Mobilization of Metabolites from Leaves to Grains as the Cause of Monocarpic Senescence in Rice

SUBRATA RAY AND MONOJIT A. CHOWDHURI
Plant Physiology and Biochemistry Laboratory, Department of Botany, University of Burdwan, Golapbag: Burdwan-713104, India

ABSTRACT
The pattern of senescence was studied by following the changes in chlorophyll and protein in the leaves and by measuring 32P retention and export from source to sink during development of the rice plant (Oryza sativa L. cv. Jaya) subjected to different manipulative treatments. With the advance of reproductive development, the chronological sequence of leaf senescence was changed, so that the flag and third leaf senesced earlier than did the second leaf. In presence of the daughter shoot of defruited plants, senescence was delayed in all three leaves of the mother plant, as compared to the same leaves of intact plants. Senescence of all three leaves was further delayed when both panicle and daughter shoots were removed from the plant. The above manipulative treatments caused the initial sequential pattern of senescence of leaves to persist. Removal of both panicle and daughter shoots caused little export of 32P between leaves. In the presence of daughter shoots of defruited plants, export of 32P was maximum from leaves of the mother plant to the nearest daughter shoots. This led to earlier senescence of such mother plant leaves than that of plants from which both panicle and daughter shoots were removed. The pattern of senescence and export of 32P in the flag and the second leaf of the daughter shoot was essentially the same as that of the intact plant. Based on these findings, it was concluded that mobilization of metabolites from source to sink is the primary cause of monocarpic senescence in rice.

Although much attention has been paid to whole plant senescence in recent years (6-10, 15, 17), neither the mechanism nor the physiological basis for this phenomenon is clearly understood (14). Previous reports from this laboratory pointed out that, in monocarpic rice plants, mobilization of metabolites from leaves to grains results in a deprivation stress in leaves, followed by an increased ABA accumulation, leading to senescence (4). There is a good deal of evidence which indicates that cytokinin-like substances produced by roots may regulate senescence of leaves in intact plants (7, 16). Available reports suggest that a rise in ABA (4, 11) and a depletion of cytokinin in leaves (2, 7, 18) during advanced stages of reproductive development might be the prime factors in the control of monocarpic senescence of a number of plants at the hormonal level. Early work (13) strongly supported the concept of mobilization of nutrients as the cause of monocarpic senescence, though it has been questioned by several workers (6, 9-10, 15). The present study provides further evidence in support of the hypothesis that mobilization of metabolites from source to sink is the primary cause of the onset of senescence in monocarpic rice plants.

MATERIALS AND METHODS
Cultivation of Plants. Certified seeds (having 100% germinability) of rice (Oryza sativa L. cv. Jaya) were collected from the Crop Research Farm, University of Burdwan, Burdwan, India. These were surface-sterilized in 0.1% HgCl₂ for 1 min and then washed well in distilled H₂O several times. Thirty-day-old seedlings were transplanted, one seedling per hill, in 1-m² plots containing 20 seedlings each. The soil was prepared by mixing with farmyard manure. Each plot was regularly watered.

Measurement of Leaf Senescence. Experiments were carried out with leaf samples collected from the flag, second, and third leaves of plants during the reproductive (104 to 125 days) and manipulative (where the plants were subjected to different surgical alterations) stages. Biswas and Choudhuri (4) established that the decline in Chl and protein contents could be taken as a reliable index of leaf senescence in rice. Hence, in the present study, the changes in the contents of Chl and protein in the flag, second, and third leaves at intervals of 7 days were taken as a senescence index. Chl was extracted from 50 mg tissue with methanol and was estimated following the method of Arnon (1). The residue of this methanol extract was digested with 0.5 N NaOH at 80°C for 1 h and centrifuged at 4,000g for 5 min. The supernatant was analyzed for protein content (12).

Defruiting Experiments. It was observed in the present study that, when the panicles were removed from the plant, a daughter shoot developed from the axil of the second and third leaves. After a period of extension growth, a new panicle was produced by this daughter shoot bearing two leaves, the flag and the second (and, occasionally, a third), simulating an intact plant (Fig. 1). In one set of experiments, panicles were completely removed from 10 plants, and the daughter shoots were allowed to grow. In a second set of experiments, both panicle and daughter shoots were removed at regular intervals in order to prevent the formation of any additional sink strength. The senescence pattern was noted at intervals of 7 days, both in surgically altered plants and in daughter plants.

32P Translocation Experiments. The three leaves of the mother plants were fed separately for 48 h with radiophosphorus (5.2 mCi/mmol), and the radioactivity (cpm/g dry weight) retained by the fed leaf and translocated to the sinks was recorded in a Geiger-Müller counting system. The detailed methodology was described in another paper (3).

The results were replicated three times and were statistically analyzed following Duncan's multiple range test at 95% confidence limits (5). Standard error around the mean was also calculated (5).

RESULTS
Senescence Pattern in Control and Two Types of Surgically Altered Plants. Table I shows that both Chl and protein contents decreased gradually in the three leaves during the progress of reproductive development. In control plants, these degradations were fastest from day 111 onward (grain-developmental stage) in the flag leaf. A steady degradation of these two components was
by the flag and minimum by the third leaf (Table II).

$^{32}$P Mobilization Pattern from the Flag, Second, and Third Leaves to Different Daughter Shoots after Panicle Removal. From the experiment shown in Table III, it is clear that the amount of export of $^{32}$P from the three leaves of the mother plant to different sinks was different. Maximum export of $^{32}$P was recorded at the early stage from all three leaves of mother plants, and then it decreased gradually with plant age. Both the second and the third leaves of the altered plant showed a high rate of $^{32}$P export to newly developed daughter shoots at their axils, whereas the flag leaf was least active in this regard.

Senescence Pattern of the Flag and Second Leaves of the Daughter Shoots. After 7 days, the daughter shoot emerged on a plant from which the panicle was removed; at maturity (24 to 26 days after panicle removal), it contained a prominent flag and a second leaf (and, occasionally, a third leaf) bearing a daughter panicle at the top. The senescence pattern of these leaves was similar to that of intact control plants, and here, also, the rate of Chl and protein degradation was maximum in the flag leaf at the time of grain developmental stage (between 35 to 42 days) (Table IV).

$^{32}$P Mobilization Pattern from the Flag and Second Leaves to Grains at the Reproductive Stage in Daughter Shoot. The export pattern of $^{32}$P from the two leaves to grains of daughter shoots showed that maximum export of radioactivity took place in the daughter flag leaf at the time of grain developmental stage (note data for day 35). In contrast, the second leaf showed a steady mobilization of $^{32}$P up to grain maturation period (Table V).

DISCUSSION

Biswas and Choudhuri (3) demonstrated that the flag leaf of rice undergoes an early senescence in comparison to the other two leaves. In their subsequent analysis (4) of the mechanism of whole plant senescence in rice, they concluded that senescence in rice is presumably induced by ABA-like substance(s) formed following a deprivational stress developing in leaves as a result of considerable export of metabolites from leaves to grains and a possible depletion of the supply of cytokinin in the leaves. The experimental evidence presented here further extends the idea that diversion of nutrients and metabolites from leaves to grains is one of the prime factors of monocarpic senescence in rice.

When plants enter the reproductive stage, senescence starts according to the chronological sequence of the leaf, but, with further development, this sequence becomes quite disturbed. Thus, at 11; there is a distinct sequential pattern of senescence in the three leaves, i.e. the youngest leaf (the flag leaf) contains the maximum amount of Chl and protein followed by the second and third leaves. This sequence is changed at the 118th day (grain-maturation stage), when a greater degree of senescence (Chl and protein loss) is observed in both the flag and the second leaves than is observed in the second leaf. Interestingly, when the panicle is removed and daughter shoots are allowed to grow, senescence is delayed and the sequential pattern of senescence is maintained by the three leaves up to 125 days. This indicates that the removal of sink (panicle) has a distinct delaying effect on senescence of leaves, particularly on proximal flag leaf. This can be further substantiated by the experiment where the daughter shoots are also removed from the mother plant.

When the panicle is removed, the newly developed daughter shoots at the leaf axes form additional sinks, and, hence, the removal of daughter shoots causes delay in senescence of the three leaves. This is quite clear when the senescence pattern of the three leaves between the plant from which the panicle is removed is compared to that from which both panicle and daughter shoots are removed. The results, thus, seem to suggest that drawing out of nutrients by the sink from the source leaf may cause the onset

---

**Fig. 1.** Development of daughter shoots from the axils of the second (L₂) and third (L₃) leaves. D₁, daughter shoot at the axil of second leaf; D₂, daughter shoot at the axil of third leaf; FM, flag leaf of mother plant.
Table I. Changes in the Contents of Chl and Protein in the First, Second, and Third Leaves in the Control and the Two Surgically Altered Plants during the Progress of Reproductive Development

The estimations were made at intervals of 7 days. Values in a column followed by the same letter are not significantly different, and values without letters are nonsignificant at the 5% level, as determined by Duncan's multiple range test. Each value is the mean of three replications.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Leaf Position</th>
<th>At 104 days</th>
<th>At 111 days</th>
<th>At 118 days</th>
<th>At 125 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chl</td>
<td>Protein</td>
<td>Chl</td>
<td>Protein</td>
<td>Chl</td>
</tr>
<tr>
<td>Control</td>
<td>Flag</td>
<td>3.71 ± 0.01</td>
<td>97.01</td>
<td>4.11 ± 0.12</td>
<td>64.06 ± 3.22</td>
</tr>
<tr>
<td></td>
<td>2nd</td>
<td>3.53 ± 0.01</td>
<td>91.95</td>
<td>3.01 ± 0.01</td>
<td>74.57 ± 2.10</td>
</tr>
<tr>
<td></td>
<td>3rd</td>
<td>3.88 ± 0.01</td>
<td>88.42</td>
<td>2.87 ± 0.01</td>
<td>63.15 ± 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-Panicle + daughter</td>
<td>Flag</td>
<td>3.73 ± 0.01</td>
<td>97.72</td>
<td>3.83 ± 0.01</td>
<td>65.01 ± 3.12</td>
</tr>
<tr>
<td></td>
<td>2nd</td>
<td>3.52 ± 0.01</td>
<td>91.45</td>
<td>3.07 ± 0.01</td>
<td>78.92 ± 2.10</td>
</tr>
<tr>
<td></td>
<td>3rd</td>
<td>3.90 ± 0.01</td>
<td>87.94</td>
<td>2.98 ± 0.01</td>
<td>70.53 ± 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-Panicle - daughter</td>
<td>Flag</td>
<td>3.70 ± 0.01</td>
<td>98.12</td>
<td>3.52 ± 0.01</td>
<td>86.12 ± 3.12</td>
</tr>
<tr>
<td></td>
<td>2nd</td>
<td>3.53 ± 0.01</td>
<td>92.08</td>
<td>3.09 ± 0.01</td>
<td>78.62 ± 2.10</td>
</tr>
<tr>
<td></td>
<td>3rd</td>
<td>3.89 ± 0.01</td>
<td>89.10</td>
<td>2.97 ± 0.01</td>
<td>70.47 ± 0.01</td>
</tr>
</tbody>
</table>

Table II. The Pattern of 32P Retention and Mobilization among Three Leaves at the Plant Age of 105 Days in Defruited Plants

Two ml of buffered 32P (5.4 mCi/mmol, 0.1 M sodium citrate buffer, pH 6.5) were fed separately through the tip of each leaf for 48 h, and radioactivity was measured thereafter.

<table>
<thead>
<tr>
<th>32P Fed through Leaf</th>
<th>Retention by the Fed Leaf</th>
<th>Mobilization to Other Leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flag</td>
<td>2nd</td>
</tr>
<tr>
<td></td>
<td>cpm/g dry wt</td>
<td></td>
</tr>
<tr>
<td>Flag</td>
<td>3,198 ± 152.2</td>
<td>30 ± 3.0</td>
</tr>
<tr>
<td>2nd</td>
<td>2,842 ± 156.4</td>
<td>28 ± 4.1</td>
</tr>
<tr>
<td>3rd</td>
<td>2,340 ± 143.4</td>
<td>22 ± 3.7</td>
</tr>
</tbody>
</table>

of senescence in that leaf.

The above results can be further corroborated by studying the mobilization pattern of 32P from source to sink. Biswas and Choudhuri (4) demonstrated that maximum 32P was exported from the flag leaf to grains during grain-development stage. The present experiment shows that, when both panicle and daughter shoots (sinks) are removed, 32P fed through the tip of each leaf shows very little mobilization to other leaves excepting the third leaf, which still shows some mobilization of 32P to the second leaf. This clearly indicates that, if the sink strength is removed, there will be little mobilization out of leaves, resulting in a significant delay in senescence.

Lindoo and Noodén (10) have shown that dephosphorylation of soybean plants results in a delayed senescence, and these authors have mentioned that the lack of transmission of senescence signal from the seeds is the cause of such delay. But the situation is quite different in rice where the sink is situated at the top, unlike soybean where sinks are present at different nodal positions. We are not aware as to whether these authors have studied the mobilization pattern from source to sink in soybean. Our experiments clearly indicate that the removal of panicle from the rice plant destroys the active centers of mobilization from the plant and the leaves, thus allowing them to retain their metabolites to a greater extent and to live longer.

The most interesting aspect of the defruitting experiments in rice plant is that a daughter shoot, with all the potentiality of a complete plant, develops from the axils of the second and third leaf after a week, from the action of mother panicle removal. This daughter shoot provides an additional mobilization center for the leaves of the mother plant. The mobilization of 32P fed through each leaf of the mother plant shows that maximum export of metabolites takes place from the nearest fed leaf to the proximal daughter shoot, resulting in the early senescence of that leaf. At the early stage of daughter-shoot development, the third leaf of the mother plant showed maximum mobilization of 32P to the daughter shoot, followed by the second and the flag leaf; later, the amount of 32P export was considerably reduced. In their experiments with soybean, Noodén et al. (15) have demonstrated that, when the pods are on top, the leaf frequently seneces, whereas it does not when the pods are below the leaf. But in rice, the allowance of development of new sinks in the form of daughter shoots at the leaf axil below the flag leaf of the defruited plant caused senescence not only of that leaf but also of the flag leaf of the mother plant. This clearly shows that senescence in rice is not associated with the transmission of senescence signal from seeds.

As already discussed, the daughter shoot, which develops at the axil of the second and the third leaf, also possesses two leaves subtending a panicle at the top, which produces fertile grains. Thus, it simulates the mother plant in this respect. The analysis of senescence behavior of the daughter flag and the second leaf reveals that the daughter flag leaf seneces earlier than does the daughter second leaf. Thus, the senescence pattern of the flag and the second leaf of the daughter shoot is identical with that of the intact mother plant. The distribution pattern of 32P from the fed leaf of the daughter shoot during seed formation also follows a similar pattern, as has been observed in the case of intact mother plant (4). Evidently, the early senescence of the daughter flag leaf may be the result of metabolite depletion from the nearest flag leaf to grains of the daughter shoot.

The experimental evidence presented here strongly supports the hypothesis that mobilization and removal of minerals and organic compounds from the vegetative part by the reproductive part is the direct cause of monocarpic senescence in rice. Clearly, the diversion of photosynthate and other metabolites from the leaves or cytokinin from the roots by the growing reproductive organs is detrimental to the vegetative organs. Such a depletion of metabolites may well form a deprivational stress in leaves and result in an accumulation of ABA in them (4), inasmuch as it has been observed that senescence of rice leaves is always preceded by a considerable amount of 32P export from the source leaf to the sink. In rice, ABA hastens senescence, whereas kinetin delays it (data not shown). Our unpublished data also indicate that the cytokinin decreases to its minimal level and ABA increases to its maximal
level just prior to senescence. All these facts strongly support the idea that the drainage of metabolites is the primary cause of initiating an imbalance in the hormonal status of the plant (particularly ABA and cytokinin), and a higher ratio of endogenous ABA to cytokinin is perhaps of paramount importance for the onset of monocarpic senescence in rice. Thus, it appears, from the present results and those of other workers (9–11, 15), that monocarpic senescence may be caused by different mechanisms in different species.

Acknowledgment—M. A. C. gratefully acknowledges the financial support by the C. S. I. R., New Delhi, during this investigation. The authors are thankful to Dr. P. Bhanja, reader in Botany, and to Dr. M. M. Mukhopadhyay, reader in Zoology, University of Burdwan, and also to Jagatpatty Tah and Rupkumar Kar for their kind help and cooperation.

LITERATURE CITED

8. LEOPOLD AC 1975 Aging, senescence and turn-over in plants. Bioscience 25: 659-752