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Changes in soluble sugar content and respiration rate in methyl jasmonate-treated rice leaves

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Abstract. The changes in respiration rate and soluble sugar content of detached rice (*Oryza sativa*) leaves were followed during methyl jasmonate (MJ)-promoted senescence. Increase in the respiration of detached rice leaves was observed as a consequence of methyl jasmonate (MJ) treatment for 48 h. However, MJ had no effect on photosynthetic rate in rice leaves. Sucrose and glucose contents, but not fructose content, were lower in MJ-treated rice leaves than in control leaves. KCN, an inhibitor of cytochrome *c* oxidase, was effective in inhibiting MJ-increased respiration rate, in reducing MJ-decreased sucrose and glucose contents, and in retarding MJ-promoted senescence of rice leaves. Exogenous addition of sucrose to MJ-treated rice leaves increased endogenous sucrose and glucose contents, but did not prevent MJ-promoted senescence of rice leaves. The role of respiration and sucrose in regulating MJ-promoted senescence of rice leaves is discussed.

Keywords: Fructose; Glucose; Methyl jasmonate; *Oryza sativa;* Respiration; Senescence; Sucrose.

Introduction

Jasmonic acid and its methyl ester, methyl jasmonate (MJ), have been proposed as naturally occurring plant growth regulators because of their wide natural distribution (Meyer et al., 1991) and their effects on many physiological processes in plants (Sembdner and Parthier, 1993). Jasmonates have been shown to be powerful promoters of leaf senescence (Ueda and Kato, 1981; Weidhase et al., 1987; Cuello et al., 1990). We also reported that MJ was effective in promoting the senescence of detached rice leaves in the dark (Chou and Kao, 1992; Tsai et al., 1996; Hung and Kao, 1997; Chen and Kao, 1998).

The relation between respiration and leaf senescence is not a simple one. If senescence were viewed as a general decline in function (Thimann, 1980), a 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 a higher respiration rate (Thimann, 1980). Studies on respiration changes (determined either by CO_2 evolved or O_2 uptake) during senescence have been made on either attached leaves or detached leaves of several plant species (Rhodes, 1980). In general, respiration rate increases during senescence in various plant materials. Kinetin, a synthetic cytokinin, at a concentration which retards senescence of detached oat leaves, completely prevents the respiration rise (Tetley and Thimann, 1974; Satler and Thimann, 1983). Recent experiments by Dufour et al. (2000) demonstrated that the respiration function is a key factor contributing to a shorter lifespan for the filamentous fungus *Podospura anserine*.

Several reports show that the soluble sugar content often goes up, not down, in senescing leaves (Shiroya et al., 1961; Trippi, 1965; Egli et al., 1980; Lazan et al., 1983; Crafts-Brandner et al., 1984), and sugar accumulation in leaves suppresses the expression of some genes related to photosynthesis (Gan and Amansino, 1997; Jang et al., 1997). It has even been proposed that an elevated sugar content may actually cause senescence (Lazan et al., 1983).

MJ treatment has been shown to stimulate respiration of apple fruit (Miszczak et al., 1995; Fan and Mattheis, 1999), but it did not change the normal climacteric rise in the respiration of mango fruit (Gonazalez et al., 2000). However, to our knowledge, no one has studied the effect of MJ on the change in respiration and soluble sugars in leaves. The work reported here, therefore, explores the relations between respiration, soluble sugars, and the MJ-promoted senescence of rice leaves.

Materials and Methods

Rice (*Oryza sativa* cv. Taichung Native 1) seeds were sterilized with 2.5% sodium hypochlorite for 15 min and washed extensively with distilled water. These seeds were then germinated in Petri dishes with wetted filter paper at 37°C in the dark. After 48 h incubation, uniformly germinated seeds were selected and cultivated in a 500 ml beaker containing half-strength Kimura B solution as described previously (Chu and Lee, 1989). The hydroponi-

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cally cultivated seedlings were grown for 12 days in a Phytotron with natural light at 30°C day/25°C night and 90% relative humidity. The apical 3 cm of the third leaf was used in all experiments. A group of ten segments was floated in a Petri dish containing 10 ml of distilled water or test solution. Incubation was carried out at 27°C in the dark.

The senescence of detached rice leaves was followed by measuring the decrease of chlorophyll and protein. Chlorophyll content was determined according to Wintermans and De Mots (1965) after extraction in 96% (v/v) ethanol. For protein extraction, leaf segments were homogenized in 50 mM sodium phosphate buffer (pH 6.8). The extracts were centrifuged at 17,600 *g* for 20 min, and the supernatants were used for determination of protein by the method of Bradford (1976).

For sugar determination, aliquots of boiling 80% ethanol extracts (0.1-0.2 ml) were evaporated to dryness. The contents of glucose, fructose and sucrose were determined using an enzyme-coupled assay based on hexokinase (EC 2. 7. 1. 1, 0.14 unit) and glucose-6-phosphate dehydrogenase (EC 1. 1. 1. 49, 0.07 unit), and using phosphoglucoisomerase (EC 5. 3. 1. 9, 0.2 unit) and invertase (EC 3. 2. 1. 26, 80 units) for fructose and sucrose interconversions (Cairns, 1987).

Photosynthetic rates were measured by using a Hansatech leaf disc oxygen electrode system to monitor the amount of O_2 evolved (Delieu and Walker, 1981). Respiration rates were determined either by measuring CO_2 evolved using a modified CO_2 analyzer (LI-6250 Gas Analyzer, LI-COR, Inc., Nebraska, USA) equipped with LI-2000 Console or by measuring O_2 uptake using Hansatech leaf disc oxygen electrode (Delieu and Walker, 1981).

Each experiment was performed four times with similar results. All values are shown as the means ± SE of four replicates obtained from four independent experiments.

Results and Discussion

Detached leaves or leaf segments are often used in senescence studies even if the senescence-induced changes in detached leaves may differ from the changes occurring in leaves attached to a plant. The detached leaves, however, represent a simple system in which the effect of the rest of the plant (including relocation of assimilates, water, and nutrients) is eliminated, and they are easy to handle and incubate under controlled conditions. Thus, detached rice leaves were used in this study.

The most obvious character of leaf senescence is yellowing. Chlorophyll loss has been the principal criterion of senescence in most reports. The protein degradation during leaf senescence has been realized from the earliest studies. In the present study, the senescence of detached rice leaves was followed by measuring the decrease of chlorophyll and protein. It is clear that MJ significantly promotes senescence of detached rice leaves (Figure 1). These results are in agreement with our previous reports (Chou and Kao, 1992; Tsai et al., 1996; Chen and Kao, 1998).

To determine the role of soluble sugars (sucrose, glucose, and fructose) in MJ-promoted rice leaf senescence, we determined the changes in their contents. In control leaf segments, the contents of sucrose, glucose, and fructose decreased with the increase of incubation time (Figure 2). Sucrose and glucose contents in MJ-treated detached rice leaves were observed to be lower than in water-treated controls throughout the entire incubation (Figure 2). However, MJ had no effect on fructose contents (Figure 2). Results refute the suggestion that sugar accumulation may cause leaf senescence (Lazan et al., 1983). Figure 3 shows that exogenous application of sucrose increased the contents of sucrose and glucose in MJ-treated rice leaves, but had no effect on senescence. These results seem to suggest that MJ-promoted leaf senescence of rice is unlikely due to the decrease in sucrose and glucose contents. The decline of sucrose possibly occurs in the cytosol of MJ-treated rice leaves. However, we do not know whether the added sucrose reaching the

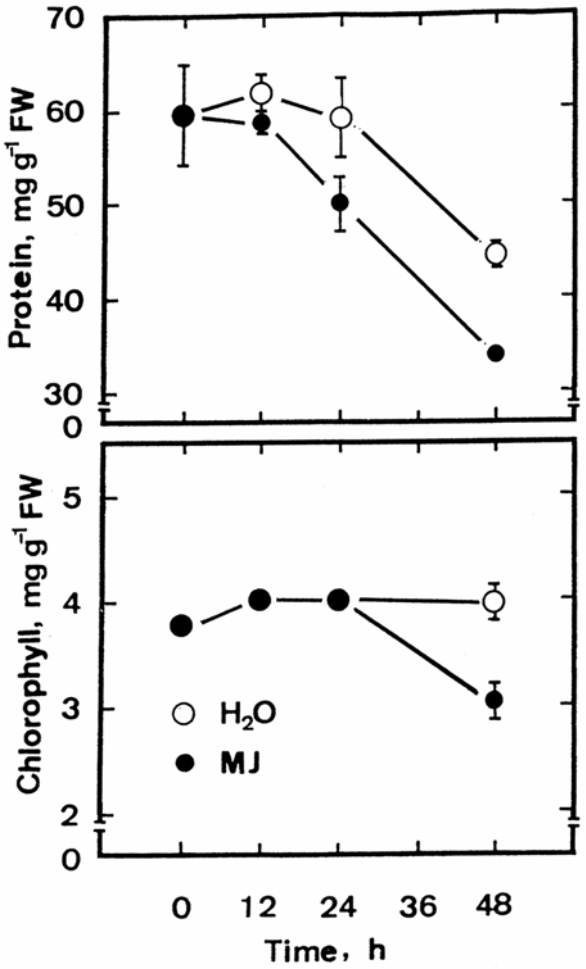

Figure 1. Time courses of protein and chlorophyll contents in detached rice leaves floating on water or 45 μM MJ in darkness. Bars indicate SE, $n = 4$.

cytosol is sufficient to play a regulatory role in these leaves. In this regard, we acknowledge that caution should be exercised when drawing conclusions about the role of sucrose in MJ-promoted senescence of rice leaves.

Sucrose and glucose could be used as key substrates of respiration. In this regard, it is of great interest to know the effect of MJ on the respiration rate of detached rice leaves. We measured respiration by CO_2 evolved and O_2 uptake in MJ-treated rice leaves. Respiration rates measured either by CO_2 evolved or O_2 uptake were found to be promoted by MJ (Figure 4). This is consistent with the case of apple fruit, in which MJ was found to increase respiration rate (Miszczak et al., 1995; Fan and Mattheis, 1999). Figure 4 also shows that MJ had no effect on photosynthetic rates in rice leaves, which is consistent with the results in *Pisum sativum* (Velitchkova and Fedina, 1998). These results indicate that the decrease in sucrose and glucose contents in MJ-treated rice leaves is not attributable to the decrease in photosynthetic rate. It seems that sucrose and glucose are most likely utilized as substrates of respiration in these leaves.

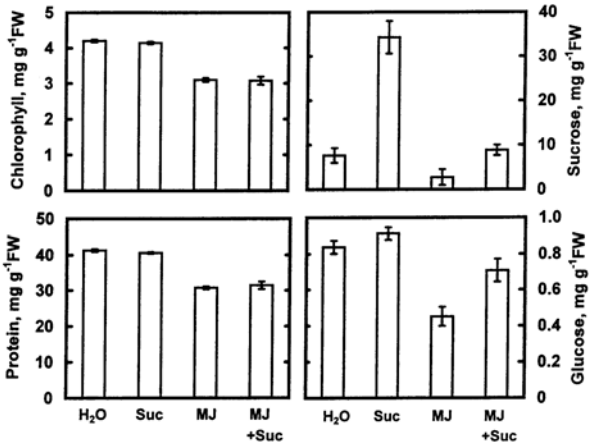

Figure 3. Effect of exogenous sucrose on the contents of chlorophyll, protein, sucrose, and glucose in rice leaves treated with MJ. The concentrations of MJ and sucrose were 45 µM and 50 mM, respectively. For all treatments, 0.25 mg l^{-1} chloramphenicol was added to prevent bacterial growth. All measurements were made 48 h after treatment. Bars indicate SE, n = 4.

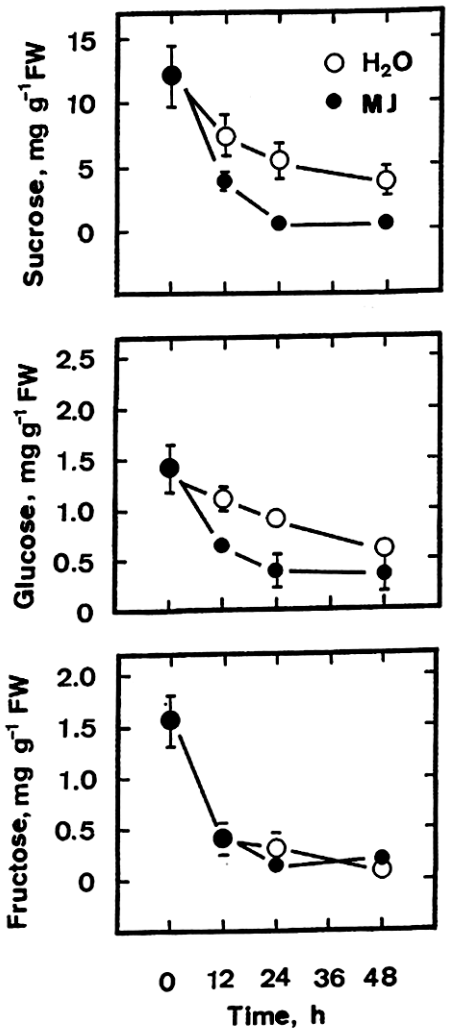

Figure 2. Time courses of sucrose, glucose, and fructose contents in detached rice leaves floating on water or 45 µM MJ in darkness. Bars indicate SE, n = 4.

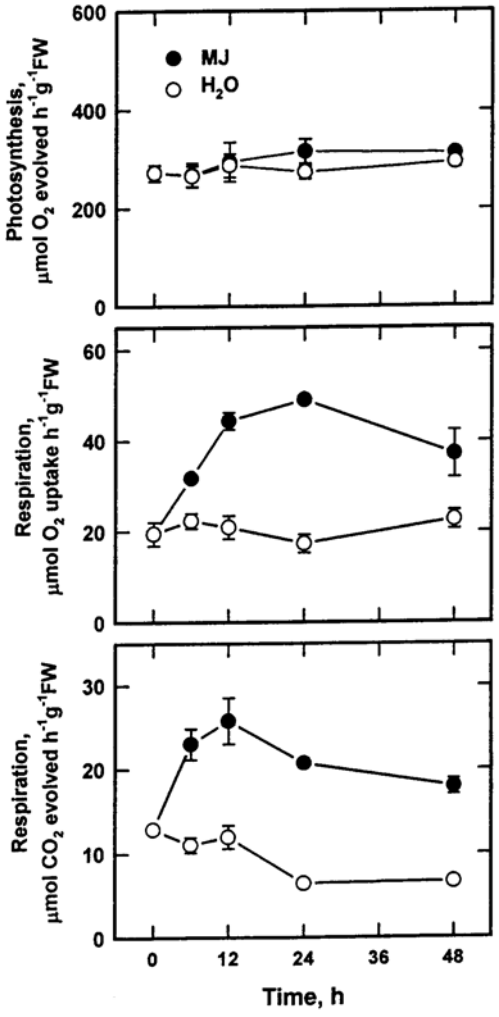

Figure 4. Time courses of respiration rates and photosynthetic rates in detached rice leaves floating on water or 45 µM MJ in darkness. Bars indicate SE, n = 4.

As is well known, the respiration of many plant tissues is sensitive to cyanide. Clearly, treatment with KCN inhibited MJ-increased respiration rate and reduced MJ-decreased sucrose and glucose contents (Figure 5), suggesting that a cyanide-sensitive pathway is operating and that sucrose and glucose are utilized as substrates of respiration in MJ-treated rice leaves. However, *n*-propyl-gallate and salicylhydroxamic acid, inhibitors of alternative oxidase (Schonbaum et al., 1971; Josse et al., 2000), had no effect on MJ-induced increase in respiration rate or the decrease in sucrose and glucose contents (data not shown). It seems that no alternative respiration pathway is operating in MJ-treated detached rice leaves. Recently,

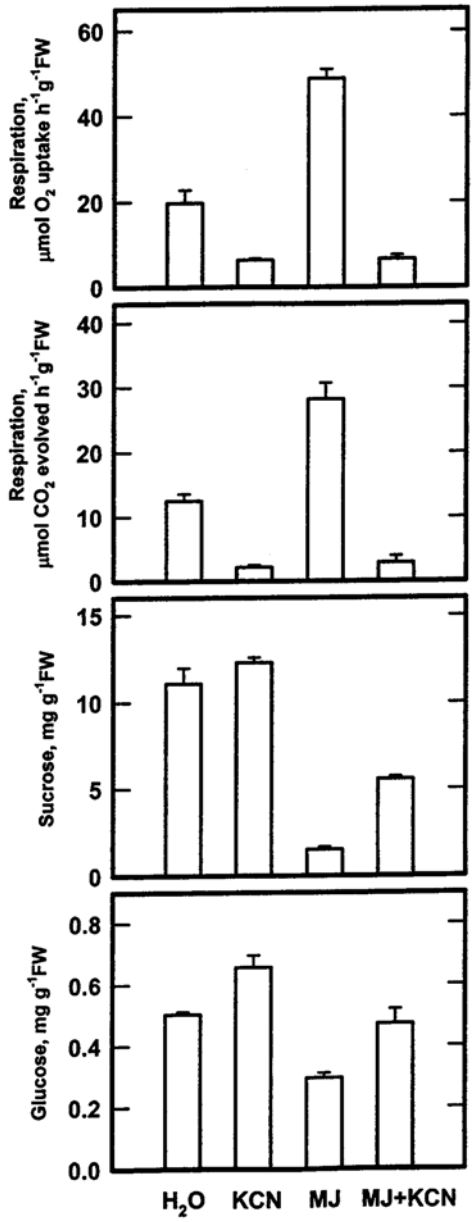

Figure 5. Effect of MJ (45 μM) on respiration rate, and the contents of sucrose and glucose in detached rice leaves in the presence or absence of 5 mM KCN. All measurements were made 24 h after treatment. Bars indicate SE, *n* = 4.

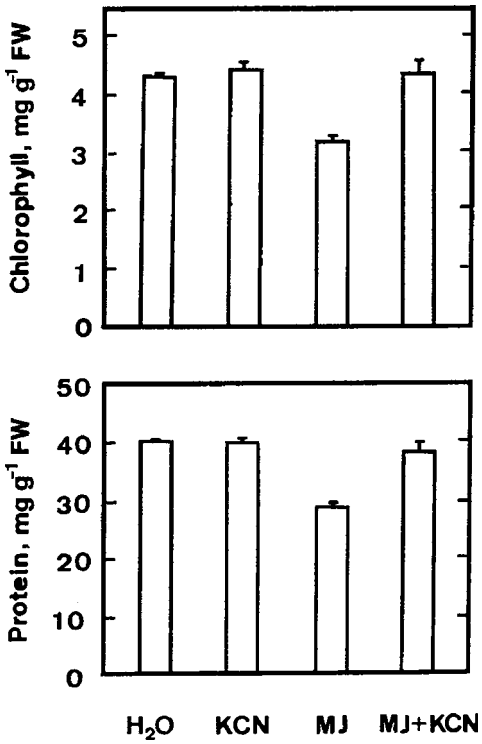

Figure 6. Effect of MJ (45 μM) on the contents of chlorophyll and protein in detached rice leaves in the presence or absence of 5 mM KCN. All measurements were made 48 h after treatment. Bars indicate SE, n = 4.

Josse et al. (2003) asserted that *n*-octyl gallate is a far better inhibitor of alternative oxidase than *n*-propyl gallate. They suggested that *n*-octyl gallate should replace *n*-propyl gallate for *in organello* or *in vivo* studies. Thus, further confirmation of the lack of an alternative respiration pathway in MJ-treated rice leaves by using *n*-octyl gallate seems justified.

Also noteworthy is that KCN reduced the senescence of rice leaves promoted by MJ (Figure 6). Results presented here seem to suggest that respiration is causally associated with senescence of rice leaves induced by MJ, implying that mitochondria contribute to the progress of the senescence process. Basically, mitochondria, the primary function of which is to produce energy, play a central role in cellular metabolism in the control of vital functions. They also represent the major source of reactive oxygen species (Kowaltowski and Vercesi, 1999; Tiwari et al., 2002). Very recently it was shown in *Podospura anserina* that inactivation of the nuclear *cox5* gene encoding subunit V of the cytochrome *c* oxidase complex leads to the exclusive use of the alternative respiratory pathway and to a decrease in production of reactive oxygen species, which results in a striking increase in longevity (Dufour et al., 2000). We have shown that MJ-promoted senescence of rice leaves is mediated through the generation of reactive oxygen species (Hung and Kao, 1998, 2004). However, we do not know whether reactive oxygen species generated by MJ in rice leaf cells are mainly located in mitochondria. It has been shown that

cell wall NAD(P)H oxidase-peroxidase is another potential enzymatic source of reactive oxygen species (Papadakis and Roubelakis-Angetakis, 1999; Blee et al., 2001). KCN is known to inhibit NAD(P)H oxidase-prosidase (Papadakis and Roubelakis-Angetakis, 1999) and superoxide dismutase. Thus, the possibility that KCN-reduced MJ-promoted senescence of rice leaves is mediated through the inhibition of cell wall NAD(P)H oxidase-peroxidase or superoxide dismutase cannot be excluded.

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茉莉酸甲基脂對水稻葉片可溶性糖與呼吸速率之影響

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本研究主要探討茉莉酸甲基脂加速水稻葉片老化過程中呼吸速率與可溶性糖含量之變化。水稻葉片經茉莉酸甲基脂處理 48 小時，呼吸速率增加，但不影響光合作用速率。茉莉酸甲基脂會降低蔗糖與葡萄糖含量，但不影響果糖含量。Cytochrome *c* oxidase **之抑制劑氰化鉀可抑制茉莉酸甲基脂所促進之呼吸速率，所降低之蔗糖與葡萄糖含量及所加速的葉片老化。外加蔗糖處理可增加茉莉酸甲基脂所降低之蔗糖與葡萄糖，但不能延緩所加速之葉片老化。顯示茉莉酸甲基脂所加速之葉片老化，不是由於蔗糖與葡萄糖含量降低所造成。文中並就茉莉酸甲基脂所加速葉片老化過程中，呼吸作用與蔗糖可能扮演之角色加以討論。**

關鍵詞：果糖；葡萄糖；茉莉酸甲基脂；水稻；呼吸作用；老化；蔗糖。