Chapter-IV
DISCUSSION

D I S C U S S I O N

Effects on Growth and Yield

Most researchers in the field of air pollution in the past, and still to some extent today, have been interested in the symptomatology of acute visible injury caused by specific pollutants when test plants are exposed to relatively high pollutant concentrations. The consequence has been a well documented and ever increasing compendium of injury symptoms, eventhough such injury is not widely occurring, and is frequently associated with episodes of local high concentrations. The situation is very different in the case of chronic injury, resulting from long-term exposures to subacute levels of pollutants, which may appear as retardation or disturbance of normal growth and development (resulting in reduction of growth, yield or quality of agricultural crops), or slow discolouration (chlorosis) leaf tip necrosis and, ultimately, total die back of plant organs. As the ambient pollutant levels in most of the industrialised areas are found to be low, the present study has been carried out under chronic air pollution stress. Moreover, an accurate prediction of economic losses in crops can only be made by exposing plants to pollutants **in situ**. The results in the current study indicated that at the age of 15 days plant height of all the cultivars were equally and sharply reduced but the reduction were minimised later to varying degrees. It would suggest that the low levels of air pollutants present during the growth period could reflect upon the growth of crops at their final height and further it would signify the differential response of the cultivars to the chronic air pollution. The suppression of plant growth by air pollutants has been an interesting subject since many decades (Hill and Littlefield, 1969; Taylor and Eaton, 1966; Thompson **et al.**, 1967; Taniyama, 1972; Bennett **et al.**, 1974; Reich and Amundson, 1984). The second year results too kept in line with the above studies as the plant height was inhibited in rice.

Further, the artificial fumigation of SO_2 also clearly produced significant reduction on all the cultivars of rice, which was identical to the report of Nandi **et al.**, (1985) on rice. The growth can be curtailed by number of mechanisms that include reduction in the net photosynthetic rate or loss in photosynthetic capacity through leaf injury. Even in the absence of visible leaf injury the net photosynthesis was reduced by sulphur dioxide in rice, as proved by Taniyama (1972). However, Matsuoka (1978) indicated that the magnitude of the photosynthetic inhibition was not fully reflected in the decrease of dry weight under SO_2 fumigation. In contrast to the above reports, Bell and Clough (1973) found that the perennial ryegrass exposed to 0.067 ppm SO_2 coinciding the winter mean concentration ranges from the polluted rural areas, showed slightly but significantly greater plant height in the initial stages of the fumigation. However, they registered an inhibition in the accumulation of dry matter. Like ryegrass, rice plants showed no significant improvement in height under the influence of SO_2. Nevertheless, chronic air pollution mixture effected into the production of greater tiller numbers in the test site plants, accompanied by more fresh and dry weight of shoot than the controls. The second year study also witnessed significant increase in the tiller numbers but no change in the fresh and dry weight of shoot system of normal polluted plants. Such improvements brought about by chronic air pollutants were not uncommon in the history of air pollution effects on plants. Plants as widely different as lemon (Thompson and Taylor, 1969) and orchard grass **(Dactylis glomerata)** produced more wet and dry matter in low fluoride atmospheres than in filtered air. Hitchcok and others (1971) also proved this fact with their 14 years study using alfalfa and orchard grass. Conversely, it has been reported that under SO_2 pollution shoot dry weight of ryegrass was reduced with a proportionate decrease in the number of tillers (Bell and Clough, 1973; Bell **et al.**, 1979) and in certain cases without changes in the tiller numbers (Ashenden and Mansfield, 1977; Davies, 1980).

The number of leaves in the rice cultivars at the test site was found to be very high, maintaining the photosynthetic leaf area, inspite of much reduced area per leaf. It could be alluded that the increased number of smaller leaves and increased tiller production would be the characters of adaptability in rice cultivars to chronic air pollution effects. In the fumigation studies, NH_3 fumigation effected a profound increase in the shoot length and dry matter, TLA and leaf dry matter of cv GR 3 which could reflect the paramount ability of this cultivar to grow under ammonia pollution. The reason could be due to an ability of this cultivar to absorb and incorporate the nitrogen from the air pollutants as observed in corn seedlings by Porter **et al.**, (1972) for their benefit and thereby ultimately bringing an increase in the grain yield as observed in the first year study. Porter and others (1972) observed that the corn seedlings absorbed NH_3 equivalent to 30 % out of the exposed 10 ppm. At low concentrations, NH_3 produced no significant effect on the growth of black gram (Raza and Bano, 1981). However, in the present study cvs CO 43 and TKM 9 were appeared to be sensitive to NH_3 as depicted by the depression in most of the studied parameters. NO_2 fumigation was found to be more toxic to cv GR 3 showing reduction in growth characters than the other cultivars providing differential response to specific pollutants.

Interaction of pollutants were common in plants as reported in marigold (Sanders and Reinert, 1982) unless one of the pollutants produced an excessive amount of injury to mask the effect of other pollutants. Reports on most studies involving mixtures included or implied an assessment of the interaction of pollutants in terms of additive effects, greater than additive (synergistic) effects, or lesser than additive (antagonistic) effects (Tingey and Reinert, 1975). In the present study, artificial fumigation of SO_2 and NH_3 mixture did not produce much disparity between the control and treated plants, which would suggest that SO_2 and NH_3 mixture were antagonistic to the growth of rice plants. It was also clear that under SO_2 + NO_2 mixture, reduction in shoot length, TLA, leaf drv matter associated with reduction in the dry matter of shoot and root were evident. The cv TKM 9 showed a marginal increase in the shoot length and TLA under NO_2 fumigation alone but experienced a reduction in the above characters to SO_2 + NO_2 exposure. Two interesting inferences could be drawn in the interaction of pollutants from these findings. First, the data constitute a valuable confirmation of the synergistic sensitive activity of SO_2 and NO_2 mixture. Secondly, the TLA and leaf dry matter of cvs CO 43 and TKM 9 were increased to NO_2 and reduced to SO_2 + NO_2 mixture. However, cv GR 3 had a drastic reduction to NO_2 showed half of its reduction to SO_2 and NO_2 mixture. It would suggest that the differences between cultivars might reside in the capacity of any particular cultivar to inactivate the toxicity. In rice, dry matter production depends on the photosynthetic rates per unit leaf area, leaf area duration and sink capacity (spikelet numbers to receive the photosynthates). Further, the efficiency of unit leaf area depends on the translocation of the photosynthates out of leaf to the developing panicle (Murty,1986).

Since SO_2, NO_2 and NH_3 were present in the test site it was more likely that the increased or unaffected dry matter of shoot could be due to the beneficiary effects of SO_2 at low concentrations (Cowling and Lockyer, 1978; Lockyer **et al.**, 1986), as SO_2 could be converted into essential organic sulphur compounds (Seigel, 1975). Likewise, oxides of nitrogen and NH_3 could also behave as atmospheric nutrients (Anderson and Mansfield, 1979; Porter **et al.**, 1972). This could be supported by the results based on a total of 43 years of examination on the vegetation in the vicinity of a copper smelter in Arizona (USA) by Haase **et al.**, (1980) who concluded that even after leaf injury the SO_2 exposures could result in beneficial effects on both growth and yield.

In the present study (I year) air pollution significantly increased the number of panicles and the percentage of sterility as the main effects. Moreover, it was interesting to note that the panicle initiation was delayed in polluted cultivars. The delay was about 13 days in cvs GR 3 and TKM 9 while it was 35 days in cv CO 43, when compared to control. As expected there was a wide variability among the cultivars in the yield components in response to air pollution. These differences might have been due to physiological differences in the plants. The pollution and cultivar interaction was found to be favourable to rice cv GR 3 as depicted by its number of panicles and filled grains. In contrast, pollution reduced the production of filled grains in cv CO 43, leading to an adverse increase in the sterility index. However, cv TKM 9 experienced a mixed effect. Kats **et al.**,(1985) found that in a rice cultivar exposed to SO_2 and O_3 fumigation, the increased sterility of spikelets was offset by increased number of panicles and they found a reduced growth and total yield. Paradoxically, in the present study a significant increase in the number of panicles and filled grains brought about a remarkable increase in the production of biological and economic yields, in the plants of cv GR 3 grown near the factory. Conversely, in the cv CO 43, though there was a considerable increase in the biological yield, a drastic reduction in the number of filled grains and increased sterility percentage rendered a meagre production of the economic yield under the polluted condition. The reason could be the limited translocation of photosynthates to the developing spikelets out of the leaves due to pollution. The cv TKM 9 showed no significant effect on its biological and economic yields. Differences in yield between control plots and the experimental plots were statistically significant when the sulphation rates exceeded 0.80 mg/(100 cm² d^{-1}) in studies elsewhere on potato, beet and barley (Wateresiewicz and Szalonek, 1972) in the vicinity of single point source. In the present study though the sulphation rate was increased above 0.82 sometimes, the presence of other pollutants should not be neglected for the effects.

It was interesting to note that there was no significant interactive effect due to environment alone, as depicted from the mean squares of ANOVA on the economic yield. But the interaction of cultivar and environment on the economic yield turned out to be significant and highly detrimental to the cv CO 43 and favourable to the cv GR 3. The adverse effect on CO 43 was overwhelmed by the favourable effect on GR 3 therebyultimately showing no significant effect between the overall control and polluted site plants. The above situation conformed the discussion of Oshima and Bennett (1979) on the use of treatment contrasts for air pollution mixture studies, where the studies did not require a statistical significance as a pre-requisite and could be applied regardless of the results of overall analysis of variance. The compilation of results of I year study on growth and yield suggested that the cv GR 3 as tolerant, TKM 9 as moderately tolerant and CO 43 as sensitive to chronic air pollution of the fertilizer plant.

In the light of I year study, among the three cultivars of rice, cv GR 3, which showed the harvest index above 0.3, a high yielding respone in rice (Murty and Venkateswarlu, 1979), was tested for another year, at the same polluted site. The yield performance, however, did not strictly suDDort the first year study. Though the chronic air pollution could produce a high number of panicles at the test site, it drastically reduced the economic yield of cv GR 3. However, an increased sterility index, unlike the I year study, possibly suggested that the pollution events would have affected during the period of anthesis. The monitored sulphation rate and fluoride concentrations were found to be increased during the month of September, 1986, at the time of anthesis which could be the possible cause for the ill-effect. However, a positive conclusion to this effect was not possible as the dry deposition of pollution was monitored on monthly basis and not daily. Alternatively the other possibility could be due to the climatic factors, since most of the growth as well as the yield parameters were showing lesser performance than the first year plants, regardless of the site of growth (It should be remembered that the Western-India faced a severe drought at that time). Comparatively the rain fall seemed to be very meagre and the average temperature had risen to 34.7°C from 32.7°C of the previous year during the growth period in the second year (Fig.3). Warm temperature during plant growth in general enhanced the sensitivity of plants to air pollutants (Dunning and Heck, 1977). In barley and rape, inhibition of photosynthesis tended to be greater at 20°C than at 10°C and in rice over the range of 25-40°C. In contrast, Matsuoka (1979) found that within the range of 18-28°C, the lower temperature the greater was the SO_2 -induced inhibition of photosynthesis in rice. Alternatively temperature might also interact with SO_2 to modify the photosynthesis by changing the rates of detoxification or the biochemical processes themselves, as was suggested by Davies (1980) for irradiance. Although only the response of SO_2 with the temperature factor was implicated here, the remaining meteorological factors and the pollutants should not be forgotten for any speculation.

It would be reasonable to conclude that in the usual monsoon season under the chronic air pollution the cv GR 3 was suitable to grow, however, the stressful season with meagre rainfall (which had also reduced the yield at the control site) made the cultivar to be prone to air pollution, showing clearly a delicate balance between the climate and air pollution resistance in this cultivar.

Mitigation of Air Pollution Effects

As the response of plants to air pollutants is complex and involves processes at the sub cellular, cellular, organ, whole plant, and plant community levels, we must know the influence of factors such as the micrometeorogical, soil, and physiological status which would allow us to provide cultural, protectant, and genetic manipulations in order to control air pollution damage at the whole plant level. Environmental factors including humidity, light intensity, day length, and temperature influence plant response to air pollutants (Ormrod and Adedipe, 1974), however, they are generally difficult to control under field conditions, as observed in the earlier discussions. On the other hand, the edaphic factors which alter the physiological status of the plants may be easily modified by soil moisture, mineral nutrition and plant water balance.

Studies in the past on the effects of nutritional status on air pollution injury have shown varying and contrasting responses. Generally plants that were given an adequate supply of nutrients, were sensitive to air pollution injury than plants with a deficient supply although there were some exceptions. Notably, Leone and Brennan (1972) working with tobacco (**Nicotiana tabacum** L.) and tomato (**Lycopersicon esculentum** Mill) found that sensitivity to SO_2 was greatest in plants with an adequate supply of nitrogen, and that it decreased in plants either with a deficient, or luxury supply of this element. In ryegrass (**Lolium perenne** L.) high N application showed an indication in the severity of injury due to SO_2 fumigation (Ayazloo **et al.,** 1980). In the present study only the panicle numbers was found to be improved as depicted by the interaction due to nitrogen alone (I year study). However, nitrogen with cultivar and environment produced a significant effect in all the parameters (Table 17). Moreover, the effect was mostly favourable to the cv GR 3. It was favourable to cv CO 43 to increase the biological yield, but the economic yield was drastically reduced. The reduction in the economic yield was due to an enormous increase in the sterility index. Though the cv GR 3 also showed a significant increase in the sterility percentage under NN, its effect was offset by the larger increase in the number of panicles, which was not so in the cv CO 43. The much delayed panicle initiation might have rendered the cultivar CO 43 to experience a high pollution stress. The HN application had influenced the performance of cv GR 3 in the polluted environment as indicated by the harvest index above 0.3.

Imposition of high N regime in the second year study also produced no significant improvement in the plant height to ambient air pollution stress, as observed in the first year. However, it minimised the reduction in their photosynthetic leaf area and leaf dry weight. At the later stages the accumulation of fresh and dry matter of culm was increased thereby minimising the reduction in the economic yield, unlike the drastically reduced normal polluted plants.

So, it was interesting to note that though the cv GR 3, tested for another year at the polluted site experienced a drastic reduction in the economic yield of plants that were untreated or treated with chemical protectants, it exhibited an insignificant reduction under high N when compared to the control, suggesting that the cv GR 3 could be cultivated in the polluted conditions under high N regime. Nevertheless, the lesser performance than the I year yield regardless of site of growth could be due to the effects of prevailed non-conducive environment.

Imposition of low N and high N regimes showed a differential response among the cultivars under artificial fumigation studies. SO_2 fumigation mostly inhibited the growth of the rice cultivars under both N levels. Such inhibition could be explained in the light that SO_2 could participate both as oxidant and as reductant and it might conceivably interfere with electron flow in both PS I and PS II, eventually inhibiting the growth (Nieboer **et al.**, 1976). Under the fumigation of NO_2 it would be interesting to note that under high N level all the cultivars showed increased shoot length, TLA and also leaf dry weight, which confirmed that the N uptake from the nutrient medium could successfully ameliorate the ill-effect observed mostly under low N. Further, results in rice indicated the reverse of what was reported earlier - a general enhancement of growth by exposure to NO_2 in tomato plants under low N supply (Troiano and Leone, 1977). Contrarily, Matsumara **et al.**, (1979) reported that the dry weight of tomato plants grown at three rates of nitrogen, decreased with exposure to NO_2 at 565 $\mu g/m^3$. Srivastava **et al.**, (1975), however, had reported that high rates of NO_2 absorption occurred in nitrogen-starved leaves.

Besides the modification of N nutrition, there were reports that water stressed tomato (Khatamian **et al.**, 1973) and tobacco (Mac dowell, 1965) were less sensitive to air pollutants, due to induction of stomatal closure by water stress which in turn would modulate the effect of gaseous pollutants (Bell, 1980). However, imposition of water stress generally produced an adverse effect to the rice cvs CO 43 and TKM 9 under polluted environment. Though cv GR 3 experienced a favourable interaction due to H_2O stress in the polluted environment it was less when compared to normal watered plants. But the observation by Menser and Street (1962) under O_3 pollution led to the conclusion that excess soil moisture caused conditions most favourable for tobacco fleck and withholding water during air pollution stress might be generally effective control method for reducing air pollution damage in irrigated crop regions (Kender and Forsline, 1983). The adverse effect in the water stressed rice cvs CO 43 and TKM 9 could be explained in terms of leaf photosynthetic capacity as observed in the case of **Picea abies** under moisture stress and thus the moderate levels of SO_2 interacted with water stress to produce higher inhibition than the plants grown in clean air (Cornic, 1987). Though there was no interaction between the cultivars and water regimes, the interaction was only significant along with environment in all the yield attributes. It could suggest that the environment played an important role in determining the interaction. The interaction of all the factors (environment, cultivar, nitrogen and water) depicted significant effect in all the studied characters. All produced favourable effect to the cv GR 3 followed by cv TKM 9 but were detrimental to cv CO 43.

Recent evidence suggested that the polyamines—putrescine, cadaverine, spermidine and spermine can function as regulators of growth in plants (Galston, 1983). In the current study exogenous application of putrescine, spermidine and spermine slightly mitigated the inhibition in the plant height, shoot and leaf dry weight and photosynthetic leaf area as affected by air pollution. The foliar injury was also found to be minimised. However, the yield of rice cultivar was sharply reduced in all the polyamine treated plants under the polluted environment. The entry of pollutants in the leaves would produce different ionic species. The ability of polyamines to minimise foliar injury indicated that it could be due to the polycationic nature in binding large quantities of cations to the cellulosic walls with high negative densities (De Marty **et al.**, 1977). Though the cv GR 3 showed better grain filling rate to the application of polyamines under control condition (Anbazhagan **et al.**, 1987), polluted environment reduced the ability for a better yield. Plants treated with the growth promoter-kinetin also produced no improvement in the yield of rice cv GR 3 to the polluted environment. Lee (1966) had earlier observed ozone susceptibility with 6-furfuryl aminopurine (kinetin). Perhaps there was a need for frequent applications in our studies as the reductions in the most of the parameters were minimised after the treatments. Among the physiological detoxificant of pollutants, ascorbic acid had been suggested to be a reliable one (Freebairn, 1960; Keller and Schwager, 1977; Nandi **et al.**, 1981). The exogenous application of ascorbic acid had minimised the effects of the ambient air pollution on the growth of rice but with significant reduction in the economic yield. The low effectiveness of ascorbic acid in the present study could be attributed to the lack of specific effect on growth alterations as indicated by Lee (1966). There was no consistent decrease in the endogenous levels of ascorbic acid in response to air pollution ruling out the possibility of induction of autooxidation of ascorbic acid in the plants exposed to pollution. The ineffectiveness of exogenous application of chemical protectants to increase their endogenous levels (polyamines and ascorbic acid) at the polluted site raised the doubt whether the uptake by the leaves was limiting or any other factor involved in this.

In the light of the studied remedial measures to mitigate air pollution effects on rice plants it was clear that the prime importance should be the cultivar selection for any study. Among the tested strategic measures it was clear that high nitrogen applications under normal watered conditions could be suggested to predispose the chronic air pollution effects but were cultivar specific. Application of chemical protectants which would require more frequency in application warranted further investigation.

Physiological and Biochemical Effects

A reasonal progression of air pollution research on plants would be the linkage between the biochemical and physiological processes and growth/ biomass, yield and foliar injury. Some of the metabolic responses and biochemical mechanisms involved in the air pollution effects on plants were well documented by earlier workers recently (Jaeger **et al.**, 1985; Wellburn, 1987).

The air emissions of the fertilizer plant increased the amount of Chl 'a', Chl 'b' and total Chl at early stage of growth and decreased at later stage in rice. The reduction was less in the air pollution tolerant cv GR 3. The increased contents under air pollution could be due to the hormoligosis phenomenon, where subharmful amounts of many stress agents might be helpful when presented in suboptimal environments to the living organisms (Luckey, 1959). Alternatively applied high nitrogen improved the status of Chl pigments in cv GR 3. In the past, industrial emissions containing SO_2 were implicated in the destruction of chlorophyll in the field (Zalenakova and Polek, 1982). Though the field studies confirmed an increase in the early active growth followed by a decrease, the fumigation studies indicated a variability in chlorophyll degradation to each pollutants. The process of degradation of chlorophylls was described as a biological enigma (Hendy **et al.**, 1987). Reports of decreased chlorophyll concentration in plants fumigated with SO_2 often recorded the degradation of Chl 'a' rather than Chl 'b' (Ricks **et al.**, 1975). In the present ambient air exposure study the increase or decrease was parallel in the Chl 'a' and Chl 'b'. Under fumigation of SO_2, Chl 'a' was found to be affected and the per cent of inhibition was less under HN regimes. Some workers were of opinion that toxic ions, formed on the dissolution of SO_2 in water inside the leaf tissues were preferentially incorporated with thylakoid membrane (Ziegler, 1977) and induce chloroplast swelling (Wellburn **et al.**, 1972) or disintegration of membrane (Malhotra, 1977). The degradative effect of SO_2 was however, confounded by reports of the promotion of chlorophyll synthesis by NO_2 (Horsman and Wellburn, 1976). The degradation might be attributed to the lamellae changes as observed in **Pinus** chloroplasts (Godzik and Knabe, 1973) or increased chlorophyllase activity (Malhotra, 1977) or it might or might not involve attach by free superoxide radicals (Shimazaki **et al.**, 1980; Merzylak and Kovrizhnikh, 1986). Although by no means proved here the reduction in growth under SO_2 and NO_2 fumigation could be explained that any detrimental acidification due to products of SO_2 and NO_2 pollution might consequently have an inhibitory effect upon the process such as CO_2 fixation in the chloroplast metabolism (Wellburn, 1987). The cv GR 3 appeared to be sensitive to NO_2 and cv CO 43 and TKM 9 to NH_3 fumigation as depicted by their diminished levels of chlorophyll contents. Plastid envelopes were permeable to nitrite, ammonia, and unionised nitrous acid but not to ammonium ions (Heber and Purezeld, 1978) and the ammonia might move across the envelope freely in either direction, the ionization relationship with ammonium in stroma for example, would be disturbed as the protons were left behind causing an acidification of the space vacated. However, when both an anion and its neutral protonation product (e.g. NO_2^- and HNO_2) could permeate a membrane barrier, shuttle transfer of protons would abolish the pH gradient across membrane. Probably this might be a partial explanation for some of the known inhibitory effects of nitrite upon CO_2 fixation (Hiller and Bassham, 1965). The spermidine application to cv GR 3 rendered in a modest increase in the Chl 'a' and carotenoids contents, suggesting that the spermidine could stabilize the chlorophyll membrane thereby preventing the loss of chlorophyll (Cohen, 1971).

The chlorophyll temperature stability index of pine needless had been used as a method for determining drought resistance (Buxton **et al.**, 1985). An attempt in the present study to compare the air pollution tolerance of rice leaves showed generally a negative relation of decreased stability index with increased tolerance nature. Mostly to the fumigation of NH_3 and SO_2 + NH_3 cv GR 3 experienced a decrease in the index but to NO_2 it developed an increase in the index. The cvs CO 43 and TKM 9 in response to NH_3 were found to increase the stability index. The mixture of SO_2 + NO_2 generally increased the index in cvs CO 43 and GR 3. To understand this fully, detailed experimentations are necessary. The pigments —carotenoids of SO_2 exposed rice plants seemed to be less sensitive than the chlorophylls, in cvs GR 3 and TKM 9 because of the higher resistance of carotenoids to destruction than the chlorophylls under adverse conditions

(Kramer and Kozlowski, 1979). The inhibition in cv CO 43 and other cultivar depending upon their sensitivity to specific pollutants might also make the chlorophyll more susceptible to photooxidation. Since chlorophyll and other pigments are necessary to harnessing light energy by Photosystem I and II, the effects of air pollutants would directly impair photosynthesis.

The results of this investigation indicated that in rice cultivars the increased endogenous ascorbic acid levels did not strictly point to the tolerant nature of cultivars to the chronic air pollution of the fertilizer plant. However, Varshney and Varshney (1984) indicated a possible correlation between ascorbic acid content and SO_2 sensitivity in three crop species viz. **Brassica, Phaseolus** and **Zea Mays.** The fumigation studies in rice to SO_2 and SO_2 + NH_3 exposure, however, indicated the maintenance of the same levels of ascorbic acid as the control, whereas to the exposure of other pollutants it was inhibited. The endogenous ascorbic acid could have a role in regulating the pigment levels, as the former being a powerful reductant, responsible for the photoreduction of protochlorophyllide as it occurred primarily in chloroplasts (Rudolph and Bukatsch, 1966) and also an important electron donor in photosynthesis (Mapson, 1958). Such a positive regulation called for a linear correlation between endogenous ascorbic acid levels with chlorophyll content and a trend amounting to this was observable in the active growth period of rice. However, interference of senescenece could have masked it at later growth stage. Under stress conditions ascorbic acid replaced water in photosynthetic light reaction II (Bohme and Trebstz, 1969) and addition of ascorbic acid in isolated chloroplasts stimulated CO_2 fixation (Champigny and Gibbs, 1969). This could bring out a possible involvement of ascorbic acid in the pollution stress protective mechanisms of crops under chronic air pollution.

The inorganic form of applied nitrogen to plants could be absorbed and subsequently assimilated by the plants into many important nitrogen containing compounds such as amino acids, proteins and nucleic acids (Bray, 1983). The cv GR 3 showed an all time increase while cvs CO 43 and TKM 9 indicated decrease or marginal increase at later stages of growth in the toal nitrogen content in their shoot portions towards the ambient air polltion exposure. This could be due to the incorporation of applied nitrogen from the soil and/or from ambient sources, because there were unequivocal examples for the utilization of oxides of nitrogen and NH_3 by plants as atmospheric nutrients (Anderson and Mansfield, 1979; Porter **et al.**, 1972; Faller, 1971; Rowland **et al.**,1987),which were available at the test site. The reduction in the total N of the shoot system at the later stages could be due to the premature senescence effect in rice as pointed out by Malcolm and Garforth (1977) in conifer foliage as a response to atmospheric pollution with sulphur dioxide. Nevertheless, fumigation experiments brought about different but interesting results throwing light on the N_2 use from the polluted environment/medium to be cultivar specific. Rice cv GR 3 exhibited an inhibition in the accumulation under SO_2 fumigation but improved when NH_3 was given alone or in combination with SO_2. The cvs TKM 9 and CO 43 responded to certain extent positively in the presence of NO_2 independently or in combination. Elkiey and Ormrod (1981) noticed an increase in total N of bluegrass with NO_2 exposure with out any effect on total S, while SO_2 and NO_2 in combination increased both. Rice plants fumigated with SO_2 + NO_2 strongly support the latter report, by increasing both nitrogen and sulphur in their shoot system.

Rabi and Kreeb (1979) used the diminution of soluble protein as an indicator of low level of SO_2 pollution. In contrast, the total protein content in the cultivars of rice was found to be increased in the polluted environment.

The increase was normally due to increase in insoluble protein in cv GR 3 and soluble protein in the cvs CO 43 and TKM 9, though both the type of proteins were increased generally in all the cultivars. Sardi's (1981) studies too pointed out to an increased amount of soluble protein under low level of SO_2 pollution. The increase in protein content could be due on hand to an increased **de novo** synthesis and on the other hand to a decreased decomposition into amino acids. Jaeger and Grill (1975) observed increased amino acids in response to pollution from high industrial emission regions. Though the increased amount of free amino acids under the polluted environment did not prove the second possibility, one had to judge in terms of the turn over rate rather than the free pool availability at a given time. The inconsistent increase/decrease observed in rice probably confirm this. The changes in the amino acids concentrations could be due to the readjustment of metabolic potentialities of the cell to achieve a mechanism of stabilization of the cellular pH, through an increase in organic bases —alkaline amino acids, polyamines (Pierre and Queiroz, 1981). Another reason for the increased amino acids as a response to pollution could be due to exogenous ammonia, available in the polluted site, as confirmed by the ^{14}C incorporation into amino acids causing an increase usually at the expense of the neutral fraction during CO_2 fixation in studies elsewhere (Kanazawa **et al.,** 1970). Added to the above it had been known that the chloroplast were able to incorporate ^{14}C amino acids into acid insoluble products and much of the incorporation was into insoluble or membrane protein (Eisenstadt, 1966). Since cell wall could contain a significant amount of structural protein, the accumulation of increased insoluble protein could be attributed to the membrane stability of rice cv GR 3 against air pollution. Moreover, the structural protein appeared to be particularly rich in amino acid proline and hydroxy proline (Jensen, 1960), the osmotic protectants.

The increased free amino acid pool in rice as a response to ambient air pollution had free proline as a major fraction in their leaves at early stages. The accumulation was high in cv GR 3, moderate in cv TKM 9 and decreased in the cv CO 43. Fumigation studies also showed more or less the same pattern of increase in the rice cultivars. There was a controversy as to whether the accumulation of proline was an adaptive response in saline resistance (Watad **et al.**,1983). An earlier report on cvs CO 43 and TKM 9 under NaCl stress indicated to their tolerance by the accumulation of high proline content in their leaves (Krishnamurthy **et al.**, 1987). Other environmental stresses like soil moisture, mineral deficiency etc. also increased the proline content as a response to tolerance (Greenway and Munns, 1980), and the accumulation was found to be due to an increased synthesis of protein-bound proline (Tan and Halloran, 1982; Krishnamuthy **et al.**,1987), as a source of solute for intracellular osmotic adjustments (Stewart and Lee, 1974) or as a protective agent for cyto-plasmic enzymes and cellular structures (Schobert, 1977). Eventhough cv CO 43 was found to be tolerant to NaCl stress, showing a high amount of proline (Krishnamurthy **et al.**, 1987) it was very sensitive to the chronic air pollution of the fertilizer plant, as depicted by the yield and accumulated less proline under pollution stress. The cultivars tolerant to air pollution accumulated high amount of proline under HN dosage. It would be reasonable to conclude that cultivars like CO 43 might differ in their tolerance to air pollution and salinity, but under all stress conditions, accumulation of proline was consistently associated as a biochemical marker for stress tolerance.

In addition to the internal counter measures by amino acids in the plants the exogenous application of polyamines could help the plants by providing positive ions to stabilize the membrane from foliar injury, as gradual or abrupt loss of normal membrane functions might be the common mechanism leading to the various stress related phenomena. The spermidine application rendered a modest increase of Chl 'a', carotenoids and insoluble protein in the test site plants, suggesting that sprmidine could stabilize the chlorophyll membrane thereby preventing the loss of chlorophyll (Cohen 1971) and could increase the protein content as observed in oat, indicating a role in the cell division and growth as well (Kaur-Sawhney **et al.**,1980). In the present study the endogenous levels of polyamines were found to be reduced sharply on the fresh weight basis when compared to the control, in contrast to the report on pea plants exposed to SO_2 where polyamine putrescine was found to be increased (Priebe **et al.**, 1978). Paradoxically, the foliar application also did not influence the internal uptake of polyamines under the polluted environment. However, there was a linear correlation between the total endogenous polyamines and plant height and leaf area independently. The reduction in the plant height under the polluted environment could be due to the less synthesis of polyamines at the intercalary meristem as observed in submerged rice plants, where the content of polyamines rose upto high level to increase the internodal elongations (Cohen and Kenda, 1986).

The accumulation of certain organic compounds in the cytoplasm of lower plant cells was to bring about osmotic adaptation to low extracellular water potentials as reviewed by Hellebust (1976). The major water soluble quaternary ammonium compounds (QAC) in many cereal crops was betaine and the choline was usually present in minor amount (Grieve and Gratten, 1983). Between these two QACs, although a precise physiological or adaptive role was not assigned to betaine, there was wide spread conjecture that the QAC might function as an organic cytoplasmic osmoticum (Wyn Jones and Storey, 1981). Some preliminary reports on cereals indicated that once synthesized betaine (QAC) might not be further metabolised and might be mobile within the plant (Ahmad and Wyn Jones, 1979). The gradual increase in the QACs of polluted site rice plants showed an inverse relation to their tolerance nature, based on their yield pattern. However, it was interesting to note that under NaCl stress these three rice cultivars depicted a positive relation of QACs to their salt tolerance (Krishnamurthy **et al.**,1988 a and b). Though cv CO 43 showed more or less same pattern of accumulation of QACs under pollution stress, it did not enjoy the same relationship as it was sensitive to pollution stress, unlike salinity stress.

Increased amount of free carbohydrate might serve as readily accessible substrate for respiratory/repair metabolism (Koziol, 1984) and they form important indicators of energy status. The accumulation of soluble sugars by the cv GR 3 in the ambient air exposure study did not strictly verify the above statement as in the active growth stage, the monitored sugars were low in the test site plants. Probably, it was due to an over utilization of these energy rich sugars to prevent the pollution inflicted injury. Thereafter, the same cultivar accumulated a higher amount of soluble sugars. However, water stress seemed to have enacted responses with increased sugars in the cv GR 3. In contrast, the pollution sensitive cv CO 43 accumulated mostly a free pool of soluble sugars. The observations could make the formulation of the hypothesis indicating a positive correlation of stress resistance with the accumulation of sugars to air pollution difficult. There was a drop in the amount of starch in the shoot system of cv GR 3 under WN-HN (whereunder it showed a tremendous increase in the yield), while other cultivars showed marked increase in the starch content. In rice if a large amount of starch and sugar remains in leaf sheaths and culms at harvest it was an indication that either translocation of photosynthates to the developing spikelets or storage was limiting (Murata, 1969). It would be reasonable to conclude that cvs CO 43 and TKM 9 would have experienced a limitation in the translocation or storage at the final harvest due to air pollution, thereby ultimately showing an increased sterility index.

Evidence is growing in support of the critical role of pH gradient across membranes and proton related membrane energization in driving ATP synthesis, in the translocation of cations, anions and neutral metabolites, and even in regulating enzyme activity (Mitchell, 1977; Williams, 1979; Smith and Raven, 1979; Beever and Burns, 1980). Significant alterations in cytoplasmic pH and buffering capacities were likely to be inhibitory and detrimental to plants (Nieboer **et al.**,1984). Since protonmotive forces assume such an importance in plant metabolism, the reduced buffering capacity found in rice would most likely be damaging. Tolerance of pollution stress might involve increased buffering capacity (Braun, 1977) with costs including synthesis of additional buffer ions and perhaps the detrimental effects of increased ionic strength on enzyme function. Buffering capacities of leaf extracts of plants exposed to SO_2 had been reported by Grill (1971) and Jaeger Klein (1977) in Norway spruce and pea plants. Keller **et al.**, (1976) however failed to detect differences in buffering capacities of another species of Norway spruce **Picea excelsa** fumigated with SO_2 in out door chambers during a winter period. Information about the buffering capacity under other pollutants was not available.

Biological systems have evolved protective scavenging or buffering mechanisms for free radicals (Fridovich, 1975). Sulfhydryls would apparently serve as free-radical scavengers. The increase in the content of glutathione (GSH), unbiquitous form of SH compound, in the rice plants grown near the factory was in as discussed early under nitrogen. The content of fluoride in the shoot of all the rice cultivars was increased as a response to air pollution. Sharma and Rao (1985) observed a depression in the accumulation of fluoride content in **Phaseolus aureus** Roxb in the presence of other pollutants under fumigation studies. However, in the mixed environment near the fertilizer plant, rice cultivars accumulated a marginally higher amount of F content than the control. Under water stressed condition only cv GR 3 showed less accumulation. The origin of F for uptake could be from the atmospheric source, since the same soil and water were used at both the sites. Nevertheless, the accumulation pattern of S and F provided no significant variation among the cultivars, since the level of pollutant was chronic and a mixed situation to mask any specific action.

The leaf absorption spectra of the leaf of rice cultivars exposed to SO_2, NH_3 and NO_2 mixture showed higher absorption peaks in rice cv GR 3 under all NN regimes and lower absorption in cv TKM 9 than the control. The cv CO 43 could show reduced spectrum under HN. Leaf reflectance characteristics (opposite of leaf absorption) has been used to identify O_3 injury on plants (Runeckles and Resh, 1975). These could largely be attributed to changes in the concentrations of leaf pigments, 65-75 % of which were chlorophylls. Nowak and Lautman (1974) also advocated the use of spectral reflectivity measurements for diagnosing sub-visual changes follwing fumigations with SO_2 or O_3 using bean and tobacco leaves. The increased absorption in the given mixture of pollutants in cv GR 3 could be compared to its tolerance nature. A comparison with chlorophyll contents might show a positive correlation in the two cvs GR 3 and TKM 9.

Few enzymes which were selected in the present study have on one hand a well defined role in the metabolic processes and on other hand were directly of indirectly indicated their altered activity by pollution. Peroxidase activity was claimed to be a sensitive assay to indicate pollutant doses which would subsequently give rise to visible injury (Curtis and Howell, 1971). The activity of peroxidase was increased in the cvs GR 3 and TKM 9 under WN while decreased in the cv CO 43. The increase in the activity observed might be an indication of stressed situation within the plants, with general increase in the oxidation process occurring. Elevated enzyme activity had been reported after exposure to SO_2, fluorides, ozone and traffic exhaust (Horsman and Wellburn, 1975; Lee **et al.**, 1966; Curtis **et al.**,1976; Sarkar **et al.**, 1986). SO_2 induced higher level of activity might reflect the initiation of sulphite oxidation by peroxidase and hydrogen peroxide (Fridovics and Handler, 1961). In rice cultivars, activity was increased under HN as observed by Keller (1976) in needles of spruce subjected to HF, particularly, when trees were also fertilized with nitrogen. The necessary elements for peroxidase catalysed oxidation of chlorophylls by H_2O_2 appeared to be present within the chloroplast at one time or another (Huff, 1982). Nevertheless, the experiments with rice indicated an increased amount of chlorophylls did not strictly increase the peroxidase activity in all the cultivars.

The enzyme acid phosphatase (APH) was considered to be the second to peroxidase as the enzyme most commonly studied for possible use as bio-indicator of air pollution. The inhibited activity of APH was found to be minimised under HN regimes of cvs GR 3 and CO 43 in the polluted environment. Since APH was produced in cell walls and occurred in the cystosol of higher plants

(Hasegawa **et al.**,1976) it was concerned with the biosynthesis of fibrous protein (Johnson and McMinn, 1958), and as a regulator of intracellular phosphate concentration (Gutman, 1959). The reduction in the activity minimised by high N regime would explain the utility of N for the improvement of plants under air pollution stress.

The activity of polyphenol oxidase increased under NN in cvs CO 43 and TKM 9 while it decreased in GR 3 under air pollution stress. However, the response was **vice versa** under HN. Increase in the activity of polyphenol oxidase involved in phenol biosynthesis and oxidation was reported in response to biotic pathogens and O_3 pollution (Goodman **et al.**,1967; Tingey **et al.**, 1975). Interestingly in the present study the total phenolic compounds were accumulated in the polluted site plants at 85 days under normal watering. Though the sensitive cultivars of **Arachis hypogea** accumulated high phenolic contents as compared to the tolerant ones to O_3 pollution (Howell, 1974), in the present study on rice plants, no such varietal difference were inferred in finding out their sensitivity/ tolerance to air pollution.

The activity of amylase appeared to be promoted in the plants grown near to the fertilizer plant. The percentage of increase over the control was found to be greater in the cv TKM 9 followed by cvs CO 43 and GR 3. The changes in the amylase activity was positively correlated with accumulation of starch and sucrose in the leaves of cotton, sensitive to salinity (Rathert, 1982). In the current study cv TKM 9 showed more or less similar type of correlation with the accumulation of starch content. The activity of invertase was appeared to be maximum in the cv GR 3 under the polluted environment and it was remarkably high under WN-HN. The activity of phosphorylase was appeared to be promoted in all the cultivars in the polluted environment under NN. But it showed a decrease in the other regimes. The increased activities of amylase, invertase and phosphorylase in the rice under pollution stress was in conformity with the reports on barley (Dzhanibekova, 1972) to salinity stress.

The activity of nitrate reductase, NR, a cytoplasmic enzyme was the first enzyme in the pathway of incorporation of nitrate into reduced form within the plant and bulk of the nitrate was reduced in the plant foliage. The chronic air pollution exposure to rice cultivars inhibited the activity of NR in their leaves under both N levels. The cv GR 3 showed a lesser inhibition in the NR activity when compared to other cultivars in the polluted environment. The pollutants NO_3, NO_2, NO and NH_3 present in the environment were all extremely reactive and hence toxic, but sufficient enzyme was normally present to prevent their accumulation by incorporation into glutamic acid. Unless there was a severe inhibition of enzymes in the pathway, the effect of these pollutants should be small (Parry and Whittingham, 1984). It was also known that NR activity would be very high under upland (aerobic) conditions since the leaf blades of rice plants grown in pots under non-submerged conditions assimilate higher levels of N (Prakasa Rao **et al.**, 1979). The latter report could be supported by the activity of cv GR 3 under water stress condition when compared to other cultivars. Individual fumigation of SO_2, NH_3 and NO_2 brought about the decreased NR activity under NN in all the cultivars. The cv TKM 9 experienced a slight increase under HN regimes to NO_2, $SO_2 + NH_3$ and $SO_2 + NO_2$ treatments. Studies with ^{15}N labelled NO_2 had confirmed that atleast a part of NO_2 nitrogen may be converted into nitrite and nitrate (Yoneyama **et al.,** 1979). The same workers went on to suggest, but not demonstrated that the reduced ^{15}N nitrogen might be assimilated into amino acid through the glutamine synthetase/glutamate synthase pathway. Wellburn **et al.,** (1980) also supported the latter mechanism. In the present study a minimised inhibition of GS activity to NO_2 showed that cvs GR 3 and CO 43 were coping with the assimilation of NO_2 under NN. The same pattern was observed in the cvs GR 3 and CO 43 to the mixture of SO_2, NH_3 and NO_2. Other regimed plants showed only the disturbed metabolism through GS activity.

Evolution of Fitness of Rice Cultivar to Air Pollution ?

There are now abundant evidences that evolution can occur within a few years or generations when plants are subjected to new stress factors. This has been most apparent for resistance to high concentrations of heavy metals in soils (Bradshaw, 1977). To assess the likelihood of evolution of particular character, such as tolerance to air pollutants necessitates the establishment of genetic variation for the character which influences the fitness. The most abundant evidence for variation in resistance was from comparisons of resistance among the cultivars of crop plants (Roose **et al.,** 1982). In the light of the I year study the rice cv GR 3 could be ranked best among the three cultivars in their tolerance to air pollution stress. Hence, seeds of the cv GR 3, particularly HN regimed plants of the polluted environment was evaluated for a subsequent generation at the same site with same regime to verify its fitness to grow in the polluted environment.

The fitness of the cultivar must be taken into account in the overall assessment of effects of pollutants on growth and productivity. The plants raised from the seeds of the previous year and normal (hybrid) ones enjoyed a healthy growth upto 45 days but later the fresh and dry matter of the culm and leaf, photosynthetic leaf area were reduced in the progeny plants. The plant height was sharply reduced from beginning. Resistance to SO_2 seemed to be accompanied by reduced growth. In discussing the costs and benefits of the evolution of pollution resistance, Roose **et al.**, (1982) emphasized that other genetic differences accompany resistance and these might confer added benefits, or atleast would not be detrimental in that particular environment. The progeny plants became more prone to the polluted environment at later stages with an increased foliar injury by 50-55 %, when compared to the normal plants, where the injury was 35-41 %. The visible injury and the production of economic yield would suggest an inverse relation. The increased number of panicles at the polluted site did not offset the loss in economic yield. Tingey **et al.**, (1972) suggested that the cultivar rankings were sometimes altered by the dosages of pollutants. Since there were no abrupt changes in the pollutant levels in the present study the influence must be due to other environmental factors, as reasoned by Heagle (1979). The meteorological variation at the sites as discussed earlier supported the latter view since the fitness of a cultivar might be influenced by genotype and environment.

The evaluated plants at the test site showed an initial increase in the chlorophyll 'a', chlorophyll 'b', and the total chlorophyll along with ascorbic acid as observed in the I year study. But the contents were reduced after 45 days. The reduction was found to be high in the progeny plants, which could suggest their inability to produce photosynthates thereby rendering loss in the dry matter. The reduction in the pigment concentrations might be due to the observed initial phaeophytin in 55 day old progeny plants at the test site. It was interesting to note that the chlorophyll degradation could be due to SO_2 induced removal of Mg^{++} ions by two atoms of hydrogen from chlorophyll molecules converting it into phaeophytin with a change in the absorption spectrum, characteristic of chlorophyll molecules (Rao and Le Blanc, 1966; Gilbert, 1968; Shimazaki **et al.**,1980). Rao and Le Blanc (1966) concluded the possible conversion of Chl 'a' to Phaeo 'a', whereas in rice under chronic polluted conditions the Chl degradation appeared to be due to Chl 'b' into phaeophytin. It had also been reported that in rice Chl 'b' was more sensitive than Chl 'a' to SO_2 fumigation (Nandi **et al.**,1986).

Reduced leaf relative water content in the polluted site progeny plants could confirm the report that treatment of pollutants such as NO_2 or NO, HF, SO_2, H_2SO_4 or HCl quickly lead to cell water imbalance. Since most symptoms of pollution injury attributed to the loss of water from the cell and effected an altered water potential on cell metabolism (Dugger and Ting, 1970), the state of water balance of cells would partially determine its tolerance to pollution stress. The inherent character of accumulation of high amount of N in the shoot system of plants in the polluted environment was positive upto 60 days in the progeny. The reduced total N content in the later part of the growth showed the premature senescence (Malcolm and Garforth, 1977) in the progeny plants thereby altering the protein metabolism and upsetting the resistance in plants. As implicated in the earlier discussions the larger increase in the amount of proline content in the progeny plants could be one of the manifestations to tolerate the air pollution effects. Levitt (1972) suggested stress avoidance (here exclusions of pollutants) and stress tolerance (repair or compensation of injury resulting from pollutant uptake) in deciding the resistance of plants to stress. However, marginal increase in the sulphur and fluoride contents of polluted site plants could be due to the incorporation of atmospheric SO_2 and fluorides (Faller, 1971; Sharma and Rao, 1985), which had brought about no relation to either of the mechanisms fully. Ayazloo **et al.**,(1982) pointed out that there was no reason to assume a '**priori**' that the same mechanism conferring resistance in response to acute exposure would operate under conditions of chronic exposures. The possible conclusion developed from this study could be a deterioration of pollution stress resistance from generation to generation in cv GR 3 but one would also consider the severe climatic conditions existed in 1986. Probably it will be more meaningful when a diverse group of genotypes of rice are available, to screen the hybrids with known genotype history rather than study on their adaptational effects to chronic air pollution.

Setting aside the genetic details about the fitness of the progeny of cv GR 3 in the polluted environment, it was clear that the relative importance seemed likely to depend on the severity of stress. In addition to the micro-meteorological differences between the first year and second year study, the differential response of the cultivar to different pollutants as depicted in the fumigation studies rendered the character of the cultivar - pollution resistance to be slower. This would be due to the fact, when several pollutants were present in the environment, the resistance of plants to one might not confer the resistance to other pollutants or to the interaction of those pollutants.