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PROLINE ACCUMULATION IN RICE LEAVES AS A CONSEQUENCE OF SENESCENCE

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ACCUMULATION of proline in plants subjected to abiotic stress conditions is a general phenomenon[1,2]. The physiological significance of proline accumulation in plant tissues during stress is not clear. Despite its possible role at high concentrations as a storage compound of reducing equivalent and nitrogen for rapid growth after the stress[3,4], an osmoticum[5,6], a protective agent of enzymes and cellular structures[7] and as an organic solute[8] has been speculated, these proposals have not been convincingly proved. On the other hand, Hanson *et al*[9] considered proline accumulation as a pathological consequence in water-stressed barley leaves. Nevertheless, this view has been questioned[8]. Studies from this laboratory have shown that proline accumulates in tungro (a virus complex)-diseased rice leaves[10]. We demonstrate in this study that proline accumulates as a consequence of leaf senescence in rice.

Five cm apical segments of fully expanded third leaf blades from 20-day-old rice seedlings (cv. Taichung Native 1) were floated with their adaxial side up in groups of five on 20 ml of either distilled water or 2×10^{-5} M kinetin (Loba Chemie, Austria), gibberellic acid (GA_3; Polfa-Kunto, Poland) or abscisic acid (ABA, Sigma Chemical Co., USA) in 10 cm petri dishes. The petri dishes were incubated in the dark at 28 ± 2°C for three days. Duplicate samples, each replicate consisting of five leaf blade segments totalling approximately 75 mg, were collected at 24 hr intervals for 3 days. The concentrations of total chlorophyll pigments extracted from these segments in 80% (v/v) ethanol and of free proline extracted from identical and parallel samples in 3% (w/v) aqueous sulphosalicylic acid were determined spectrophotometrically[11].

As a measure of leaf senescence, the total chlorophyll content of senescing and detached leaf blade segments was determined. During the three days of incubation of the detached leaf blade segments in darkness, the leaf blade segments

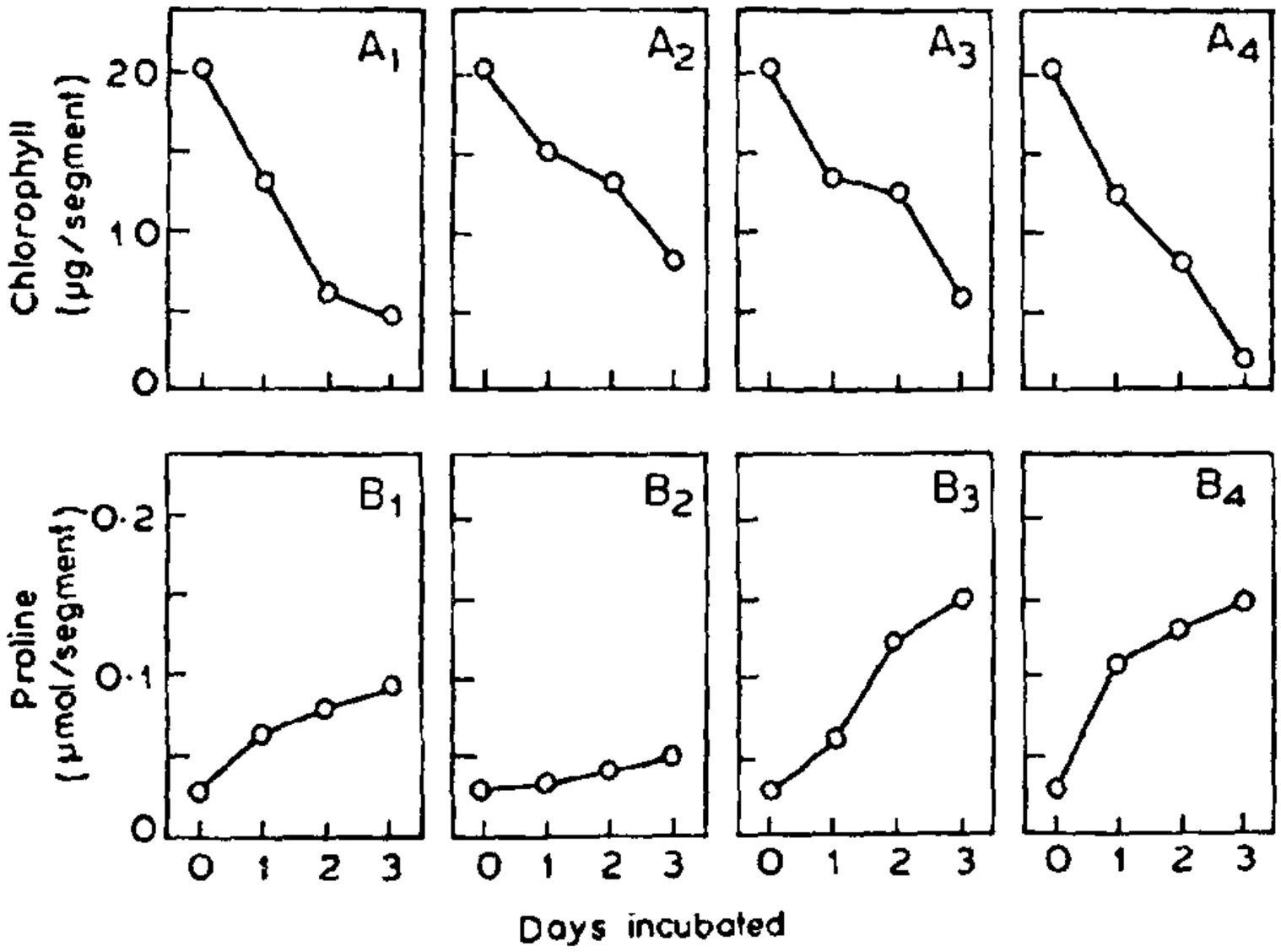

Figure 1. Time course of total chlorophyll (A) and free proline (B) contents of leaf blade segments (cv. Taichung Native 1) floated on water (1) and on 2×10^{-5} M kinetin (2), GA_3 (3) and ABA (4) solutions in the dark. The individual values were always within ±5% of the mean.

floated on water, kinetin, GA_3 and ABA solutions; lost 76, 59, 68 and 88% of their initial total chlorophyll content, respectively (figure 1, A_1–A_4).

Leaf senescence is regulated by hormones[12,13]. During senescence, the levels of cytokinins and gibberellins decrease, while the level of ABA increases. The effect of applied growth regulators on leaf senescence is well-supported by the established fact that cytokinins retard and ABA accelerates senescence[14]. Gibberellins have been shown to delay senescence in a few plant species[15]. However, in rice, GA_3 showed a weak effect in delaying senescence.

Correspondingly, the proline content increased greatly in detached rice leaf blades during senescence. ABA further accelerated senescence (figure 1, A_4) with a concomitant increase in the amounts of proline (figure 1, B_4). Although GA slightly retarded the senescence of leaf blade segments as shown by its ability to retain chlorophyll pigments to some extent (figure 1, A_3), GA was equally effective as ABA in enhancing proline concentration in the senescing leaf blade segments (figure 1, B_3). In contrast, kinetin retarded the leaf senescence (figure 1, A_2) and arrested the rise in proline content (figure 1, B_2). The level of endogenous ABA increases in rice in response to disease[16], temperature[17] and water[18] stresses. Changes in endogenous ABA levels are usually accompanied by changes in the levels of free proline[10,19,20]. Stimulation of proline accumulation by exogenous ABA[19–21] has been demonstrated.

Three metabolic causes for proline accumulation have been implicated: (i) stimulated synthesis from glutamate, (ii) inhibition of proline oxidation, and (iii) slowed incorporation of proline into protein[22]. Since applied ABA does not affect either proline oxidation or incorporation into protein in barley leaves[21], presumably proline accumulation is due to its augmented synthesis from glutamate. One possible source of glutamine in senescing leaf blades is from breakdown of host proteins. Admittedly, accumulation of proline is induced by changes in endogenous ABA in senescing leaf blades and it reflects a metabolic symptom of injury.

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