our rice system is unlikely due to the decrease of the uptake of cyanide and malonate, since membrane permeability increased during senescence (unpublished data).

The respiration rate was lowered by CHI, DP, Ni^{2+} and CO_2 treatments, which delayed dark senescence (Table 3). However, light and low temperature, which also delayed dark senescence, promoted the respiration rate (Fig. 1 and Table 3). It seems that not all treatments (or chemicals), which delay dark senescence, inhibit respiration rate, suggesting different treatments (or chemicals) exert its effect on respiration via different mechanisms. Satler and Thimann (1983) recently reported that lower aliphatic alcohols delayed dark senescence and yet promoted respiration rate.

Inhibition of respiration by CO_2 may possibly be due to the closure of stomata. Generally, low temperature decreases the metabolism of leaves, which in turn

would cause the leaf segments having higher level of respirable substrates as compared with the control. This would explain why low temperature treated leaf segments tended to have higher respiration rate than the control.

Cytokinins were shown to inhibit respiration rate during oat senescence under dark condition (Tetley and Thimann, 1974; Satler and Thimann, 1983). We here reported that BA also inhibited respiration rates during senescence of rice leaves under both dark and light conditions (Fig. 3). In excised stems and flowers, cytokinins have also been shown to inhibit respiration (Dedolph *et al.*, 1961, 1962; Kastsumi, 1963; Maclean and Dedolph, 1962; Sugiura, 1963; Wittwer *et al.*, 1962). However, none has been reported on the effect of ABA on respiration during leaf senescence. In our system, we found that ABA substantially promoted respiration (Fig. 3). The stimulating effect induced by ABA may be related to the results obtained by Hemberg (1978) with potato tuber discs.

DNP uncouples phosphorylation from respiratory electron transport in almost all known tissues and therefore is regarded as uncoupler *par excellence*. Rice leaves that had senesced for three days under both light and dark conditions showed drastically the effect of DNP (Table 4), suggesting that in senescent leaves respiration and phosphorylation are still coupled. These results are in contrast with those of Teltley and Thimann (1974), who reported that in senescent oat leaves respiration became uncoupled from phosphorylation. The mode of action of BA seems to keep respiration and ATP production coupled much tighter, while that of ABA seems to make these two become uncoupled. This conclusion was based on the observations (under both light and dark conditions) that (a) BA treated leaves was more senestive to DNP than senescent leaves and (b) ABA treated leaves showed relatively little effect of DNP (Table 4). The responses to DNP of BA and ABA treated leaves would explain why BA inhibited but ABA promoted respiration rate.

Acknowledgements

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水稻葉片老化之研究

(十四) 切離葉片老化過程呼吸速率之變化

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本研究主要探討切離之水稻葉片在光照與黑暗下老化時呼吸速率之變化與呼吸作用之一些特性。老化初期呼吸速率降低，而後期增加。最大呼吸速率（約處理後三天）爲最小呼吸速率之二倍。老化後期呼吸作用對氰酸與 malonate 之敏感度降低。

一些可以延緩老化之藥劑與處理如 cycloheximide, α, α'-dipyridyl, Ni^{2+} 與二氧化碳抑制呼吸速率之增加，光線與低溫雖亦可延緩老化，但促進呼吸速率之增加。

不論在光照或黑暗下，BA 抑制而 ABA 促進呼吸速率之增加。可能的原因是 BA 使 ATP 形成與電子傳遞 couple 在一起，而 ABA 則使 ATP 形成與電子傳遞 uncouple。