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BRIEF COMMUNICATION

# The relation between accumulation of abscisic acid and proline in detached rice leaves

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## Abstract

The relation between abscisic acid (ABA) and proline accumulation was investigated in detached rice (*Oryza sativa* L.) leaves. In darkness, proline content increased about 2-, 2.5- and 6-fold after 24, 48 and 72 h. ABA content reached maximum after 48 h. In the light, proline content remained almost unchanged until 48 h and subsequently increased slightly. ABA content in the light was lower than in darkness, but the maximum was also after 48 h. During 12-h exposure to decreased air humidity, proline content gradually increased, but ABA content increased about 25-fold after 4 h and declined thereafter. Exogenous application of ABA resulted in an increase in proline content in detached rice leaves under both light and darkness.

*Additional key words*: *Oryza sativa*, water stress.

It is well established that proline accumulates in plant tissues under water stress, salt stress, and dark-induced senescence (*e.g.* Kao 1981, Moftah and Michel 1987, Munns *et al.* 1979, Stewart and Voetberg 1985, Venekamp and Kost 1988, Wang *et al.* 1982). Proline accumulation can be induced by abscisic acid (ABA) in plant tissues (Aspinall *et al.* 1973, Chou *et al.* 1991, Pesci 1987, Stewart 1980, Stewart and Voetberg 1985). However, the lack of proline accumulation in response to ABA treatment has also been reported for spinach, pearl millet, tobacco and sunflower leaves (Aspinall and Paleg 1981, McDonell *et al.* 1983). ABA is known to increase rapidly not only in intact but also in detached leaves under water stress (Hsiao 1973). In darkness, it has been shown that the content of ABA in leaves of oats and rice increased during dark-induced senescence (Gepstin and Thimann 1980, Wang *et al.* 1999). Therefore, proline accumulation in response to water stress or dark-induced senescence may be a response to accumulated ABA. In fact, Ober and Sharp (1994) reported that increased content of ABA is required for proline accumulation in maize primary roots at low water potentials. However, proline accumulation in the absence of ABA accumulation has also been reported (Stewart and Voetberg 1985, 1987).

Previously, we have reported that proline accumulates in detached rice leaves during dark-induced senescence (Kao 1981, Wang *et al.* 1982) or exposed to water stress (Kao 1981). In this paper, we have examined the relation between accumulation of ABA and proline in detached rice leaves.

Rice (*Oryza sativa* L. cv. Taichung Native 1) seedlings were planted on a stainless net floating on modified half-strength Johnson's nutrient solution in a 500-cm$^3$ beaker (Kao 1980). The nutrient solution (pH 4.5) was replaced every 3 d. Rice seedlings were grown in a greenhouse with natural daylight at day/night temperature 30/25 °C and relative humidity (RH) about 95 % (for detail see Kao 1980). The apical 3 cm segments were excised from the third leaves of 12-d-old seedlings. Groups of twenty leaf segments were exposed to slightly decreased RH above a solution of 0.5 M NaCl. Under this condition, relative water content of leaf segments after 4, 8, and 12 h was 80, 62, and 60 %, respectively (Yang *et al.* 2000).

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*Abbreviations*: ABA - abscisic acid; f. m. - fresh mass.

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Similar segments of leaves were floated on 20 cm$^3$ of distilled water or a solution of 45 µM abscisic acid (ABA) in a Petri dish to serve as controls and ABA-treated samples, respectively. All samples were incubated at 27 °C in darkness or under irradiance 40 µmol m$^{-2}$ s$^{-1}$. Proline and ABA were extracted and quantified as described previously (Kao 1981, Chen and Kao 1993). For proline utilization, detached rice leaves were pretreated with 50 mM ornithine [since addition of ornithine has been observed to be more effective than that of glutamic acid or arginine in increasing proline content in rice leaves (Yang *et al.* 2000)] to increase the endogenous proline content and then transferred to distilled water, 45 µM ABA, or decreased humidity for 4, 8, and 12 h in the light. Change in proline content were then monitored. The decline in proline content will be considered as that proline is utilized (Yang *et al.* 2000). All data were expressed on the basis of initial fresh mass.

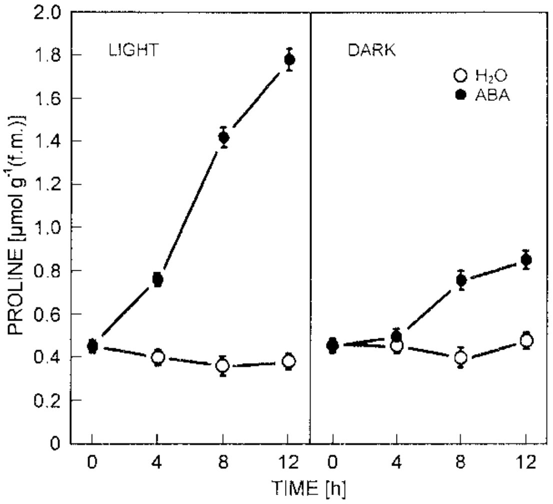

Fig. 1. Changes in proline content of detached rice leaves floated on distilled water or 45 µM ABA solution under dark or light for 12 h. Means ± SE, *n* = 4.

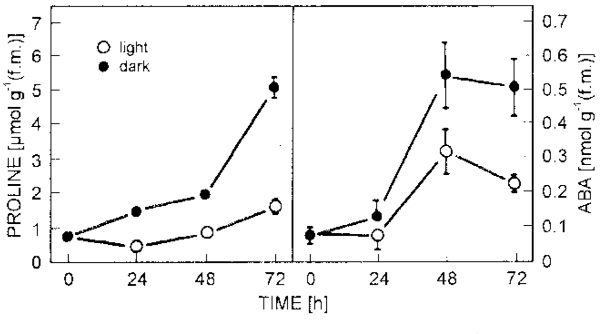

Fig. 2. Changes in proline and ABA contents of detached rice leaves floated on distilled water under dark or light for 72 h. Means ± SE, *n* = 4.

All experiments were repeated three times; within each experiment, treatments were replicated 4 times. Similar results and identical trends were obtained in all experiments. The data reported here are from a single experiment.

To establish the relation between accumulation of ABA and proline in detached rice leaves, we have to know whether proline accumulation can be induced by ABA. Under both light and darkness, ABA treatment indeed resulted in an accumulation of proline in detached rice leaves (Fig. 1).

In darkness, proline content in detached rice leaves incubated in distilled water for 24, 48 and 72 h, increased about 2-, 2.5- and 6-fold, respectively (Fig. 2), which confirmed our previous results (Wang *et al.* 1982). Proline content, however, in detached rice leaves incubated in the light remained relatively unchanged after 48 h and subsequently increased slightly (Fig. 2). It is apparent that proline accumulates more in darkness than in the light in detached rice leaves. In darkness, ABA content did not increase 24 h after incubation, but increased 48 h and remained unchanged 72 h (Fig. 2). ABA content in detached rice leaves incubated in the light increased in a similar pattern but to a lesser extent than in darkness (Fig. 2). It is clear that the increase in ABA content in detached rice leaves in the dark was observed after proline accumulation.

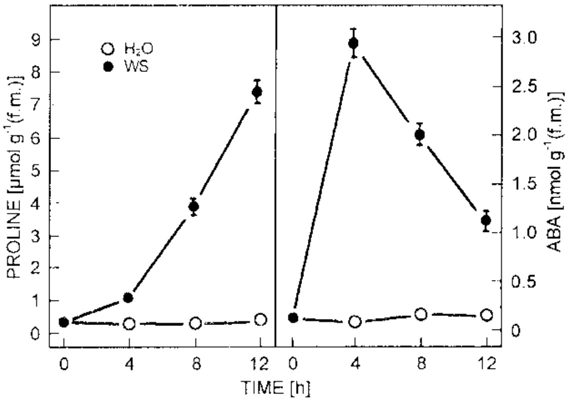

Fig. 3. Changes in proline and ABA contents of detached rice leaves floated on distilled water ($H_2O$) or exposed to slightly decreased RH above 0.5 M NaCl (WS; RWC 80, 62, and 60 % after 4, 8, and 12 h, respectively) in the light. Means ± SE, *n* = 4.

In control leaves, proline and ABA contents remained relatively unchanged during 12 h of incubation in the light. Treatment with decreased humidity resulted in a significant increase in proline content in the light (Fig. 3). ABA content in detached rice leaves incubated at decreased humidity increased rapidly from a control level of about 0.1 to over 2.5 nmol g$^{-1}$ (f. m.) in a period of 4 h, and declined thereafter (Fig. 3). Similar time course has been observed previously for pearl millet, rice and barley (Henson and Quarrie 1981, Stewart and Voetberg 1985). It is clear that ABA content reached maximum at the time when proline accumulated slightly.

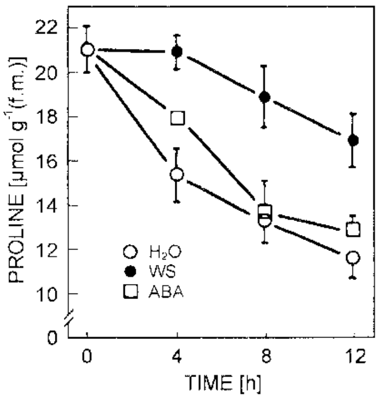

Fig. 4. Changes in proline content in detached rice leaves pretreated with 50 mM ornithine for 3 h in the light and then floated on distilled water ($H_2O$), 45 µM ABA solution (ABA), or exposed to decreased RH (WS) in the light. Means ± SE, *n* = 4.

Proline accumulation in plant tissues has been suggested to be resulted from *1*) a decrease in proline degradation, *2*) an increase in proline biosynthesis, and *3*) a decrease in proline utilization for protein synthesis (Yoshiba *et al.* 1997). Recently, we demonstrated that proline in detached rice leaves in the dark is less utilized than in the light (Yang *et al.* 2000). It is possible that less proline utilization may also contribute to the accumulation of proline in detached rice leaves exposed to decreased humidity. The decline in proline content was slower at decreased humidity than in water-treated controls (Fig. 4). If there is a cause and effect link between ABA and proline accumulations in detached rice leaves, then proline in ABA-treated detached rice leaves is also expected to be less utilized. Contrary to our expectation, proline in ABA-treated rice leaves was utilized as effective as in distilled water-treated rice leaves (Fig. 4). Based on the results reported here, we conclude that application of ABA can induce accumulation of proline, however, accumulation of endogenous ABA did not seem to be involved in dark- or water stress-induced accumulation of proline in detached rice leaves.

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