

SUMMARY AND CONCLUSION

S U M M A R Y A N D C O N C L U S I O N

Paddy is one of the most important cereal crops grown in India. It occupies about 25 per cent of the total cropped area and accounts for about 40 per cent of the total food grain production. Success in improving the food situation in India depends upon an early breakthrough in food production, resulting from a rice revolution. One of the methods by which maximization of agricultural production could be achieved is by bringing more acreage under cultivation.

In India, extensive areas of soil are rendered unfit for cultivation due to salinity. Salinity in the language of the decade is an environmental problem and has, therefore, evoked considerable interest. The major salt content of saline areas especially those of Gujarat has been found to be sodium chloride. One of the ways suggested for reclamation of moderately saline soils successfully and economically is by prolonged leaching followed by the cultivation of salt tolerant crops. The use of manures and fertilizers also have a great bearing on the yield of rice. Nitrogen fertilizers are well known to improve the growth and yield of rice. Nitrogen is supplied to paddy mainly in the form of ammonium or nitrate. Whether paddy prefers nitrogen in the ammoniacal or nitrate form especially during the growth of seedlings is a question of controversy. Keeping in view the importance of paddy and

the problems of salinity a detailed investigation was undertaken with a view to finding out the following

1. The best source of nitrogen for the growth of paddy seedlings.
2. Effect of varying concentrations of salt (sodium chloride) on growth of seedlings of paddy.
3. Effect of varying concentrations of salt on growth, uptake of nitrate and activity of nitrate reductase.

Nitrogen, in the form of potassium nitrate at a concentration of 25 mM was found to be the best source of nitrogen for paddy. Seeds raised with $(\text{NH}_4)_2\text{SO}_4$ showed a marked decline in the rate of emergence. The growth of the shoot and root systems was highly enhanced by KNO_3 whereas $(\text{NH}_4)_2\text{SO}_4$ significantly inhibited their growth. Seedlings raised with KNO_3 also showed a marked increase in the dry weight right from day 3 to 21. The total nitrogen content of the axes of seeds subjected to KNO_3 was highly significant up to day 9. The extremely favourable vegetative growth, dry weight and total uptake of nitrogen obtained in the case of KNO_3 treated seedlings up to day 21 may presumably be favouring further growth, available tillers and increase in yield.

The percentage of germination observed at 48 hours with salt at a concentration of 1% was only 60 when compared to control. However, at 96 hours 89% germination was recorded. Salt at a concentration of 1.5% showed deleterious effects on

germination. Sodium chloride at 0.5% level showed a 10% decrease in the growth of the shoot and it did not show any inhibitory effect on the growth of the root system. At 1 and 1.5% levels of salt, the growth of the shoot and root systems was markedly inhibited. KNO_3 at 25 mM greatly enhanced the growth of shoot and root systems under the influence of 0.5% salt. Parallel to the increase in the growth of shoot and root systems with 0.5% NaCl and KNO_3 there was an increase in the dry weight of the axis and a rapid depletion in the dry weight of the endosperm. The uptake of nitrate by seedlings and the activity of nitrate reductase in first leaves was adversely affected by salt at 0.5% and 1% levels.

It has been found that growth of seedlings was greatly stimulated by GA_3 at all concentrations tried. Enhanced growth was also observed at all concentrations of GA_3 under the influence of 0.5 per cent of salt. Growth of seedlings was also stimulated by GA_3 and KNO_3 under the influence of 0.5 per cent of salt. The dry weight of seedlings registered a marginal increase at all concentrations of GA_3 , GA_3 plus NaCl (0.5%) and KNO_3 . The uptake of nitrate and the activity of nitrate reductase was adversely affected by 0.5% of salt. This inhibitory effect of salt was partially nullified by 10 mg/l of GA_3 . Under the influence of 1% salt, the percentage of germination of seeds, growth of seedlings and their dry weight was enhanced by the application of GA_3 (tried at all concentrations). However, treatment with GA_3 failed to bring

about stimulation of uptake of nitrate and activity of nitrate reductase under a salinity level of 1%.

Succinic acid at all concentrations tried brought about a marginal increase in the percentage of germination of seeds. Growth of seedlings was slightly influenced by succinic acid. The growth of the shoot and root systems under the influence of 0.5% of salt was stimulated by succinic acid. Enhanced growth of shoot and root systems by succinic acid (5, 10 and 20 mg/l) was observed under the influence of 0.5%^{salt} along with 25 mM KNO_3 . Succinic acid at all concentrations tried slightly increased the dry weight of axes under 0.5% of salt alone and salt and 25 mM KNO_3 . The uptake of nitrogen and activity of nitrate reductase was stimulated more by 10 mg/l succinic acid than by other concentrations tried under the influence of 0.5% of NaCl. At 1% concentration of NaCl, succinic acid at all concentrations tried, increased the percentage of germination but failed to increase the growth of seedling and their dry weight. The uptake of nitrate and the activity of nitrate reductase, however, showed a marginal increase.

Cycocel at all concentrations tried (500, 1000, 1500 mg/l) increased the percentage of germination but inhibited the growth of the shoot and root systems. However, a increase in the dry weight of the seedlings was observed. Cycocel did not stimulate the uptake of nitrate and activity of nitrate reductase under saline conditions.

Nitrate reductase is considered to be an enzyme of prime

importance in the production of grain and grain protein. Increase in the substrate concentration results in an increase in nitrate reductase level. From the present studies it has been found that KNO_3 is a good inducer of the enzyme nitrate reductase. Nitrate reductase showed a striking increase in its activity in fully expanded first leaves of seedlings under the influence of KNO_3 . Roots of seedlings failed to show activity of nitrate reductase. The nitrate reductase activity assayed at different stages of seedling growth at a 3 day interval showed a very low activity on day 6. Highest activity was recorded on day 9, and a decline in the activity of nitrate reductase was observed on day 12. Increase in the concentration of KNO_3 from 10 to 25 mM led to an increase in the activity of nitrate reductase. KNO_3 at a concentration of 50 mM showed a marked decline in the activity of nitrate reductase. The enzyme nitrate reductase showed a preferential if not absolute requirement for the cofactor NADH.

Nitrate reductase is an enzyme induced by its substrate nitrate. The induction of nitrate reductase also has light requirements. Experiments conducted to find out the role of light on the induction of nitrate reductase by the inducer (NO_3^-) showed the following results. The activity of nitrate reductase was not detectable in leaves of 9-day-old etiolated seedlings of rice supplied with 25 mM KNO_3 . Activity of nitrate reductase was observed in leaf tissues of etiolated

seedlings raised in the presence of 25 mM KNO_3 and exposed to light. Leaf tissues of etiolated seedlings raised in the presence of no_3^- showed enhanced levels of nitrate reductase activity when floated in a no_3^- medium in the presence of light. Activity of nitrate reductase was not detected in leaf tissues of etiolated seedlings raised with 25 mM KNO_3 and floated in the dark on 100 mM glucose. Nevertheless, activity of nitrate reductase was observed when leaf tissues of 9-day-old etiolated seedlings were incubated in the dark in a medium containing glucose and nitrate.

When leaf tissues of etiolated seedlings grown in water were floated in varying concentrations of KNO_3 and exposed to light, activity of nitrate reductase was found to increase with increase in the concentration of KNO_3 and the duration of the exposure to light.

When leaf tissues of etiolated seedlings grown in water and supplied with exogenous nitrate (at varying concentrations) and glucose (at varying concentrations) and incubated in the dark for a duration of 3, 6, 12 and 24 hours, activity of nitrate reductase was found to increase with increase in the concentration of KNO_3 , glucose and the duration of incubation. The activity of nitrate reductase obtained with 100 mM KNO_3 and 200 mM glucose almost equalled the activity obtained under the influence of light at a substrate concentration of 100 mM. The activity of nitrate reductase obtained with 150 mM sucrose and 100 mM KNO_3 was similar to the activity obtained under the influence of 200 mM glucose and 100 mM of the substrate.

The activity of nitrate reductase obtained in the case of leaf tissues incubated in complete darkness with ATP at a concentration of 5 mM and 100 mM of the substrate was comparable to the activity obtained under the influence of light at the end of the 3rd and 6th hour of incubation. However, at the end of 12 hours there was a 37% decrease in the activity of nitrate reductase.

Over and above the said sources of energy kinetin was found capable of inducing the enzyme in etiolated leaf tissues in the presence or absence of the inducer in darkness.

Nitrate reductase was induced by certain D-amino acids like aspartic acid and serine and also by DL-amino acids leucine and threonine.

It was also concluded from the present studies that actinomycin D at 40, 80 and 160 ug/ml along with the inducer (NO_3^-) in the presence of light inhibited the activity of ? NO_3^- nitrate reductase. The inhibition was partial at 40 and 80 ug/ml concentrations of actinomycin D and about 33% inhibition was observed at a concentration of 160 ug/ml. However, when the incubation period was prolonged to 12 hours, 50% inhibition was observed with actinomycin D at a concentration of 80 and 160 ug/ml.

Cycloheximide (at all concentrations tried) in the presence of the substrate at a concentration of 100 mM resulted in total inhibition of the induction of nitrate reductase both

in light and darkness.

Induction of nitrate reductase was marginal when leaf tissues were incubated with chloramphenicol at 0.5, 1 and 2 mg/ml concentrations. When the tissues were incubated with chloramphenicol at a concentration of 1 mg/ml along with the inducer KNO_3 in the presence of light an increase in the activity of nitrate reductase was observed. Chloramphenicol at a concentration of 2 mg/ml resulted in a marked inhibition of nitrate reductase.

It is concluded from the present studies that seedlings of paddy showed a preference for KNO_3 . Nitrate stimulates the germination and growth of seedlings of paddy whereas $(\text{NH}_4)_2\text{SO}_4$ inhibits the said processes. NaCl at 1 and 1.5% levels inhibit germination, growth of seedlings, uptake of nitrate and activity of nitrate reductase. The inhibitory effect of salt on growth of seedlings could be reversed by the application of GA_3 . The adverse effect of 0.5% of salt on the uptake of nitrate and activity of nitrate reductase of seedlings was partially nullified by GA_3 at 10 mg/l. The toxic effect of salt on growth, uptake of nitrate and activity of nitrate reductase of seedlings was reversed by the application of succinic acid. Cycocel at all concentrations tried failed to overcome the toxic effects of salt on germination, growth, uptake of nitrate and activity of nitrate reductase of paddy. The role of light on the induction of nitrate reductase by the inducer nitrate could be

substituted by glucose, sucrose, ATP and kinetin. Kinetin could substitute not only the role of light but the role of the inducer also. Nitrate reductase could be induced by D-amino acids like aspartic acid and serine and also by DL-amino acids leucine and threonine.

The induction of nitrate reductase by the inducer could be completely blocked by cycloheximide and partially by actinomycin D. Chloramphenicol, in the absence of the inducer nitrate, induced the activity of nitrate reductase. Chloramphenicol at 1 mg/ml concentration along with the inducer nitrate enhanced the activity of nitrate reductase. The stimulation of nitrate reductase observed in the present studies with chloramphenicol may be due to the nitro group in which the oxidative level of the nitrogen atom may be the same as in nitrates.
