DISCUSSION

Although a great deal of experimental results has been accumulated, the exact mechanism of monocarpic senescence, particularly at the molecular level, still remains unsettled. However, the evidence suggests that monocarpic senescence is correlatively controlled and there is no doubt that reproductive parts exert regulatory influence in the manifestation of monocarpic senescence. Among monocarpic plants, there are many different patterns in the relation of the time course of leaf senescence (287). In the present investigation, the patterns of leaf senescence have been studied in several rice and wheat cultivars in order to get an insight into the correlative control and the possible cause of monocarpic senescence in rice and wheat plants. To analyse the cause(s) of different patterns of senescence in these cultivars and to explore the role of mobilization of metabolites in the development of these senescence patterns, some physiological, biochemical, anatomical and hormonal studies were carried out in intact as well as surgically altered cultivars of rice and wheat.

Degradation is an essential feature of the senescence process and some quantitative measures are essential for understanding the developmental events occurring during
the senescence process. Previous studies from this laboratory indicated that the loss of chlorophyll and protein (32, 35, 257, 339) and the loss of mineral nutrients, particularly nitrogen and phosphorus might serve as reliable indicators of the progressive senescence during reproductive development, at least in rice (256) and wheat (111) plants. Hence, in the present investigation the loss of above parameters was taken as indicators of senescence and an index of mobilization of metabolites from source to sink.

2.4.1. Studies in the leaf senescence behaviour of rice and wheat:

2.4.1.1. Two distinct modes of senescence patterns during whole plant senescence of rice and wheat cultivars have been reported (35, 257). In the sequential mode of senescence the leaves senesce in a chronological order which means that leaf senescence is a function of leaf age. Whilst in the non-sequential mode, such age-related senescence could not be observed as in some rice cultivars, where the younger flag leaf senesced earlier than the older second leaf (257, 339). The results of the present study with different rice and wheat cultivars reveal that the flag leaf of short-statured Rasi and Sashyasri rice cultivars demonstrated a nonsequential mode of senescence. Thus, there was a dramatic change in the decline of chlorophyll and protein in the flag leaf at the grain maturation stage (21 DAA). In these two rice
cultivars the loss of chlorophyll and protein and the percentage of leaf yellowing were larger in the flag leaf than in the second leaf (Figures 1, 2 & 4), and they became more prominent at the senescent stage (28 DAA). On the other hand, in medium-statured Kalojira and Badsabhog rice and Sonalika and Kalyansona wheat cultivars, a sequential mode of senescence was observed, where the loss of chlorophyll and protein and increase in percentage of leaf yellowing were proportional to the leaf age (Figures 1 to 6) and the higher metabolic activity in terms of maintenance of chlorophyll and protein was noted in the younger flag leaf than in the older second leaf. These observations further confirm the earlier findings from this laboratory (257, 258). But the present study also included two other rice cultivars which showed an intermediate mode of leaf senescence. Thus, in tall-statured Patnai and Kalma rice cultivars, the loss of chlorophyll and protein and the percentage of leaf yellowing were more or less equal in the flag and the second leaf at the senescent stage and the two leaves senesced almost simultaneously (Figures 1, 2 & 4). Hence, these two rice cultivars could not be categorized under either sequential or nonsequential mode as reported earlier and they showed an intermediate mode. The existence of these different patterns of leaf senescence in rice and wheat cultivars indicates that the senescence signal or death hormone may not be involved in inducing different patterns of leaf senescence in these cereal cultivars.
There is a distinct delimitation between reproductive parts and vegetative parts in cereals, and thus, any signal or death hormone originating in the panicle and moving downward would have first induced senescence in the nearest flag leaf in all the cultivars of rice and wheat reported here. Had it been the case, all rice and wheat cultivars would have shown a nonsequential mode of senescence, i.e., the flag leaf would have invariably senesced first regardless of species and cultivars due to its closest proximity to reproductive parts. But this was not true. The fact that both sequential and intermediate modes of leaf senescence also occur in rice and wheat cultivars apparently suggests that the senescence signal was not coming from the panicle, at least in rice and wheat cultivars.

2.4.1.2. If it is assumed that nutrients are mobilized from the nearer leaves to the developing seeds, there will be a gradual decrease of the mineral status of leaves during the progress of reproductive development. Also, a correlation between declining mineral status and the leaf senescence may be expected if deprivation of minerals and metabolites is governing the manifestation of senescence syndrome. In fact, the present study, where two most important mobile nutrients, nitrogen and phosphorus, were taken as indicators of mineral status of leaves (256), points in that direction. Thus, in short-statured Rasi rice cultivar, which represents
a non-sequential mode of whole plant senescence, the loss of these nutrients was more pronounced in the flag than in the second leaf at the senescent stage (28 DAA) (Figures 8 & 9). Interestingly, an opposite trend was observed in medium-statured Kaloljira rice and Sonalika wheat cultivars, which are representatives of sequential mode of whole plant senescence. In fact, in these cultivars the loss of nitrogen and phosphorus in the three uppermost leaves below the panicle was exactly proportional to their leaf age (Figures 8 & 9). On the other hand, in the tall Patna1 rice cultivar, which represents an intermediate type, the loss of these nutrients is more or less equal in the flag and the second leaf of different maturity status. Although Deerman et al.,(89) observed transfer of nitrogen from leaves to fruits in soybean before visible leaf yellowing, they failed to correlate such withdrawal of nutrients with the onset of senescence in soybean. However, the present study clearly reveals that loss of nutrients such as nitrogen and phosphorus from the leaves could be well correlated with the patterns of whole plant senescence in different rice and wheat cultivars. These results, thus, seem to suggest that loss of nutrients from leaves exerts regulatory influence on the manifestation of different modes of senescence observed in different rice and wheat cultivars.
2.4.1.3. To elucidate further the possible relation between mobilization of nutrients from leaves to grains and modes of leaf senescence demonstrated by different rice and wheat cultivars, the pattern of \(^{32}\text{P}\)-phosphate export from leaves to grains and retention of the same by the fed leaves were analysed. Apparently, the data favour the view that mobilization of nutrients from leaves to grains is an important factor in determining different modes of senescence manifested by different rice and wheat cultivars. The export of \(^{32}\text{P}\)-phosphate per leaf basis was significantly greater from the flag than from the second leaf in all the rice and wheat cultivars, which reached a maximal point at the grain development stage (14 DAA), declining thereafter (Figure 10). This suggests that flag leaf is the main exporter of nutrients to the grains, perhaps due to its greater proximity to the sink (Figure 10). When measured per unit leaf area basis (Figure 11), the export of \(^{32}\text{P}\)-phosphate was maximal from the flag leaf of Rasi followed by that of Kalojira rice and Sonalika wheat and Patnai rice cultivars. The leaf area data depicted in Figure 4 further reveals that the export of \(^{32}\text{P}\)-phosphate was inversely proportional to the area of the flag leaf in different cultivars. The area of the flag leaf of Rasi was minimal, but the export of phosphate isotope was also maximal in this cultivar. However, this was just opposite in other cultivars. Thus, the flag leaf of Rasi undergoes greatest nutrient deprivational stress at the
grain development stage (14 DAA) and registered the earliest senescence syndrome. Conceivably, the difference in export between the flag and the second leaf was more pronounced in Rasi than in either Kalojira or Patnai and the difference was much less in the latter cultivars suggesting that both the flag and the second leaf equally participated in the process of nutrient export and the second leaf, being older than the flag leaf, senesced earlier. The pattern shown by Sonalika wheat cultivar exactly followed the same trend as that in Kalojira. Working with the Vigna radiata, Rao and Ghildiyal (335) have suggested that sink itself is instrumental in hastening the declining photosynthesis and the leaf senescence by effecting directly the mobilization and reutilization of nitrogen. The results of the present study also suggest that leaf became exhausted owing to increasing sink demand of the developing grains.

The data of retention capacity of $^{32}\text{P}$-phosphate of fed leaves (Figure 12) reveal that the isotope retention capacity remained greater in the second leaf than in the flag leaf of Rasi even at the senescent stage. This suggests that the metabolic activity remained greater in the second than in the flag leaf of this cultivar at the senescent stage. On the other hand, the capacity for retention of $^{32}\text{P}$-phosphate in Kalojira rice and Sonalika wheat was identical and always greater in the flag than in the second leaf which was in accordance with the maturity status of the
leaves (Figure 12). However, in Patnai, although the pattern of retention of phosphate isotope was similar to that of Kalojira rice and Sonalika wheat cultivars particularly at the earlier stages of reproductive development, the retention capacity of the isotope by both flag and second leaf of this cultivar became more or less equal at the senescent stage (Figure 12). If we consider \(^{32}\text{P}\)-phosphate retention capacity as an indicator of leaf metabolic activity, it will be evident that the retention capacity was proportional to the leaf maturity status in the cultivars Kalojira rice and Sonalika wheat, which were showing sequential mode of senescence. Interestingly, the situation was quite opposite in Rasi showing non-sequential mode of senescence. In the cultivar Patnai, where both the flag and the second leaf senesced almost simultaneously, also registered an equal \(^{32}\text{P}\)-phosphate retention capacity by the flag and the second leaf.

If we cast a glance at the \(^{32}\text{P}\)-phosphate mobilization data shown in Figure 10, it will be revealed that the export of \(^{32}\text{P}\)-phosphate from the leaves was greatest in Patnai followed by Kalojira and Rasi and least in Sonalika. That this export was directly proportional to the sink strength can be substantiated from the data of total weight of panicle in different cereal cultivars studied (Table 1). Thus, it is clear from the data that among the rice cultivars the export of \(^{32}\text{P}\)-phosphate was maximal in Patnai where the
sink strength (panicle weight) was also maximal. On the contrary, the export of $[^{32}\text{P}]$-phosphate from leaves to grains was minimal in Rasi where the sink strength was also minimal. These results thus suggest that sink demand of different rice and wheat cultivars exerts a regulatory influence on the export of nutrients from leaves. Whether active sinks, such as developing fruits, function only in a passive manner by consuming soluble nutrients thereby driving a mass flow system (73) or whether sinks send some hormonal regulatory signals to the supply organs to regulate their activity (289, 290) cannot be said from these results with certainty. Nevertheless, it is certain that the rate of senescence has a relationship with mobilization of nutrients which seems to be proportional to the sink size in rice and wheat cultivars. MacKown et al. (218) have recently reported that labelled N in wheat is translocated to the reproductive parts according to the sink strength and this is in full agreement with the $[^{32}\text{P}]$-phosphate export data presented here.

2.4.1.4. It is well known that supply of water, mineral nutrients and cytokinins from roots to leaves influences the rate of senescence (115, 276, 278, 450). During monocarpic senescence in soybean several authors have shown that root growth ceases, degenerative changes occur and net mineral accumulation declines (89, 281, 283). Roots generally provide
cytokinins and minerals required for maintenance of foliar function and pod development. It has been suggested that changes in the rate of delivery of xylem solutes have regulatory effect on leaf senescence and regulate soluble protein and chlorophyll level (234), as also photosynthetic gas exchange and stomatal resistance (143). In the present study the export of $^{32}$P-phosphate from the isotope-fed roots to the leaves was taken as an indicator of mobilization of nutrients from roots to leaves. The data presented here reveal that there was a gradual decline in the export of $^{32}$P-phosphate from roots to leaves in all rice and wheat cultivars (Figure 13). An analysis of the pattern of mobilization of this isotope in Rasi rice cultivar shows that $^{32}$P-phosphate was exported significantly at a greater extent to the second than in the flag leaf and least so in the third leaf. Thus, not only greater export of metabolites from the flag leaf to the grains, but also lesser entry of nutrients (and possibly root-originated cytokinins) into this leaf might possibly be the major reasons for earlier senescence of the flag leaf than the second leaf. In contrast, the export of $^{32}$P-phosphate from roots was larger in the flag than in the second leaf (Figure 13) in Kalojira rice and Sonalika wheat cultivars and it seems clear that the flag leaf of these cultivars received greater supply of nutrients (and also cytokinins) from the roots compared with the second leaf which enabled it to acquire greater sustenance
capacity and hence senescing later than the second leaf. In Patnai rice cultivar, the export of $^{32}$P-phosphate from roots to the flag and the second leaf did not differ appreciably (Figure 13) and senesced almost simultaneously. The above results, therefore, point out that the supply of nutrients from roots to the leaves varies in different cultivars and possibly plays a role in modifying the leaf senescence behaviour. Such differential export patterns from roots to leaves not only seems to depend on the metabolic status of roots and leaves, but also on the vascular architecture of the specific plant concerned. This will be evident from the studies that follow.

2.4.1.5. To substantiate the above view, the number of vascular bundles at the juncture of leaf lamina and leaf sheath, the area of xylem vessels in a bundle and the lumen of the largest vessel were recorded in three rice and one wheat cultivars (Table 2). Kishitani and Tsunoda (187) could obtain a positive correlation between total cross-sectional area of xylem vessel at the leaf base and leaf area with the photosynthetic rate and water supply. Thus, in short-statured Rasi rice cultivar, showing non-sequential mode of leaf senescence, the above xylem parameters were greater in the second than in the flag leaf and the data of the previous experiments also provided evidence of greater nutrient supply to this leaf. It can, therefore,
be concluded that supply of nutrients (and also cytokinins) from roots to leaves can be regulated and restricted by the vascular architecture prevailing at the juncture of leaf lamina and leaf sheath, which plays an important part in regulating leaf senescence behaviour of the plant. This will be further clear if we analyse the xylem parameters of Kalojira and Patnai rice and Sonalika wheat cultivars (Table 2) where the data of $^{32}$P-phosphate export from roots to leaves and the mode of leaf senescence could also be well correlated. It can be documented in the literature that the fibrovascular tissue system of a plant regulates supply of water, mineral nutrients and cytokinins from roots to the leaves which can eventually modify leaf senescence and that the hydraulic architecture of the shoot also plays a major role in the regulation of leaf senescence (269,273, 277,281,291).

2.4.2. Effect of phytohormones on whole plant senescence of rice and wheat:

2.4.2.1. It is well known that phytohormones as foliar sprays can modify senescence of leaves attached to the plant depending upon the species and leaf maturity status (32,35, 87,281,297,351,339). Among the phytohormones, cytokinins almost universally retarded senescence of leaves. Next to cytokinins, GA$_3$ has been found to retard senescence in many cases (249,272). However, auxin is the least effective
hormone in this respect (167). Nooden (285) also reported
that a combination of (BA + GA₃) synergistically delayed
senescence of soybean plants. On the other hand, ABA and
ethylene promote senescence of the whole plant in a ma-
jority of the cases. In the present investigation, BA, GA₃
and their combination (BA + GA₃) and ABA were used as foliar
sprays to examine their effects on the modification of non-
sequential and sequential modes of senescence (two extreme
types) as observed in rice and wheat cultivars. The study
reveals that none of these hormones could change the pattern
(i.e., non-sequential and sequential) of senescence charac-
teristic of a particular cultivar. However, BA, GA₃ and
their combination retarded senescence of all the leaves over
their control, the combination treatment being most effec-
tive, whereas ABA accelerated senescence in these cultivars
(Table 3 to 6). The most interesting feature of this study
is that, although the hormones could modify the senescence
of leaves, they failed to modify the pattern of whole plant
senescence shown by a particular cultivar. It is to be noted
that the application of BA, GA₃ and their combination
(BA + GA₃) could reduce the difference in the contents of
chlorophyll and protein between flag and second leaf in
all the cultivars which became most pronounced at the sene-
escence stage in untreated control plants. ABA treatment,
on the other hand, markedly increased such difference in
the levels of above cellular components in the flag and
second leaf over the untreated control plants in these cultivars (Table 5). It is noteworthy that effects of all these phytohormones, regardless of their senescence retarding or promoting effects, became evident only at the later stages of reproductive development suggesting that the sensitivity of attached cereal leaves to these phytohormones was much less at the earlier stages of reproductive development. It may be assumed that since the effects of these phytohormones on the total longevity of these plants were rather small, being only effective at the very late stage of reproductive development, they failed to impose any modification on the mode of whole plant senescence observed in these cultivars.

2.4.2.2. Since depletion of mineral nutrients from leaves during reproductive development is an important characteristic of monocarpic senescence (56,89,132,268,291,383), it will be worthwhile to study the effects of above phytohormones on the changes in nitrogen and phosphorus contents of the leaves during the progress of reproductive development in rice and wheat cultivars (Tables 7 to 10). The study reveals that the application of senescence retarding hormones, such as BA, GA₃ and their combination, also retarded the loss of these nutrients from the leaves in accordance with the senescence behaviour of leaves of each cultivar of rice and wheat (Tables 7 to 10). Expectedly, the senescence
promoting hormone ABA rather accelerated the loss of these nutrients, thereby creating greater depletion in the leaves and consequently their earlier senescence. Even though the application of these phytohormones could delay or accelerate the loss of nitrogen and phosphorus from leaves of these cultivars, they failed to alter the specific trend and pattern of depletion of these nutrients shown by the leaves of control plants of each cultivar. Thus, the depletion of these nutrients could be correlated with the mode of senescence displayed by a particular cultivar, notwithstanding that such depletion was either delayed or accelerated by phytohormone application.

2.4.2.3. In order to critically assess the role of senescence retarding and promoting hormones on mineral redistribution, the pattern of export of labelled phosphate from leaves to grains and the retention capacity of the leaves were studied in BA- and ABA-treated rice and wheat cultivars (Tables 11 & 12). The results reveal that BA significantly increased export of [^{32}P]-phosphate as well as the retention capacity of the fed leaves (Table 11), whereas ABA caused an opposite effect in all the cultivars (Table 12). It is interesting to note that although BA and ABA exerted an influence on the export and retention capacity of [^{32}P]-phosphate, the pattern remained essentially the same as that of leaves of untreated control plants in all these
cultivars. This is reminiscent of the observations made by Mondal and Choudhuri (257) in some other rice cultivars. The higher export and retention capacity of $[^{32}\text{P}]$-phosphate by the leaves treated with BA may be attributed to higher synthetic and metabolic activity of BA-treated leaves (91, 198, 304, 423), while the lower export and retention capacity of radiophosphate in ABA-treated leaves may be interpreted as due to lower metabolic activity and higher degradative processes induced by ABA in treated leaves (104, 289, 304, 370).

2.4.3. Role of certain biochemical and enzymatical changes in the senescence development in rice and wheat:

It is now well recognized that gene action is mediated by gene products which characterize the particular tissues and particular stages of development (43, 203). Thus, the appearance and then disappearance of gene products are characteristic features of developmental cycles (92). However, senescence is regulated by a set of genes acting in concert and it is a carefully orchestrated phenomenon resulting in the controlled dismantling of specific cell structures (43, 291, 410, 455). Implicit is the idea that senescence results from programmed changes in gene products. Results of the present study provide evidence of changing patterns of RNA and histone protein in different leaves of rice and wheat cultivars. These two biomolecules seem to
play an important role in the execution of programmed senescence (191,376,388). The present experiment shows that there was a decrease in RNA and increase in histone protein and decrease in the ratio between RNA and histone in the leaves of all the rice and wheat cultivars according to their mode of senescence (Figures 15 & 16). It may be emphasized that there exists an inverse relationship between changes in RNA and histone protein. And the decrease in the ratio of RNA/histone seems to be a consistent indicator of ageing and senescence rather than the changes in their individual component. Furthermore, their changes were most easily discernible prior to senescence of a particular leaf, implicating their active involvement in the senescence process. It is documented in the literature that chromosome-associated histone protein increases and RNA decreases during ageing and senescence of leaves (43,94,252,351,368,410,452,455) which further corroborates the present findings. Application of BA and ABA to the leaves on these cultivars appropriately modified these changes (Table 13), pointing-out their regulatory effects on genes and gene products associated with senescence development. It is now well known that histone protein inhibits the ability of DNA to serve as a template for RNA synthesis (180,204,390). Petel and Berlyan (317) have also shown a close relationship between DNA/histone ratio and RNA content, and the decrease in H1 histone has also been correlated with transcriptionally poised chromosome
structure (69). From the observations of decline in RNA and RNA/histone ratio and slow rise in histone protein in the leaves approaching senescence in different rice and wheat cultivars, it can be assumed that the major synthetic activities are perhaps gradually switched off and the degradative processes switched on during ageing and senescence.

Another spectacular manifestation of switching on of the degradative processes during ageing and senescence is the protein breakdown resulting from high protease activity and the consequent rise in free amino acids in leaf tissues. The liberated free amino acids along with other nitrogenous substances produced during senescence are eventually translocated to the developing grains to meet their demand for nitrogen assimilation (323). The present study also confirms this. The loss of protein in leaves of different rice and wheat cultivars could be correlated with the rise in protease activity with the concomitant accumulation of free amino acids. This was also found to be a function of senescence pattern of leaves. Thus, in the cultivar Rasi, which shows non-sequential mode of leaf senescence, the changes in protease activity and accumulation of free amino acids in leaves were also found to be non-sequential (Figures 17 & 18). Whereas, in the sequential mode of senescence, manifested by Kalojira rice and Sonalika wheat cultivars (Tables 17 & 18), their changes were also sequential, i.e., proportional to the leaf age. The greatest accumulation of free amino acids
in leaves due to proteolysis could be observed at the grain maturation stage, but there was a decline afterwards suggesting that both demands for nitrogen by the sink (panicle) and the supply by the source (leaves) are perhaps reduced prior to senescence. The maintenance of high protease activity until complete senescence of leaves might suggest that protein breakdown occurred till very late stage of senescence. However, lesser accumulation of free amino acids in leaves during this period may apparently appear to be conflicting. But this apparent contradiction may be interpreted as due to known involvement of free amino acids in the synthesis of different hydrolytic enzymes associated with senescence (396) and also in meeting the nitrogen demand of other vegetative parts which are yet to senesce (351).

The deferment of senescence by the application of BA and its promotion by ABA are also reflected in protease activity and free amino acid accumulation (Table 14), implicating a regulatory role of these phytohormones in protein turnover (289, 423). Regardless of the particular pattern of senescence displayed and the initiating and controlling factors involved, the senescence of individual leaf of the cereal cultivars seems likely to be similar at the cellular level. And the present study is indicative of the fact that a balance between senescence retarding and promoting hormones at the cellular level may be one of the important controlling factors of senescence development.
Cellular membranes are selective, dynamic barrier that play an essential role in regulating biochemical and physiological events and the deterioration of membrane is an early fundamental feature of the senescence process (411). Thus, during senescence there is progressive loss in membrane integrity resulting in a rise in membrane permeability. Most of the proteolytic enzymes are known to be located in the vacuoles, while majority of the proteins are located in the chloroplasts (112,220,221,402,438). It follows, therefore, that membrane damage is necessary for protein breakdown and senescence development. It has been postulated by Wierskov and Thimann (424) that senescence process is facilitated by gradual impairment of vacuolar membrane, allowing proteases to enter into the cytosol and attack the proteins there and in the organelles. Free radical-mediated lipid peroxidation is an important mechanism causing membrane deterioration during senescence (411). The accumulation of free radicals during ageing and senescence is also known to augment lipid peroxidation, and one of the reasons for their accumulation is the gradual loss of activities of free radical scavenging enzymes such as superoxide dismutase (SOD), catalase and peroxidase. The present study with different cultivars of rice and wheat also supports the above hypothesis. Thus, there was senescence-related proportional increase in the level of malondialdehyde (MDA), which is the oxidation product of polyunsaturated membrane fatty acids and a reliable
indicator of membrane deterioration, in leaves of different rice and wheat cultivars (Figure 19) pointing out that membrane deterioration is the function of ageing and senescence in these cultivars. That such membrane deterioration is induced by free radicals can be indirectly substantiated from the observations of the decline in the activity of SOD and catalase in leaves with the approach of senescence (Figures 19 & 20) favouring accumulation of oxy free radicals which might lead to membrane deterioration. It has been amply demonstrated by a number of workers that H$_2$O$_2$ is the stable product of oxy free radicals generated in tissues (96) and the involvement of H$_2$O$_2$ in the senescence of leaves has been demonstrated in various plants (61). The rise in peroxidase activity during senescence along with H$_2$O$_2$ may also facilitate oxidative degradation of cellular macromolecules and such a rise in peroxidase activity was also noted in leaves of rice and wheat cultivars (Figure 20). The accumulation of MDA and changes in free radical scavenging enzymes were most pronounced at the senescent stage of rice and wheat cultivars. All these results seem, thus, to indicate that progressive loss of free radical scavenging enzymes, which favour accumulation of free radicals and H$_2$O$_2$, may lead to membrane deterioration in leaf tissues and consequently senescence.
The external application of BA significantly retarded MDA accumulation and enhanced the activities of free radical scavenging enzymes (Tables 15 & 16) as mentioned above, while ABA caused an opposite effect. Thus, it may be assumed that one of the mechanisms of phytohormones retarding or promoting senescence of plant tissue is mediated by regulating free radical scavenging enzymes such as SOD, catalase and peroxidase and modulating free radical-induced lipid peroxidation and membrane damage.

2.4.4. **Role of transpiration in the regulation of senescence**: Transpiration seems to play an important role in the regulation of monocarpic senescence because the supply of cytokinins, nutrients and water to the aerial parts is partly dependent on the transpiration pull along the stem axis (115, 157, 174, 281, 289, 384). The present study shows that rate of transpiration by the whole plant (main tiller) gradually decreased in rice and wheat cultivars during their reproductive development (Table 17). The rate of transpiration also decreased in accordance with the mode of senescence in these cultivars. Such decrease in the rate of transpiration may be related with age of leaves, since stomata are known to fully or partially close in aged tissue of many plants (403, 405). While treatment of plants with BA significantly increased the rate of transpiration by the whole plant as well as by
individual leaf, ABA spraying strongly inhibited the same in all the cultivars studied (Table 18). It is documented in the literature that cytokinins keep stomata open, while ABA causes stomata to close (160,171,231,333,403). The effects of BA and ABA in the present experiments can be interpreted on the basis of their regulatory influence on stomatal opening and closing. There are also reports that a decrease in cytokinins and an increase in ABA level take place during ageing and senescence in plants (85,118,300, 360,436). The occurrence of such a situation under natural condition may strongly favour stomatal closure that presumably be responsible for the reduction of transpiration in different rice and wheat cultivars during progressive ageing.

The accumulation of proline in stressed tissues is a widely reported observation (46,324,393,395). Proline accumulation during ageing and senescence has also been reported in isolated (394,430) as well as in intact leaves (260,392) of many species. Furthermore, it has been postulated by Biswas and Choudhuri (32) that mobilization of metabolites from leaves to grains in rice during reproductive development causes a deprivalional stress in leaves. It was subsequently demonstrated by Ray and Choudhuri (339) that there was an increase in ABA-like substances due to such deprivalional stress in rice leaves. That stress-induced ABA can, in turn, enhance proline accumulation in leaves is also well documented
(12,324,394). Like ABA, proline can also promote stomatal closure (333,334). Thus, the accumulation of both ABA and proline with age may serve as reliable indicators of whole plant senescence. In the present study it was observed that proline accumulation gradually increased in the leaves of all rice and wheat cultivars up to the grain maturation stage, but decreased thereafter (Figure 21). Thus, at the grain maturation stage maximal accumulation of proline took place in the flag leaf of Rasi, while it was in the second leaf in Kalojira rice and Sonalika wheat cultivars. These results fit in well with the observed modes of senescence in these cultivars. The maximal accumulation of proline at the grain maturation stage suggests that the leaves had undergone maximal stress prior to this stage. The data of $^{32}$P-phosphate export, discussed earlier (Figure 10), show that maximal mobilization of metabolites took place at the grain development stage. Thus, it may be assumed that the leaves were subjected to maximal deprivational stress at the grain development stage (14 DAA) and such an assumption finds support from the observation of proline accumulation at the grain maturation stage (21 DAA). Our data, therefore, lend further credence to the postulation made by Biswas and Choudhuri (32) as alluded before. A large number of reports shows that the application of ABA induces proline accumulation in the treated tissue (12,324,392,394). However, the effect of cytokinin on proline accumulation has been scarcely reported (260,395). Nevertheless, the retardation of proline
accumulation by BA and its acceleration by ABA treatment in leaves of rice and wheat cultivars (Table 19) suggest that proline accumulation is one of the characteristic features of senescence syndrome and may be intimately involved in the process of senescence development. From the evidence presented here it may be assumed that retardation of the accumulation of proline by BA and its promotion by ABA treatment may presumably be mediated by decreasing or increasing the internal nutrient deprivational stress, respectively.

2.4.5. **Hormonal mechanism of regulation of senescence**

In recent years, several studies have emphasized the regulatory role of endogenous cytokinins and ABA in the senescence process of the whole plant or detached organs, particularly leaves (9, 32, 64, 278, 283, 297, 300, 351, 384, 403, 423). It is also well known that ethylene plays an important role in leaf abscission, but its role in leaf senescence is not very clear. Several workers, however, have suggested that ethylene acts as a senescence promoting hormone (1, 5, 119, 176). It has also been demonstrated that during senescence the release of endogenous ethylene increases (5, 176). Reports concerning the decrease in endogenous cytokinin-like (CK-like) substances and the rise in endogenous ABA level during ageing and senescence of leaf tissues are also not very uncommon in the literature (32, 60, 84, 118, 174, 211, 291, 300, 302, 303, 339, 436). The present study with different rice
and wheat cultivars also supports the above findings. The study shows that CK-like substances gradually decreased in all the leaves of both rice and wheat cultivars during the progress of reproductive development (Figure 22). In Rasi, the decline in CK-like substances was much greater in the flag than in the second leaf. In contrast, an opposite trend was observed in Kalojira rice and Sonalika wheat cultivars. These findings are in complete agreement with the observed mode of senescence in these cultivars. In general, the decline in CK-like substances may presumably be ascribed to the decline in root cytokinin production (85, 184, 294, 300) which may also be correlated with lowering of transpiration pull (21, 115, 150). The little cytokinins that are still produced by the roots may be translocated to the developing seeds instead of leaves because of greater sink demand of the former (211, 287, 289, 357, 436). Such a view gets its support from the observation that depodding increases CK-level in leaves and delays senescence in soybean (300). The decline in cytokinin in leaves may be also responsible for inhibiting the synthetic processes and promoting the degradative processes (32, 35, 173, 174, 259, 289, 460).

Concomitant with the decline in CK-like substances in leaves, the level of ABA gradually increased up to the grain maturation stage, declining thereafter in rice and wheat cultivars (Figure 23). The decline in ABA in the flag leaf
of Rasi was faster than that in the second leaf. Whereas in Kalojira rice and Sonalika wheat cultivars, the level of ABA increased in chronological order of the leaves (Figure 24). Thus, earlier accumulation of ABA in the flag leaf of Rasi and in the second leaf of Kalojira rice and Sonalika wheat cultivars with the simultaneous decline in CK-like substances prior to senescence development appears to be an important factor regulating monocarpic senescence of cereal plants. The external application of these two hormones also exerted their regulatory effects on the whole plant senescence of these cultivars. The effect of ABA on the whole plant senescence may presumably be mediated through retardation of many physiological and biochemical processes such as photosynthesis, protein synthesis, stomatal closure etc (118, 134, 289, 291, 345, 351, 370, 403).

It is noteworthy that the ratio of CK-like substances and ABA gradually decreased with the progress of reproductive development in both rice and wheat cultivars. The maximal decrease was observed at the grain maturation stage (21 DAA), which just preceded the senescent stage (28 DAA). All these results seem, thus, to suggest that a critical balance between these two hormones has a profound regulatory influence on the development of monocarpic senescence in cereal plants. This is in complete agreement with the view of several workers (95, 101, 351). But it is not yet
clear from these results what actually triggers the initiation of monocarpic senescence in these plants.

Among the known hormones, the role of ethylene in whole plant senescence is most conflicting, although ethylene has been suggested by many workers to act as a senescence promoting hormone in attached and detached leaves of many species (1, 5, 176, 241, 332, 458). Aharoni et al. (7) have reported that ethylene production decreased in the first phase of senescence and there was a transitory rise in ethylene production during the phase of rapid chlorophyll loss. From our data it could be stated that the release of ethylene was the function of leaf ageing and senescence development in different rice and wheat cultivars (Figure 25). Thus, with the approach of senescence, the release of ethylene also increased. The rise in ethylene production was significantly faster at the later stage of reproductive development in both rice and wheat cultivars. The difference in ethylene production between the flag and the second leaf of Kalojira (rice) and Sonalika (wheat) was not as prominent as that of Rasi. That ethylene plays a regulatory role in petal senescence has recently been demonstrated by Lawton et al. (203) in carnation. It has also been reported by Woodson (448) that flower senescence in carnation is associated with changes in gene expression involving both protein and in RNA changes. The early rise in ethylene in leaves during anthesis stage suggests that ethylene might
also play a similar role in cereal leaf senescence as has been observed in carnation petals. One interesting finding reported by Schwabe and Kulkarni (366) is that severe water stress, which was accompanied by a significant rise in ABA in detached leaves of *Kleinia articulata*, did not lead to senescence unless combined with ethylene treatment and postulated that while ABA might play an important role in leaf senescence, its lethal effect could not be realised until and unless ethylene-induced membrane changes would also synergistically assist. External application of ethylene has also been reported to enhance various senescence-related processes in plants (176,313,447). From all these observations along with those reported here, it may be concluded that a decline in CK level and a rise in ABA and ethylene levels in plants during their reproductive development are intricately involved in the senescence development of different cereal cultivars.

2.4.6. Changes in nucleic acids and proteins in the shoot apex during transition from vegetative to flowering stage of rice and wheat cultivars:

It has been observed that the transition from the vegetative to the reproductive stage is accompanied by profound physiological, biochemical, genetical and hormonal changes in plants (25,78,126,131,164,165,195,217,371). Since developmental processes are genetically programmed, the gene products are also accordingly changed for the execution of
this programme. Evidently, characteristic changes are expected to occur in nucleic acids and proteins, the main biochemical agents for the execution of the developmental programme during transition from the vegetative to the reproductive stage. In the present study we, therefore, attempted to analyse changes in some important biomolecules deemed to be associated with such transition. Thus, changes in total protein, DNA, RNA and histone protein were studied prior to this transition in two rice and one wheat cultivars. There was a significant rise in protein, DNA and RNA in the shoot apex of Rasi rice cultivar up to the plant age of 74–80 days, i.e., at least 12 days before panicle emergence and there was a decline thereafter (Figure 26). Interestingly, the histone protein increased at the plant age of 77 to 80 days when other biomolecules started declining. The same trend was also noted in Kalojira rice and Sonalika wheat, varying only in the time period (Figures 27 & 28). Histone protein is known to act as an inhibitor of gene expression (180, 388, 390). The critical rise in nucleic acids and total proteins and decline in histone protein prior to transition from one phase to the other suggests that profound gene activity may possibly be required for panicle initiation in these cereal cultivars. The decline in nucleic acids and protein and the rise in histone protein perhaps mark the end of the transition phenomenon. From all these results it can be concluded that marked changes in genetic materials as manifested by the changes in
the contents of nucleic acids and acidic and basic proteins occur in the shoot apex prior to transition from the vegetative to the reproductive stage of these cultivars.

2.4.7. **Surgical experiments**:  

2.4.7.1. Reproductive parts of monocarpic plants have been reported to be the most important agents which influence the whole plant senescence presumably by drawing metabolites from the vegetative parts (nutrient drain hypothesis) and/or supplying some as yet unknown senescence-inducing factor(s) to the vegetative parts (senescence signal hypothesis). Thus, it has been reported by the majority of the researchers that removal of reproductive parts delays senescence of the whole plant \((35,210,257,290,318,339,421)\), notwithstanding a few reports to the contrary \((8,65,137)\). The present experiments showed that removal of panicle from Rasi and Kalojira rice cultivars, either at the anthesis (0 DAA) or at the grain-filling stage (7 DAA), reduced the loss of chlorophyll and protein over the intact control plants and delayed senescence (Tables 20 & 21). The most interesting feature of panicle removal experiment is that such treatment altered the mode of senescence in Rasi, where the non-sequential mode was changed to the sequential mode, as observed in Kalojira rice and Sonalika wheat cultivars. Although removal of panicle delayed the onset of leaf senescence in Kalojira rice and Sonalika wheat cultivars, there was no change in the mode
of senescence (Tables 21 & 22). It may be noted that the delaying effect due to panicle removal was more pronounced if done at the earlier stages in all the rice and wheat cultivars. This was also true for Rasi. This is imperative because if the sink (panicle) is removed at earlier stages, less nutrients will be drawn out from the source (leaves) and evidently there will be delay in leaf senescence. And this perhaps happened in the present case. Furthermore, if any senescence factor from reproductive parts be involved in initiating senescence, the possibility of its accumulation in leaves would be less if the reproductive sink (panicle) was removed earlier. Another interesting feature of this experiment is that the delaying effect due to panicle removal was more marked in rice compared with that in wheat. One of the possible reasons of such difference may be ascribed to the greater availability of current photosynthate produced by the glumes and awns in wheat than in rice (40,100,185,356,415), which possibly made it less dependent on source leaf unlike that happened in rice. The evidence in favour of this explanation may be provided from the data presented here, which showed that the initial content of both chlorophyll and protein in glumes was significantly higher in wheat than in rice, but the decline of these components was much greater in wheat than in rice during reproductive development (Figure 29). This suggests that the contribution to grain-filling from the flag leaf of rice was greater than
that of wheat (265) and conversely, the contribution of ear parts to grain-filling was greater in wheat than that in rice (414). It has been suggested by Biswas and Mondal (35) that in wheat the glumes act as intermediaries in the transfer of assimilates from leaves to grains and the contribution of flag leaf is rather indirect. However, the situation appears to be opposite in rice (259). This suggestion could well be applied to the above observations made by us in both rice and wheat.

The analysis of nitrogen and phosphorus contents of the leaves of rice and wheat cultivars from which the panicle was removed at the anthesis (0 DAA) and grain formation (7 DAA) stage showed that their contents decreased relatively slowly in comparison to that of intact control plants with the progress of reproductive development. Interestingly, the removal of panicle after grain-filling stage (14 DAA) neither reduced the loss of nitrogen and phosphorus nor delayed senescence of leaves (Tables 23 to 25), suggesting that the maximal sink demand possibly was developed around the grain-filling stage (14 DAA) causing the greatest depletion of nutrients in leaves and promoting senescence. The delayed removal of panicle produced no effect on the mineral contents of leaves over the intact control plants suggesting that the demand of nutrients from the leaves by the developing grains had already been met by the time the panicle was
removed. The removal of sink has been shown to increase the mineral level in leaves in many species (89,294,443,444). That the rate of senescence could be altered by sink size has also been demonstrated by several workers (72,73,74,75). However, it is also suggested by several workers that drawing of a large amount of metabolites from the vegetative parts by the developing fruits may be an important factor for rapid senescence in many monocarpic plants (32,35,253, 258,339). A similar conclusion has been also drawn by Davies and his coworkers working with G2 line of pea (139,182).

The fact that the decline in minerals from leaves, particularly nitrogen and phosphorus, is due to their export to grains could be demonstrated by isotope studies. In fact, $[^{32}\text{P}]-$phosphate isotope experiments revealed that the removal of panicle at the anthesis (O DAA) or grain-filling stage (7 DAA) significantly increased the retention capacity of $[^{32}\text{P}]-$phosphate of all the three leaves and stem of rice and wheat cultivars (Tables 26 to 28). However, such an act of removal at the later stages of reproductive development (14 and 21 DAA) failed to increase the retention capacity of $[^{32}\text{P}]-$phosphate in the leaves. This suggests that export of major nutrients from the leaves to the grains became relatively insignificant after the grain-filling stage, since the maximal export of $[^{32}\text{P}]-$phosphate took place within 14 DAA after anthesis of rice and wheat cultivars. The isotopic
studies further substantiated the observation that the developing seeds are less dependent on the leaves for the metabolite supply in wheat compared with that in rice. The rise in retention capacity of $^{32}$P-phosphate in leaves and stem of plants from which the panicle was removed at the earlier stages of reproductive development is likely to be due to the absence of sink demand which remained maximal at the earlier stages of reproductive development in intact plants of rice and wheat cultivars.

2.4.7.2. Surgical alterations of the source-sink ratio may provide some interesting clues to the specific involvement of an individual leaf and the reproductive parts in the whole plant senescence of rice and wheat cultivars. Thus, in order to assess this, the plants were first subjected to surgical alteration by defoliation treatment. The senescence behaviour of the remaining leaves was studied after excision of a particular leaf. The results clearly revealed that the removal of the flag leaf had the most significant effect on the enhancement of senescence of the second leaf in all rice and wheat cultivars (Tables 29 to 31). Whereas, removal of the second leaf, although enhanced the senescence of the flag leaf, had less pronounced effect compared with that observed in the second leaf when the flag leaf was removed. The detachment of the third leaf, on the other hand, produced little effect on the senescence of either
the flag or the second leaf. All these results seem to suggest that the flag leaf plays an important role in the mobilization of metabolites to the sink in these cereal cultivars. It is also to be noted that the effect of leaf removal on the senescence of remaining leaves was more pronounced in rice than in wheat. This again suggests that the involvement of leaves in the export of metabolites was less significant in wheat than in rice. These observations are in full agreement with those of Mondal and Choudhuri (258) in rice and Biswas and Mondal in wheat (39). Analysis of leaf nitrogen and phosphorus contents of the remaining leaves as well as $[^{32}\text{P}]$-phosphate retention capacity and export data of the plants from which a particular leaf was excised, lends further credence to the above suggestion (Tables 32 to 37). These data further point out that mobilization of nutrients and metabolites from the leaves to the panicle plays an important role in the senescence development of cereal plants. It was also clear from the results presented above that when the ratio of source to sink was lowered by excision of leaves, particularly the flag or the second, the remaining leaves appeared to compensate the sink demand (312) which remained constant throughout and such a situation presumably enhanced the development of senescence in the remaining leaves due to augmented depletion of metabolites and the resultant exhaustion.
2.4.7.3. As previously stated, the reproductive structures of monocarpic plants may act as a strong sink for drawing nutrients and metabolites from leaves or may send some senescence-inducing stimulus to the leaves or both. In order to further clarify the role of reproductive structures in inducing senescence of rice and wheat cultivars, we attempted to examine the effect of removal of different percentage of spikelets from the panicle or emasculation treatment on the whole plant senescence of rice and wheat cultivars. One of the most interesting features of rice cultivars is that complete removal (100%) of spikelets induced the development of a secondary branch at the axil of the second leaf unlike that in wheat cultivar where no such branch development took place (Table 38 & 39). Furthermore, when the spikelets of the secondary branch of rice was completely removed there was development of the tertiary branch. However, no further branch development occurred when the spikelets of the tertiary branch itself were completely removed. The secondary and tertiary branches contained only two leaves. Removal of different percentage of spikelets from the panicle of the main tiller or emasculation of the panicle in rice and wheat cultivars produced some interesting results. Thus, the removal of 50, 75 and 100 percent spikelets from the panicle or emasculation of the panicle in Rasi and Kalojira rice cultivars caused an induction of secondary branch development while the removal of 25 percent spikelet had no effect on
branch development. Again, the removal of 75 and 100 percent spikelets from the panicle or emasculation of the panicle of the secondary branch was only effective in producing tertiary branch, while the removal of 25 and 50 percent spikelets from the panicle of the secondary branch produced no branch, i.e., this did not induce the tertiary branch development (Tables 38 & 39). Interestingly, even complete removal of spikelets from the tertiary branch or its emasculation treatment failed to produce further branch development (i.e. quaternary branch).

All these results seem, thus, to suggest that the sink size in rice cultivars exerts a regulatory role on the secondary and tertiary branch development. However, the failure of quaternary branch development even after the complete removal of the panicle in the tertiary branch points out that the sink size is not the only limiting factor in inducing branch production. It can be observed from the data of leaf area and seed production capacity of the main-tiller, secondary and tertiary branches that there was a gradual reduction in the number of seeds per panicle and the area of the flag and the second leaf from the main tiller to the tertiary branch (Tables 38 & 39). This leads to the suggestion that a critical balance between the size of the sink and the source was necessary for the induction of branch development in rice cultivars. It appears that such
a balance was lost in the tertiary branch and hence no further branch development occurred at the leaf axil of this branch. These interesting results however need further critical evaluation.

It is clear from the above results that the reproductive parts (panicle) exerted an inhibitory influence on side branch development in rice plants comparable with the phenomenon of 'apical dominance'. Nevertheless, the complete inhibition of development of quaternary branch from the tertiary branch clearly indicates that some factor(s), associated with branch development, became limiting in the tertiary branch. As a consequence of this, there was no quaternary branch development and the above-ground parts of the plant completely senesced and died along with the tertiary branch. It is tempting to speculate that the apical dominance-like effect, as exerted by the panicle, appears to regulate the onset of whole plant senescence. Thus, the removal of a higher percentage of spikelets was necessary for destroying the apical dominance-like effect and inducing the development of tertiary branch than what was necessary for the induction of secondary branch development in both the rice cultivars studied.

Another factor which seems to be involved in this phenomenon is the leaf area. The seed production capacity of different branches was found to be directly related with the leaf area, since leaf is the main source for supplying photosynthate and other metabolites for the seed development (44, 232). It seems, therefore, that the initiation of side branches in rice cultivars was the consequence of a balance
between the inhibitory influence of the panicle and the promotive influence of the leaf subtending the branch, possibly mediated by metabolites and phytohormones. Evidently, the failure of quaternary branch development at the second leaf axil of the tertiary branch even after complete removal of spikelets or emasculation treatment seems to point out that the branch initiation factor(s), possibly supplied by the second leaf of tertiary branch, became limiting due to the reduction of leaf area below a critical level. That the leaf provides an essential factor(s) for the side branch development can be further corroborated from the findings that complete removal of both spikelets and the second leaf from the main tiller and similar treatment of the secondary branch in both rice cultivars completely failed to induce secondary and tertiary branch development, respectively. It is to be noted here that the removal of spikelets from the main tiller of the wheat cultivar did not initiate secondary branch development, suggesting that inhibitory influence of the panicle is species specific. However, there may be another possible reason for such difference observed in rice and wheat cultivars. In rice, the grain-filling is largely dependent on the supply of photosynthate and metabolites from leaves, whereas in wheat the reproductive parts also actively participate in the grain-filling process, where the leaves often play a secondary role. Thus, the removal of total sink in wheat cultivar
possibly did not cause sufficient alteration in the cellular metabolites in the second leaf which seems necessary for branch development. A recent report by Bangerth (14) shows that polar transport of IAA from the earlier developing seeds inhibits the development of other seeds and also observed a Primigenic dominance (PP), a kind of correlative inhibition, in which the earlier developing seeds inhibits later developing organs. Such transport of IAA from the fruits has also been found to be closely related to the number of seeds present (385) which inhibits lateral bud formation. It has also been suggested by several authors that cytokinins originating from roots possibly inhibit IAA metabolism and promote lateral bud growth (154,325). The application of cytokinin or IAA and a combination of both in lanoline paste on the cut surface of the depanicled plants had no influence on secondary branch development as noted in the present study (data not shown). This suggests that the apical dominance like influence of the panicle may be exerted through some substance(s) other than IAA and/or cytokinins. Admittedly, this can not be said with certainty unless labelled IAA or cytokinins are used in such experiments.

Looking at the senescence behaviour of leaves of two rice and one wheat cultivars after removal of different percentage of spikelets from the panicle or emasculation treatment, one can easily infer that senescence was delayed
in both rice and wheat cultivars due to removal of spikelets or emasculation treatment and the delaying effect gradually increased with increasing removal of spikelets (Tables 40 to 42). Thus, the reduction of sink demand was related to the progress of senescence, although complete removal of the panicle (sink demand) did not prevent senescence completely. This observation finds support from the observations of Biswas and Mondal (39) in wheat, Valio and Polo (421) in bean, Crafts-Brandner and Egli (73) in soybean and Kelly and Davies (182) in G2 pea. Another interesting feature of the present study is that a minimum of 50 percent spikelet removal from the main tiller was necessary to induce secondary branch development at the second leaf axil of the main tiller of Rasi cultivar as well as for inducing a change from the non-sequential to the sequential mode of senescence. To achieve such results in the secondary branch of Rasi, a minimum of 75 percent spikelet removal was necessary. Although there was a delaying of senescence of leaves in Kalojira due to spikelet removal or emasculation treatment, the sequential pattern of senescence is maintained throughout the reproductive development.

If one follows the pattern of leaf senescence of secondary and tertiary branches, it will be seen that they always showed sequential and non-sequential mode according to the mode shown by the main tiller unless, of course the
spikelets were removed, suggesting that a particular mode of senescence is genetically controlled. Although the removal of 75 and 100 percent spikelets or emasculation of tertiary branch delayed leaf senescence, quaternary branch was not formed in either rice cultivar and consequently the mode of leaf senescence behaviour in Rasi was not changed in this branch, i.e., it remained non-sequential like the main tiller (Table 45) because there was no alternative sink at the second leaf axil of the tertiary branch.

It is noteworthy that the total longevity of the main tiller (i.e. the whole plant) was extended by about 42 days over the unexcised control plants owing to side branch production at the second leaf axil. Hence it may be concluded that both leaves and reproductive parts might exert regulatory role in side branch production, which, in turn, might control the longevity of the whole plant. The senescence inducing substance, if formed in the subtending second leaf, was not transmitted to the leaves of the side branch. Had this happened, the second leaf of Rasi would have then senesced earlier than the flag leaf of the side branch which was, however, not the case. Thus, in rice, the failure of further branch production on removal of spikelets marks the end of the whole plant.