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Short Communications

Proline accumulation as a reliable indicator of monocarpic senescence in rice cultivars[1]

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Summary. Proline content proportionally increased with leaf age as well as during aging in darkness of excised leaf segments collected from the flag, second and third leaves of the Jaya cultivar of rice. BA significantly suppressed, whereas ABA augmented, the rise of proline level in the leaf segments. Proline also increased in the attached flag, second and third leaves of all the 4 rice cultivars with the progress of reproductive development but the pattern of its accumulation was non-sequential in Jaya and Ratna and sequential in Masuri and Kalojira. Although proline accumulation was retarded and enhanced by treatment with BA and ABA, respectively, as foliar sprays, the mode of proline accumulation (non-sequential or sequential) remained unaltered.
Key words. Oryza sativa; rice cultivar; proline accumulation; benzyladenine; abscisic acid; senescence, monocarpic.

Several workers[2–6] have reported the striking increase in proline content in water stressed plants. Recently, it has been demonstrated that proline also accumulates during senescence of excised rice leaves[7,8]. However, relatively little attention has been paid to the proline accumulation during senescence of intact attached leaves, particularly during monocarpic senescence.

It was reported earlier from this laboratory that there are varietal differences in the pattern of monocarpic leaf senescence in rice[9–11]. Thus, for example, the cultivars Jaya and Ratna exhibited a non-sequential mode of senescence where the young flag leaf senesced earlier than the old second leaf; but the cultivars Masuri and Kalojira manifested a sequential mode of senescence where the leaves senesced in a chronological sequence. The present communication describes the pattern of changes in proline content in aging excised leaf segments as well as in intact attached leaves of 4 rice cultivars during the progress of reproductive development. In addition, the effects of benzyladenine (BA) and abscisic acid (ABA), the inhibitor and promoter respectively of rice leaf senescence[12], on proline accumulation in both excised and intact attached leaves during senescence were also analyzed.

Seeds of 4 cultivars of rice (*Oryza sativa* L.) viz., Ratna, Jaya (both dwarf and photoperiod insensitive) Masuri and Kalojira (both tall and photoperiod sensitive) were collected from the Crop Research Farm of Burdwan University. 30-day-old seedlings raised from these seeds were transplanted with 1 seedling per hill at a spacing of 25 × 30 cm in 1-m^2 plots.

Experiments were carried out with excised leaf segments induced to age in the dark, and also with the 3 uppermost attached leaves of the 4 rice cultivars during the progress of reproductive development. In one set of experiments, the effects of benzyladenine (0.5×10^{-3} M) and abscisic acid (10^{-4} M) were examined on changes in the proline content in excised leaf segments collected from the flag and second and third leaves from the tops of 4 rice cultivars when they entered into the reproductive phase. Leaf segments weighing 1 g were placed in sterile petri dishes containing 30 ml of water, BA or ABA solu-

Effects of BA and ABA treatment on proline content of 3 uppermost leaves of 4 rice cultivars over the control

Days after flowering	Cultivar	BA Flag	2nd leaf	3rd leaf	ABA Flag	2nd leaf	3rd leaf
7	Jaya	− 17.2	− 12.6	− 9.4	+ 6.0	+ 7.9	+ 10.5
	Ratna	− 14.8	− 10.0	− 7.2	+ 4.9	+ 8.1	+ 9.3
	Masuri	− 12.7	− 9.8	− 6.7	+ 7.2	+ 9.6	+ 9.9
	Kalojira	− 13.1	− 11.4	− 7.4	+ 7.8	+ 10.3	+ 11.5
14	Jaya	− 29.4**	− 21.6*	− 15.2*	+ 7.6	+ 10.2	+ 15.7*
	Ratna	− 27.5**	− 18.4*	− 12.2	+ 5.2	+ 11.6	+ 17.0*
	Masuri	− 21.1	− 16.3*	− 10.3	+ 11.1	+ 15.3*	+ 18.5*
	Kalojira	− 22.3*	− 19.0*	− 12.9	+ 12.4	+ 15.9*	+ 21.4*
21	Jaya	− 24.3*	− 22.2*	− 12.3*	+ 12.3	+ 18.6*	+ 24.3**
	Ratna	− 26.4**	− 20.5*	− 10.5	+ 13.5*	+ 20.3*	+ 27.1**
	Masuri	− 17.6*	− 13.2*	− 6.8	+ 16.0*	+ 26.5**	+ 33.0**
	Kalojira	− 18.7*	− 15.7*	− 8.8	+ 17.9*	+ 24.7**	+ 30.9**
28	Jaya	− 15.4*	− 18.7*	− 9.6	+ 18.7*	+ 26.4**	+ 16.1*
	Ratna	− 13.9*	− 17.6*	− 8.9	+ 16.0*	+ 24.8**	+ 17.4*
	Masuri	− 8.7	− 6.5	− 5.4	+ 19.6**	+ 35.2**	+ 29.4**
	Kalojira	− 9.2	− 7.0	− 6.3	+ 22.2**	+ 34.0**	+ 25.3**

Values expressed as percentage increase (+) or decrease (−) over control. Significance test was done between control and treated plants at *p = 0.05 and **p = 0.01

tion and incubated in complete darkness at a temperature of 25 °C. The leaf samples were taken out after 0, 2, 4 and 6 days of incubation, rinsed well with distilled water and assayed for proline.

In another set, 10 plants each of the 4 rice cultivars were separately sprayed with BA (0.5×10^{-3} M) and ABA (10^{-4} M) at the rate of 5 ml plant^{-1} day^{-1} for 2 consecutive days just prior to each experimental estimation of proline at intervals of 7 days starting from flowering (day 0) to the senescent (28-day) stage. Randomised leaf samples were collected from the flag, second and third leaves for each determination of proline which was done according to the method of Bates et al.[13].

Each experiment was replicated 3 times and the average values were included. The results were statistically analyzed for standard error and by the t-test (significance test) where necessary.

Proline accumulation in excised leaf segments and in intact attached leaves of 4 rice cultivars was followed during aging and senescence. Figure 1 shows the proline level of excised leaf segments collected from the flag, second and third leaves of the Jaya variety of rice during aging in darkness in presence of water, BA (0.5×10^{-3} M) and ABA (10^{-4} M) solutions. It is evident from the data that the proline content was highest in the oldest (third) leaf and lowest in the youngest (flag) leaf and that it increased significantly during aging of excised leaf segments in darkness, suggesting that proline accumulation is a function of leaf age. Treatment with BA significantly suppressed the rise of proline in leaf segments kept in the dark, whereas ABA treatment significantly augmented its rise in segments of the 3 leaves. However, proline content was always found to be proportional to leaf age. The pattern of changes in proline content was almost identical in the other 3 cultivars; hence data for these are not shown. The present study therefore confirms the observations of Wang et al.[8] who showed that proline accumulates in excised rice leaves during aging in

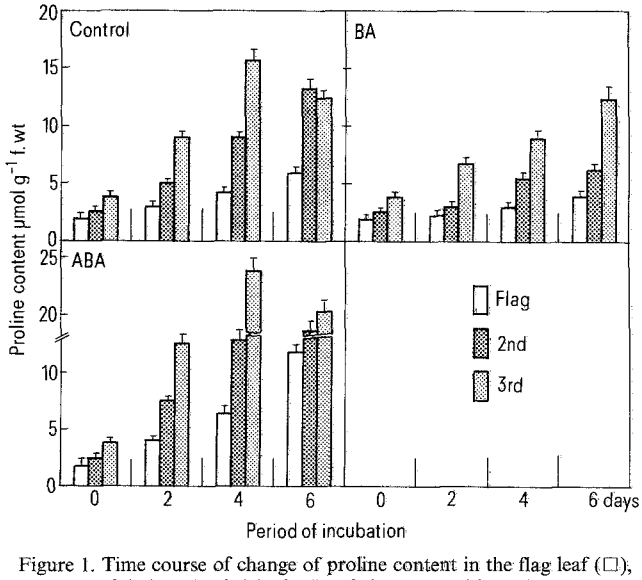

Figure 1. Time course of change of proline content in the flag leaf (□), second leaf (▨) and third leaf (▩) of the Jaya cultivar during senescence in the dark in water, benzyladenine (BA) or abscisic acid (ABA).

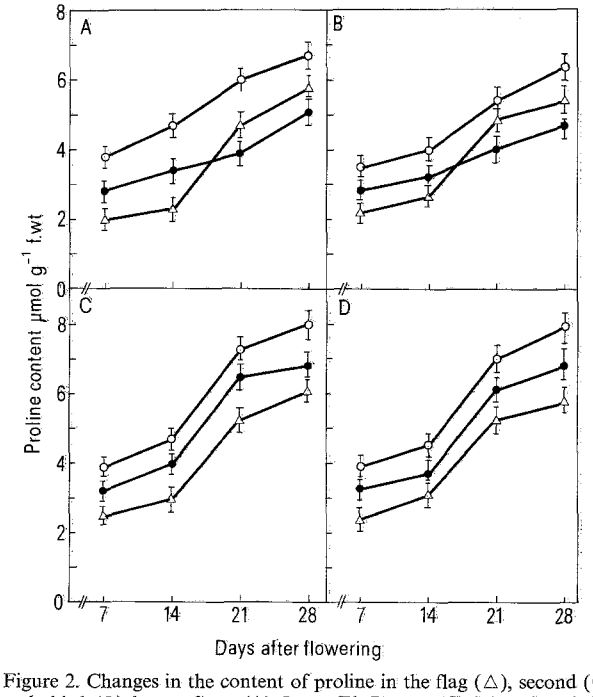

Figure 2. Changes in the content of proline in the flag (△), second (●) and third (○) leaves from (A) Jaya, (B) Ratna, (C) Masuri and (D) Kalojira during reproductive development. Data were recorded at intervals of 7 days from flowering to senescent stage. Each experiment was replicated three times and the mean values were determined. SE indicated by bars.

darkness. The present study further demonstrates that proline level also varies according to the maturity of the leaf.

In the present study, proline accumulation was also followed in the 3 uppermost leaves of 4 rice cultivars during their development after flowering, i.e., during monocarpic senescence. It was most interesting to note that in both Jaya and Ratna proline accumulation increased according to the chronological age of the leaf up to the 14th day from flowering, but on the 21st day the accumulation of proline was higher in the flag than in the second leaf which suggests that the accumulation pattern of proline was non-sequential (fig. 2). Unlike the above 2 cultivars, both Masuri and Kalojira showed a sequential pattern of proline accumulation, i.e., it was proportional to the chronological age of the leaf throughout development. Such an observation clearly fits the concept of the mode of senescence reported for these rice cultivars[9-11]. All these data seem thus to suggest that proline accumulation can serve as a reliable indicator of monocarpic senescence in rice.

Both cytokinin and abscisic acid were implicated in the regulation of monocarpic senescence in rice, the former retarding but the latter hastening the leaf senescence[12]. The idea that proline accumulation can be taken as a reliable indicator of senescence is further supported by the present observation that the retardation of leaf senescence with BA was also accompanied by a proportional decrease of proline accumulation and its acceleration with ABA was associated with a characteristic rise of proline accumulation compared with the untreated control (table). However, the pattern of proline accumulation in 3 leaves of the 4 cultivars (being non-sequential in Jaya and Ratna and sequential in Masuri and Kalojira) remained unaltered in BA treated plants of Jaya and Ratna suggesting that such treatment could not establish the sequential mode of proline accumulation in these cultivars where BA proportionally suppressed proline accumulation throughout plant development.

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Reciprocal biological activities of the cyclic tetrapeptides chlamydocin and HC-toxin

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Summary. Chlamydocin, a potent cytostatic agent against cultured mammalian cells, and HC-toxin, a host-specific phytotoxin, are cyclic tetrapeptides containing the same epoxide α-amino acid. We show here that these compounds have reciprocal biological activity; HC-toxin is cytostatic against cultured mastocytoma cells, and chlamydocin has host-specific toxin activity against maize. Chlamydocin and another related cyclic peptide, Cyl-2, are less host-specific than HC-toxin because maize tolerant to HC-toxin is more sensitive to chlamydocin and Cyl-2.

Key words. Chlamydocin; HC-toxin; phytotoxin; cytostatic agent.

Host-specific phytotoxins are compounds which lectively affect the same plant species, varieties, or genotypes as the pathogenic fungi that produce them[2]. *Bipolaris zeicola* (Nisak. and Miyake) Shoem. (*Cochliobolus carbonum* Nelson) race 1 produces a phytotoxin, HC-toxin, which specifically inhibits maize that is susceptible to the fungus; dicotyledons and other grasses and maize genotypes are affected only at concentrations at least 100 times higher than are needed to inhibit growth of susceptible maize[3]. Major resistance to *B. zeicola* race 1 and to HC-toxin is conditioned by a single dominant nuclear gene *Hm* which has been mapped on the first chromosome[4]. HC-toxin has recently been identified as cyclo(L-Aoe-D-Pro-L-Ala-D-Ala)[5], where Aoe stands for 2-amino-8-oxo-9,10-epoxidecanoic acid[6,7]. Its structure is similar to those of two other previously described fungal metabolites, chlamydocin and Cyl-2 (figure). All three compounds are cyclic tetrapeptides containing Aoe and either Pro or Pip. Chlamydocin, cyclo(L-Aoe-α-Aib-L-Phe-D-Pro), is produced by the cosmopolitan soil-inhabiting fungus *Diheterospora chlamydosporia* (Kamyschko) Barron and Onions[8]. Chlamydocin was originally isolated as a cytostatic agent against cultured mammalian cells[9]. It is more potent than several other common cytostatic drugs, including actinomycin D, amethopterin, colchicine, vincristine, and vinblastine, yet has a very low toxicity to rats[10]. Chlamydocin and HC-toxin have been synthesized[11,12]. Cyl-2, cyclo-(Aoe-D-O-methylTyr-L-Ile-L-Pip), produced by the phytopathogenic fungus *Cylindrocladium scoparium* Morgan, was discovered as an inhibitor of lettuce root elongation and rice seedling growth[13,14].

This report compares the effects of these cyclic peptides of diverse origins in two test systems: maize seedlings tolerant of or sensitive to HC-toxin, and mammalian cell cultures.

Materials and methods. HC-toxin, chlamydocin, and Cyl-2 were purified from culture filtrates of the respective fungi[6,9,13]. The compounds were tested in a root growth bioassay against two maize (*Zea mays* L.) hybrids that differ at only the nuclear *Hm* locus: Pr × K61 (genotype *hmhm*; susceptible to *B. zeicola* race 1 and sensitive to HC-toxin) and Pr1 × K61 (genotype