GENERAL INTRODUCTION

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"To give manuring advice for a crop like rice, which is grown in practically all regions of the world, under quite different conditions and varied climates, with different cultural practices, different types of rice and tremendous differences in yield is very difficult if not foolhardy". (Comhaire, 1965).

Paddy is one of the most important cereal crops grown in India. It occupies about 25 per cent of the cropped area and accounts for about 40 per cent of the total food grain production. India is only second to China in the world as far as the area under the production of paddy is concerned. Success in improving the food situation in India depends upon an early breakthrough in food production resulting from a rice revolution. Such a breakthrough has yet to become a reality. The present indications are that the country is still not close to achieving a rice revolution.

Next only to water, it is the use of manures and fertilizers and the adoption of better cultural methods that have a great bearing on the yield of rice. As a result of research work carried out in the country during the last few decades, improved methods of cultivation suitable for the conditions existing in the different areas have been evolved and the application of suitable manures and fertilizers is being advocated for increased rice production.

Of the commonly applied fertilizers (nitrogen, phosphorus and potassium) nitrogen is usually the most important for successful rice production. As reported by Dastur and Malkani (1933) and Dastur and Kalyani (1934), rice soils in India are generally deficient in nitrogen and contain, on an average, 0.04 per cent of nitrogen. The average crop of rice removes about 35 pounds of nitrogen from the soil every year and it is found necessary to apply nitrogen in one form or the other to maintain the fertility and productive capacity of the soil.

Nitrate is the most common form of nitrogen available to plants since the reduced forms of nitrogen commonly applied rapidly undergo nitrification in well aerated soils above 5°C (Thompson, 1957). Plants and soil organisms have developed an ability to utilize the anion as the nitrogen source required for their growth and development (Conn and Stumpf, 1972). The overall process whereby nitrate is reduced to ammonium with the subsequent formation of amino acids, protein and other nitrogenous cell constituents is known as 'nitrate assimilation'(Fewson and Nicholas, 1961). It is perhaps difficult to understand why in nature ammonia is readily oxidized to nitrate which in turn must be again reduced to ammonia before incorporation into amino acids. One advantage of course is that nitrate represents a more stable storage form than the somewhat volatile ammonia, although the existence of the latter as ammonium is more likely in neutral and acid soils. A second advantage is that ammonia molecule is rather toxic and therefore, cannot be stored as such in tissues whereas nitrate is relatively less toxic and can accumulate in large amounts in plant sap (Conn and Stumpf, 1972).

Although it is generally believed that nitrogen is absorbed by roots of higher green plants almost entirely as ammonium and nitrate ions, the relative availability and value to the plant of the two sources of nitrogen has long been a subject of investigation (Weissman, 1950). The ammonium form of nitrogen is usually considered as a better source of nitrogen than nitrate for rice. However, the nitrate form produces results equal to those of the ammoniacal form particularly if applied to the plants with well established root system. This indicates that the rice plants have no difficulty in using nitrogen in the nitrate form. Whether rice plants prefer nitrogen in the ammoniacal or nitrate form is a question of controversy. According to Dastur and Malkani (1933) and Dastur and Kalyani (1934) the rice plants absorb nitrogen in ammoniacal form in the earlier stages and and in nitrate form in the later stages of its growth. Bonner (1946) concluded from cultural experiments that rice plants utilized ammonium in preference to nitrate while Malavolta (1954) found that rice plants grew better in a media containing nitrate as the sole nitrogen source. Rao _et_ _al_. (1979) in their recent attempt to ascertain as to whether rice plants take up and assimilate nitrate when grown under flooded conditions and fertilized with urea, observed that the main shoot of rice plant assimilates nitrate throughout its growth and development when grown under lowland conditions.

In India young rice plants are grown in flooded fields and ammonium is the main form of nitrogen supplied as fertilizers (Shen, 1969). Nitrate has seldom been tested as a fertilizer for rice plants for the following reasons a) nitrate is scarcely absorbed to soil particles and is readily washed away in flooded water, b) nitrate is broken down to nitrite by denitrification under the reducing conditions existing in the soils under the water (Sasakawa and Yammamato, 1978). Yamasaki and Seino (1965) found that nitrate can be used in cultivation of rice plants and that when it is used in rice nurseries it increases the development of new roots after the seedlings are planted out in the paddy fields. According to them it has beneficial effects on growth and yield. They also reported increase in metabolic activity and absorption of cations such as calcium, magnesium and potassium by rice roots. Yamasaki (1964) found a better establishment of seedlings with nitrate nitrogen than with ammonium if nitrate fertilizer is applied after the flooded water is completely drained from the nursery bed 7-10 days before transplanting. Seedlings supplied with nitrate nitrogen have a higher oxidative power and respiratory ability than those given ammonia and thus accelerate the rooting activity and nutrient uptake of plants in reduced soil. He also reported high yield of paddy from soil rich in nitrate nitrogen. Ozi and Izawa (1970) found that nitrate was converted to proteins at the same rate as that of ammonium ion in young seedlings of rice. In contrast to assimilation of ammonia the uptake of nitrate was highly influenced by the level of respiratory substrates in tissues, concentration of oxygen in the soil and intensity of light. According to Yoneyama and Kumazawa (1972) the distribution of ^{15}N in the leaves of rice seedlings supplied with $(^{15}NH_4)_2SO_4$ and $K^{15}NO_3$ changed with age, and the movement of the two forms of nitrogen to leaves in the dark was less than 1/10 of that in the light. Weissman (1972) reported that under conditions of controlled pH, nitrate and ammonium are equally effective in supporting the growth of young soybean and sunflower plants. However, ammonium and nitrate as sources of external nitrogen differ in their effects on the growth, enzyme activity, free amino acid composition of root excudate and root tissue, organic acids and mineral content.

Atanasiu *et al.* (1978) reported that in submerged paddy soils ammonium and nitrate undergo different transformations. In the case of nitrate application higher nitrogen losses by leaching and denitrification can be noticed than in the case of ammonium. Ammonium containing and ammonium producing fertilizers are commonly used in rice cultivation. With the increased cultivation of high yielding varieties high nitrogen doses in split application and complex fertilizers with differing nitrate and ammonium content are used to a large extent. In reality, the nitrogen form of fertilizers has become a question of great importance.

In view of all these reports an attempt was made to ascertain as to whether paddy seedlings take up and assimilate nitrate nitrogen in preference to the ammoniacal form of nitrogen.

The salinity problem is of great interest to people of many disciplines. Saline environment poses severe problems in agriculture as year by year non-saline soils and the non-saline waters become intensively and extensively exploited. The cultivation of saline soils and the use of water with a relatively high content of soluble salts is of great importance for the maximization of agricultural production. Moreover, the rapid development of industries in many countries is causing severe water pollution of rivers and mismanagement in agriculture leads to secondary salinization of soils and sources of irrigation water (Poljakoff-Mayber and Gale, 1975). Salinity is also a challenging problem to botanists and plant physiologists, for in their study of plants with respect to growth, structural and functional adaptations and metabolism the question naturally arises as to whether plants actually thrive in a saline environment or merely tolerate it.

The response of plants to saline environments has evoked interest mainly from two points of view. One purely applied is concerned with the utilization of saline soils, the prevention of secondary salinization and the usage of slightly saline waters in agriculture. The other is concerned with the basic effects of salinity on structure and composition of soils, the interrelationships between salinity of soils and water and the plants growth, productivity and physiology. The study of the latter problems is purely a fundamental one. However, it also forms the foundation of an intelligent approach to the solution of the problem of salinity which in a way becomes beneficial to mankind. This basic research on salinity which is academic provides both an intelligent approach for solving the problem and a thorough understanding of man's effect on the environment (Poljakoff-Mayber and Gale, 1975).

Salinity is a problem which occurs all over the world. On every continent, and practically every country, very extensive areas of soil are rendered unfit for cultivation owing to primary, natural or secondary often man-made salinity. The latter in the language of the decade is an environmental problem. The excessive salts present in saline soils do not contain equal quantities of all ions present but are usually concentrated with sodium chloride, sodium sulphate or a mixture of the two. Occasionally large quantities of carbonates are present. According to Poljakoff-Mayber (1975) salinity affects many aspects of the metabolism of plants and induces changes in their anatomy and morphology. These changes are often considered to be adaptations which increase the chances of the plant to endure the stress imposed by salinity. Salinity has been found to affect the time and rate of germination, the size of plants, branching and leaf size and overall plant anatomy and the metabolic and biochemical aspects (Poljakoff-Mayber, 1975). The effects mentioned above of the high ionic concentration in the cell, are probably responsible for most of the observed changes in the biochemical pathways and enzyme activity induced by salinity. The effects may also have much to do with the extremely varied morphological, anatomical and submicroscopic structural modifications occurring in response to salinity. Most of the observed changes, metabolic and structural, are probably signs of salinity damage (Poljakoff-Mayber and Gale, 1975).

One of the most common effects of salinity is stunting of growth, often without any other modifications of growth habit, such as increased leafiness. So Gale and Poljakoff-Mayber (1970) suggested that growth hormones may be involved in response to plant salinity. One of the first hormones to have been studied from this point of view was gibberellic acid. Nieman and Bernstein (1959) were unable to find a relationship between gibberellic acid and salinity. El-Saidi and Kuiper (1972) reported that the toxic effect of sodium chloride could be partially counteracted by exogenously applied kinetin. Kinetin has also been reported to affect the pattern of distribution of Na^+ and Cl^- according to them. Itai et al. (1968) showed that in sunflower, bean and tobacco, the level of endogenous cytokinins was reduced under conditions of stress, including water shortage and salinity. This suggests that at least one reason for metabolic disturbances under saline conditions may be due to reduced level of cytokinins. Plant hormones, especially kinetin and abscissic acid (ABA) appear to play an important role in plant-water relations through their effect on stomatal regulation (Livne and Vaadia, 1965). A clear relationship has been found between kinetin, abscissic acid and various plant stresses including salinity. Mizrahi et al. (1972) working with tobacco found a lowering of the level of endogenous kinetin and an increase of abscissic acid when the plants were exposed to either mannitol or sodium chloride.

Gibberellins are a group of plant hormones which play a decisive role in plant growth and development. These bioregulants enhance or check various physiological processes taking place inside the seed leading to its germination. It is now known that in the case of cereal seeds the gibberellin synthesized in the axis during the early phase of germination controls the metabolic activities of the endosperm (Black, 1972). The literature on the physiology of seed germination and on physiological action of gibberellins has been reviewed by various workers from time to time (Koller et al., 1962; Paleg, 1965; Wareing and Saunders, 1971; Jones, 1973; Mayer and Shain, 1974). Hastening of germination has been reported by several workers like Hayashi (1940) in rice and Prathapasenan (1970) in cotton. Dramatic disappearance of reserve food materials from the storage organs of seeds leading to a marked loss of their dry weight has been observed by Halevy et al. (1964) in several species and similar results were reported in the case of cotton (Prathapasenan, 1970) and sorghum (Kamalavalli, 1969) as a result of GA_3 treatment. Significant increase in the length of mesocotyl of sorghum seedlings and hypocotyl of cotton has been reported by Kamalavalli (1969) and Prathapasenan (1970) respectively.

Succinic acid has been shown as one of the most important growth regulators by Russian scientists like Artemova (1968), and Maurina et al. (1969). Treatment of seeds with succinic acid enhanced the rate of germination of seeds of corn (Gertsuskie, 1959 and Maurina et al., 1969), of cereal seeds (Drozdov and Babuk, 1968), and cotton seeds (Blagoveshchenshii and Rakhmanov, 1964). There are several reports of increase in the percentage of germination of seeds as a result of their treatment with succinic acid (Lagutina, 1966; Siuliauskas, 1967; Kudinov, 1967). Several workers have also reported that the growth was promoted when seeds of various crops like corn and cabbage

(Kotyashkina, 1968) and cucumber (Artemova, 1968) were treated with succinic acid. Shevchenko (1957) and Koeleva (1964) have observed an increase in the dry matter production of crop plants under the influence of succinic acid. Seed yield of corn (Maurina et al., 1969) and ragi (Mansingh and Mathur, 1965) was increased as a result of treatment of seeds with succinic acid.

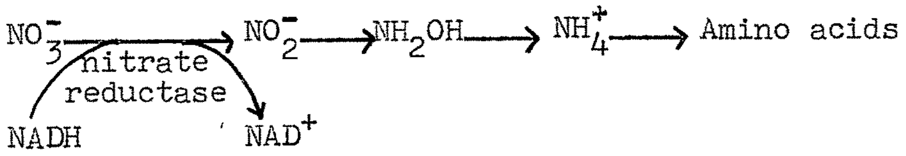
Plant growth retardents are a diverse group of chemicals which reduce stem elongation without causing malformation. Tolbert (1960) reported a new group of quaternary ammonium compounds to be effective as growth retardents. The most active substance 2-chloroethyl trimethyl ammonium chloride (cycocel) was found to limit growth of many species. The most characteristic effect of cycocel is the development of stockier plant with shorter and thicker stems (Tolbert, 1960). Shrivastava et al. (1965) have found that cycocel failed to reduce plant height. A consistent acceleration of flowering by tomato plants was obtained over a rather wide range of concentrations of cycocel (Tolbert, 1960). Haleem (1978) reported that treatment of cycocel along with foliar application of nitrogen resulted in the greatest plant height, percentage fruit set, number of berries/plant, berry weight/plant and yield/ha of solasodine. Cerans et al. (1970) and Furrer and Stauffer (1978) have observed increase in yield in wheat and Triticum spelta.

Studies on the action mechanism of plant hormones suggest that the primary reactions induced by the hormones in the cell are at the level of nucleic acid and protein metabolism (Key, 1969). Hormones act in such a way that it consequently affects the production of a specific enzyme whether the enzyme is induced, promoted or inhibited. Bearing in mind the action mechanism of plant hormones, paddy seedlings subjected to saline conditions were treated with GA_3, succinic acid and cycocel to see whether the said chemicals could stimulate the nitrogen metabolism.

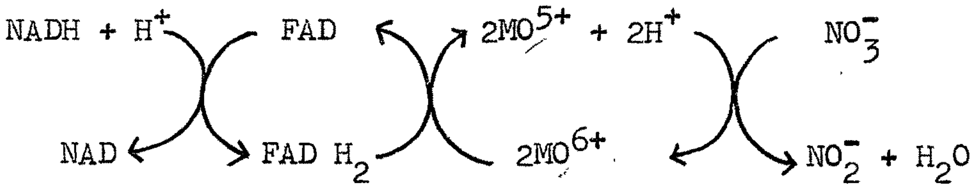
One of the major problems of irrigation in the semi-arid parts of the world is salinity. Adoption of soil amendment practices is the obvious remedy. It is not practicable to implement these due to non-availability of suitable quality of water for leaching, difficulty of adequate drainage and high cost involved in these operations. Both in coastal areas and in many inland salt desert regions, the water available for irrigation is high in dissolved salts, which makes it unsuitable for most crop plants (Moore, 1978).

In India, accumulation of salts has affected a million and half acres of agricultural land. About half of this area is being found in Punjab and Uttar Pradesh. Losses of 20 or more per cent of yield of crop can be due to salts without salt damage being apparent to farmers. Over and above the agricultural land there are several million acres of saline or salinized soils occurring in India. Chapman (1975) reported that in the Punjab area there are some five million acres of soil in which the predominant forms of salts are carbonates, bicarbonates and sulphates. Some three million acres (3/5 of the total) have become salinized as a result of irrigation and by 1960, 1.3 million had gone out of productivity because the salt concentrations were too high. In Uttar Pradesh saline soils with excess sodium carbonate and chloride are found and there has been some salinization as a result of irrigation. In Maharashtra the main salts are sulphate and chloride and there is less evidence of salinization due to irrigation. Around the rann of Kutch in Gujarat there are 800 square miles of saline soils.

The rate controlling and regulated step in the process of nitrate assimilation seems to be the conversion of nitrate to nitrite, catalysed by the enzyme nitrate reductase (NADH : Nitrate oxidoreductase E.C. 1.6.6.1. NR). The following sequence of reactions are involved in the conversion of nitrate to amino acids.



It is a metalloflavoprotein containing flavin adenine dinucleotide, molybdenum and active sulfhydryl groups with NADH serving as electron donor. During nitrate reduction, the hydrogen or electron transport goes from NADH via FAD and MO to nitrate. It apparently involves a change of oxidation state of the molybdenum between +5 and +6. Thus the reaction can be described by the following scheme.



Nitrate reductase activity was first discovered in aqueous extracts of potato tubers as early as in 1904, but more definitive reports of the enzyme came out of the outstanding studies of Evans and Nason (1953), Nason and Evans (1953). It is widely distributed in plants and has been isolated and extensively purified and its properties studied (Paneque and Losada, 1966; Shen et al., 1976). In the last review on nitrate reductase (Hewitt, 1975), the constitution and assembly of nitrate reductase and its physiological and regulatory aspects have been discussed.

A characteristic feature of nitrate reduction is its extreme sensitivity to a range of environmental conditions. It is well established that it is influenced by light, drought, mineral nutrition, hormonal treatment, age of the plant and genetic composition. The induction of nitrate reductase in various tissues in response to inducers is related to maturation and age, the capacity of protein synthesis and increasing maturity which is associated with a decreased nitrate reductase inducibility. The latter may be due either to decreased capacity of protein synthesis or due to an accumulation of soluble nitrogenous products which may repress the synthesis of nitrate reductase (Beevers and Hageman, 1969). The biosynthesis of nitrate reductase is subject to repression, induction and derepression in an independent manner. Induction by nitrate is almost universal (Hewitt, et al., 1976). Evidence that nitrate reductase is inducible by nitrate has been proved by several workers. It was demonstrated by Candela et al. (1957) in cauliflower plants. Direct evidence of the adaptive formation of nitrate reductase was obtained by Tang and Wu (1957) in five-day-old rice seedlings kept with nitrate ion. According to them the adaptive formation of nitrate reductase in rice seedlings is a clear instance of the formation of an adaptive enzyme in higher plants. Afridi and Hewitt (1964) have reported that the enzyme nitrate reductase could not be detected in the leaf tissue of cauliflower plants grown in sterile cultures if nitrate is absent. However, they report that excised leaf tissues of these plants formed the enzyme when infiltrated with nitrate. Induction of nitrate reductase has also been shown by Ferrari and Varner (1969) in barley aleurone layers and by Beevers and Hageman (1969). Extensive results have also been obtained with cauliflower, radish, tobacco callus cells, spinach and maize (Hewitt and Afridi, 1959; Afridi and Hewitt, 1964; Beevers et al., 1965; Schrader et al., 1968; Zielke and Filner, 1971; Kaplan et al., 1974).

However, Beevers and Hageman, 1969 and Hewitt 1975 reported that although the induction of NADH - NR in algae, fungi and higher plants is well established, the mechanism of regulation of this enzyme is far from clear. The regulation of nitrate reductase by ammonium, the end-product of nitrate reduction, have yielded varied results in different species. In algae and fungi, ammonium prevented the induction of the enzyme and in certain cases enhanced its disappearance in vivo (Cove, 1966; Lewis and Finchman, 1970; Losada et al., 1970; Rigano, 1971; Herrera et al., 1972). In higher plants both inhibition (Smith and Thompson, 1971; Stewart, 1972; Pate, 1973, Orebamjo and Stewart, 1975a, b) and promotion (Schrader and Hageman, 1967; Bayley et al., 1972a, b; Higgins et al., 1974; Mohanty and Fletcher, 1976) have been reported.

Light is also an important factor controlling the induced synthesis of nitrate reductase in green plants (Hageman and Flesher, 1960; Beevers et al., 1965; Jordan and Huffaker, 1972; Travis et al., 1970a, 1970b and Travis and Key, 1971). The role of light in the regulation of nitrate reductase induction is complex and multiple (Beevers and Hageman, 1969, and Hewitt, 1975). Beevers and Hageman (1969) reported that light is not an absolute requirement for induction of nitrate reductase, but is supposed to increase nitrate uptake by leaves leading to higher intercellular concentrations of nitrate for induction. Travis and Key (1971) have explained the effect of light on the basis of the close correlation between content of polyribosomes and synthesis of nitrate reductase. In etiolated corn seedlings transferred to light they found a continuous increase in nitrate reductase activity as well as in the polyribosome content of the leaves. According to them adenosine triphosphate formed during photosynthesis was necessary for maintenance of integrity of cytoplasmic polyribosomes required for protein synthesis. Since the required energy could be supplied by respiratory oxidative phosphorylation these authors suggested that the apparent requirement of light for nitrate reductase induction in etiolated leaves seems not to be specific. The work of Aslam *et al.* (1973) shows that nitrate reductase is inhibited by the addition of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and induced by photosynthetic and respiratory processes. Jordon and Huffaker (1972) suggest that photosynthetic products may be required for the induction of significant levels of nitrate reductase activity. Kannangara and Woolhouse (1967) indicate that nitrate reductase is an adaptive enzyme which requires light and nitrate for its induction. They have shown that the synthesis of this enzyme appears to depend on active photosynthesis. Sawhney and Naik (1972) suggest that non-cyclic photosynthetic electron transport is important for the induction of nitrate reductase activity. Travis and Key (1971) report that light or glucose was responsible for maintaining polyribosomal level. It has also been shown that light effect is mediated via photosynthetic reactions. Jones and Sheard (1975) reported that phytochrome may regulate nitrate reductase via both nitrate movement and a general mechanism such as enhancement of protein synthesis. Sasakawa and Yammamato (1979) found that nitrate reductase activity was greatly increased by brief exposure to red light and that this exposure did not influence nitrate uptake. However, they do not exclude the possibility that light enlarges the active metabolic pool or increases transport of nitrate from roots to shoots without enhancing total nitrate uptake. Aslam et al. (1973) reported that under aerobic conditions glucose can substitute for the light requirement. Glucose probably supplies the energy requirement for induction of nitrate reductase activity which otherwise would have been supplied by light (Aslam and Oaks, 1975). Roth-Bejerano and Lips (1970) state that the expression of the inductive capacity of nitrate in green leaves requires light or the supply of suitable respiratory substrates such as glucose or sucrose and Sluiters-Scholten (1975) reported that in etiolated leaves induction of nitrate reductase activity was possible and that the increase in activity was dependent on nitrate and sucrose. It has been shown that nitrate exists within the a cell in two pools, a storage or non-metabolic pool and an active or metabolic pool. Nitrate which accumulates in the storage pool in darkness is relatively inaccessible for induction (Ferrari et al., 1973; Heimer and Filner, 1971). Sihag et al. (1979) have shown that in Pea the stimulation of nitrate reductase induction by light involved both photosynthates and non-photosynthetic processes, whereas sugar increases RNA and protein synthesis in general.

Light and darkness appear to activate or inactivate certain enzymes (Huffaker et al., 1966). The mechanism is often obscure. Low light intensities promoted the rate of nitrate uptake by etiolated seedlings and the response was saturated at about 3000 lux. Uptake of nitrate did occur in darkness but there was no induction of enzyme. However, in maize light is not now considered necessary for induction if polyribosomes are abundant (Travis et al., 1970a, Travis and Key, 1971). The question of whether existing enzyme is activated by light and inactivated by darkness, whether the enzyme breaks down irreversibly in the dark and is stabilized by light or a photosynthetic product, or whether normal loss is continually occurring and resynthesis is dependent on product of illumination, or is inhibited by a product of dark metabolism are not answered satisfactorily by experiments so far described. Results of experiments conducted by Sasakawa and Yammamato (1979) indicate that the main role of light is to supply photosynthetic products that generate energy and supply a carbon skeleton for nitrate reductase induction. According to Jones and Sheard (1975) phytochrome seems to be involved in the action of light. Their results show that nitrate reductase induction in etiolated pea terminal buds was increased more by 5-minute exposure to red light than by similar exposure to blue or far red and that it was red/far red reversible. They seem to be of the opinion that phytochromes may regulate nitrate reductase via both nitrate movement and a general mechanism such as enhancement of protein synthesis.

Thus we see that various explanations have been given as to how light causes enhancement of nitrate reductase activity in higher plants. According to Beevers _et_ _al_. (1965) it is supposed to provide energy for nitrate uptake via photosynthesis and thereby increase the intercellular concentration of nitrate for induction. A second explanation is that light increases the amount of polyribosomes and consequently promotes nitrate reductase induction (Jordon and Huffaker, 1972 and Travis and Key, 1971), a 3rd point is that red light which is too low in energy to support photosynthesis, caused enhancement of nitrate reductase activity in etiolated plants in a direct or indirect way through phytochromes (Jones and Sheard, 1972, 1975). A fourth explanation reported by Wallace (1973) is that light depresses the nitrate reductase inactivating enzyme and consequently increases nitrate reductase (Jolly and Tolbert, 1978). Nitrate is required for light-dependent nitrate induction as well as dark-dependent induction. Accordingly the four explanations described above should also be related with the probable increase in nitrate uptake in plants by light. However, the results obtained by Sasakawa and Yammamato (1979) provide evidence for the light stimulated nitrate reductase induction without bringing about an increase in the uptake of nitrate. Nitrate reductase activity was greatly increased by brief exposure to red light and since this increase was not only due to activation of nitrate reductase, it seemed to be due to increased synthesis induction of nitrate reductase. They were unable to find a correlation between nitrate reductase induction and nitrate uptake. Continuous illumination for twelve hours with blue light was found to be more ~~a~~ffective than red light. e They reported that the greater effectiveness of continuous illumination with blue light indicates the concomitant operation of the so-called high energy reaction which is probably related to pigments other than phytochrome. However, the action of red and blue light on enzyme induction requires further study.

The effect of cytokinins on nitrate reductase formation was discovered by Borriss (1967). Roth-Bejerano and Lips (1970) and Gandhi and Naik (1974) report that the need for light could be partially replaced by cytokinins. However, Beevers and Hageman (1969) reported that cytokinin treatment did not influence nitrate reductase activity in radish cotyledons and Afridi and Hewitt (1964) were unable to influence nitrate reductase induction by kinetin in cauliflower leaf tissues. Barrie (1976) reported that the rythmic reductase activity in leaves of <u>Capsicum</u> <u>annum</u> is controlled by kinetin. The levels of nitrate moving up the stem after kinetin treatment were more dependent upon the rate of sap flow than on the concentration of nitrate in the sap. Kendre *et al*. (1971) detected induction of nitrate reductase in embryonic tissues of *Agrostemma githago* in response to cytokinins 30-60 minutes after its addition. Additive responses were obtained when embryos were treated with both nitrate and cytokinins. The same authors reported that cytokinins enhanced nitrate reductase activity in *Agrostemma* embryos even when the extracts lacked nitrate. Chantarotwong and Huffaker (1971) in their studies to correlate the increase in nitrate reductase activity with changes in polysome content and increased capacity for *in vitro* protein synthesis found that kinetin and benzyladenine markedly increased nitrate reductase activity in detached corn and barley leaves both in light and dark. However, they were unable to determine if synthetic rate or degradation rate or both are affected by the cytokinins. They reported progressive senescence in control leaves and little senescence in cytokinin treated leaves. The polyribosomal content on a fresh weight basis seemed to be greater in kinetin treated tissues. Roth-Bejerano and Lips (1970) reported that the level of nitrate reductase activity in leaves of tobacco is determined not only by nitrate availability but also by the interaction of kinetin and gibberellin. According to them kinetin seems to modulate the effectivity of gibberellin on the determination of nitrate reductase activity. The hormonal treatment therefore, replaced the requirement of light for induction of nitrate reductase. However, Gandhi and Naik (1974) specifically report that it is not so for rice. Rijven and Parkash (1971) suggested that kinetin may be removing a limitation that prevents the synthesis of RNA and the expression of the genome.

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Schrader et al. (1967) reported that in corn seedlings addition of certain amino acids to the induction medium containing nitrate stimulates, or has no effect on nitrate reductase induction. This is in contrast to the repression of induction of nitrate reductase in cultured tobacco pith cells by certain amino acids (Filner, 1966). He observed that transcinnamic acid and coumarin, metabolites derived from phenylaline are effective repressors of nitrate reductase induction in corn seedlings. Phenylalanine or intermediates leading to lignin are not repressive. Glycine fully represses nitrate reductase. In some cases the addition of another amino acid like arginine or lysine resulted in derepression. Radin (1975) reported that induction of nitrate reductase activity in root tips of cotton was regulated by several amino acids and by ammonium. Certain amino acids inhibited induction of activity synthesis by nitrate strongly and others weakly. Eleven of them were reported to have little or no effect. Aspartate and glutamate strongly stimulated induction. Since great difference was observed in root and shoot in response to the growth of amino acids, Radin (1975) suggested that nitrate reductase activity may be regulated differently. Choudary and Rao (1976) reported that a wide range of D-amino acids induced nitrate reductase synthesis in yeast. Among the 22 L-amino acids tested by them only phenylalanine induced significant levels of nitrate reductase. Oaks et al. (1977) have shown that a physiologically correct mixture of amino acids, one similar in composition to amino acids released by the endosperm could support normal growth and protein synthesis in corn embryos. When certain amino acids such as glutamine and asparagine were added to the "corn amino acid mixture" the inhibition of nitrate reductase was found to be severe. However, they reported that arginine enhanced the recovery of nitrate reductase in root tips but inhibited it in sections of matured roots. They presumed that the effect of the amino acids is apparently on some phase of the induction processes like the uptake or distribution of nitrate or a direct effect on the synthesis of enzyme and not on turnover of the enzyme. Behrend and Mateles (1975) reported that certain amino acids inhibited growth and nitrate reductase development of tobacco, tomato, carrot and soybean cell cultures when nitrate or urea was the nitrogen source but not when ammonia was the nitrogen source. Threonine was found to inhibit not only the growth of cells and ? development of nitrate reductase but also the uptake of nitrate in cultures of cells of tobacco, tomato, carrot and soybean.

The effect of inhibitors of protein and RNA synthesis has been studied by several workers. Smith and Thompson (1971) reported that induction of nitrate reductase was inhibited by inhibitors of protein and RNA synthesis. Sawhney and Naik (1972) suggested that fresh protein synthesis in light was necessary for the formation of active enzymes and that mere activation of inactive enzymes or their precursors by light was not involved. Chloramphenicol is known to inhibit protein synthesis in subcellular organelles of plant tissues, but cytoplasmic protein synthesis is relatively insensitive. Schrader _et_ _al_. (1967) found that in maize seedlings, the induction of nitrate reductase was not inhibited by chloramphenicol, an inhibitor of protein synthesis of chloroplast. They concluded that this agreed with the localization of nitrite reductase in the chloroplast (Ramirez _et_ _al_., 1966; Ritenour _et_ _al_., 1967) and of nitrate reductase in cytoplasm (Ritenour _et_ _al_., 1967). However, Coupe _et_ _al_. (1967) reported that nitrate reductase is a chloroplast enzyme. According to Ritenour _et_ _al_. (1967) the enzyme is probably attached to the outer surface of the external membrane of the chloroplast. Afridi and Hewitt (1965) found that induction of nitrate reductase was inhibited by cycloheximide, but not by chloramphenicol. Stewart (1968) has reported similar results. The unusual and profound effect of chloramphenicol on the induction of nitrate reductase in seedlings of rice led to the speculation that the nitro-group in which the oxidative level of the nitrogen atom is the same as in nitrates, induced the formation of nitrate reductase in the tissue. This postulation was supported by the fact that other nitro-compounds also induced nitrate reductase in seedlings of rice. However, Beevers et al. (1965), and Shibata et al. (1969) reported that etiolated leaves have low levels of nitrate reductase. During light-induced greening of leaves the activity of nitrate reductase increased. This rise in activity was inhibited by chloramphenicol. Travis et al. (1970) and Travis and Key (1971) suggested that the role of light in the induction of nitrate reductase may be attributed to the production of a high amount of polyribosomes. Sluiters-Scholten (1973) reported that in green leaves of *Phaseolus vulgaris* the induction of both nitrate reductase and nitrite reductase was affected by cycloheximide only and no inhibitory effect of chloramphenicol on the induction of the enzyme during the light-induced greening of the leaves was observed when the development of the chloroplast was allowed to proceed for 24 hours before the application of the inhibitor. The reason for the lack of inhibition is obscure. It appears questionable that it can be attributed only to the difference in material. At low concentration chloramphenicol stimulates the activity of nitrate reductase and nitrate reductase. However, Tripathi et al. (1977) reported inhibition of induction of nitrate reductase by chloramphenicol in maize seedlings. They further stated that even pre-treatment with chloramphenicol before inducing the enzyme with nitrate also inhibited nitrate reductase activity and that the inhibition declined with the induction period. Stewart (1968) has shown that cycloheximide completely inhibited the synthesis of nitrate reductase in Lemna minor at levels above 5 ug/ml. The addition of cycloheximide at any stage during the induction prevents synthesis of nitrate and nitrite reductase suggesting that it interferes with protein synthesis at the"translation level". Rao and Rains (1976) investigated the effect of cycloheximide on nitrate absorption and their data suggested that it interferes with nitrate absorption. Pretreatment studies conducted by them also showed that cycloheximide affects either energy transfer or nitrate reductase activity or both. Sasakawa and Yamammato (1978) reported that the uptake of nitrate was almost entirely inhibited by addition of cycloheximide. Radin (1974) reported that the early peak activity of nitrate reductase in soybean and sunflower seedlings which was dependent on nitrate was sensitive to cycloheximide, but not to actinomycin D or other inhibitors of RNA synthesis. Inhibition according to Jones et al. (1978) was more quickly brought about by cycloheximide than actinomycin D. The formation of nitrate induced nitrate reductase which occurred after giving actinomycin D suggested that mRNA already formed in the presence of nitrate allowed translation to continue for a short time.

The enzyme nitrate reductase is, therefore, considered to be a factor of prime importance in the production of cereal grain and grain protein. There is a good correlation between the activity of this enzyme and total reduced nitrogen in vegetation and grain in wheat (Eilrich and Hageman, 1973), grain yield and grain protein in Maize (Deckard, et al., 1973) as well as in several other cereal crops (Solomonson and Spehar, 1977), total dry weight accumulation in rye grass (Bowerman and Goodman, 1971) and productivity in some agricultural plants (Tokarov and Shumnyi, 1976a, b). Increase in substrate concentration brought about increase in nitrate reductase level and protein content of the plant. Results of this type are encouraging in that they indicate increased potential for food and protein production. It was therefore, of great interest to the study the activity of nitrate reductase.

Scope of the work

The present studies were taken up with a view to finding out

The best source of nitrogen and its optimum concentration for the growth of paddy.

Localization of induction of nitrate reductase in seedlings of paddy.

The duration of peak activity of nitrate reductase in seedlings of paddy.

The concentration of nitrate required for the induction of maximum activity of nitrate reductase.

The specific cofactor requirement of nitrate reductase.

The effect of varying concentrations of salt (sodium chloride) on germination, growth of seedlings, uptake of nitrate and activity of nitrate reductase of paddy.

The effect of bioregulants namely, gibberellic acid (GA_3), succinic acid (SA) and cycocel (CCC) on germination, growth of seedlings, uptake of nitrate and activity of nitrate reductase of paddy under the influence of varying concentrations of salt.

Role of light on the induction of nitrate reductase in paddy by the inducer-nitrate.

Induction of nitrate reductase in paddy by compounds other than nitrate namely L- and DL-amino acids.

Understanding the mechanism of induction of nitrate reductase in paddy by the inducer nitrate by employing various inhibitors of protein synthesis namely, chloramphenicol, actinomycin-D and cycloheximide.

The results of these studies are discussed in the light of relevant literature and are presented in this thesis in four chapters.

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CHAPTER I

CHAPTER I

EFFECT OF NITRATE AND AMMONIUM ON GERMINATION, AND GROWTH AND NITROGEN CONTENT OF SEEDLINGS OF RICE.

INTRODUCTION

Although it is generally believed that nitrogen is absorbed by the roots of higher green plants almost entirely as ammonium and nitrate ions, the relative availability and value to the plant of the two sources of nitrogen has long been a subject of investigation (Weissman, 1950). Nitrate is the principal source of nitrogen for most plants growing under normal field conditions in fertile soils. Nitrification of ammonia is usually rapid when aeration, moisture content and soil temperature are favourable (Hewitt *et al*., 1976). However, there are several reports as to whether ammonium or nitrate is the better form of nitrogen for rice plants. Shen (1969) reported that rice plants are adapted to the reductive soil conditions in the flooded field and ammonium is the main form of nitrogen supplied in fertilizers. Investigations conducted by him showed that rice seedlings can assimilate nitrate but that utilization of nitrate is completely suppressed by the presence of ammonium. According to Dastur and Malkani (1933) and Dastur and Kalyani (1934) rice plants absorb nitrogen in ammoniacal form in the earlier stages and in the nitrate form during the reproductive stages. Bonner (1946) concluded from cultural experiments that rice plants utilized ammonium in preference to nitrate, while Malavolta (1954) later found that a medium containing nitrate was more favourable for the growth of rice plants. Yamasaki and Seino (1965) found increase in the development of new roots and also beneficial effects on growth and yield when rice plants were supplied with nitrate. According to them nitrate favours the uptake of Ca, K, Mg, Si and Mn. Yamasaki (1964) states that in the case of the various techniques established for high rice yield, rice plants are expected to absorb nitrate nitrogen and Ishizuka (1964) supported this fact of Yamasaki by saying that the condition of soil which allows the existence of nitrate nitrogen will contribute towards achieving a high yield. However, enormous amounts of nitrate nitrogen becomes necessary to convert reduced paddy soil to an oxidized condition. Ponnamperuma (1964) ascertained that nitrate is as good a source of nitrogen as ammonia if iron is rendered available in the culture solution. According to Weissman (1964) the protein level of young wheat seedlings provided with a simultaneous supply of ammonium and nitrate was found to be greater than in the case of those treated with ammonium or nitrate alone. Blackwood and Miflin (1976) reported that nitrate feeding increased the incorporation of ^{14}C into malate whereas ammonium nutrition resulted in a lesser incorporation of ^{14}C into malate in young maize plants.

Atanasiu et al. (1978) from their studies on the effect of different nitrogen sources on the yield and yield formation of rice reported that the highest yields were obtained with split nitrogen dressing. Rao et al. (1979) observed that the main shoot of rice plant can assimilate nitrate throughout its growth and development. Generally, plants show physiologically different responses to different nitrogen species. Kato (1980) suggested that these differences probably are the result of differences in metabolism between the nitrogen species.

Whether paddy prefers nitrogen in the ammoniacal or nitrate form especially during the growth of seedlings is still a question of controversy. Experiments were, therefore, conducted to find out the best source of nitrogen for the growth of paddy seedlings and the results are presented in this chapter.

MATERIALS AND METHODS

Selected seeds of paddy (*Oryza sativa* L. var. Bhura Rata) of uniform size (obtained from Gujarat State Dept. of Agriculture) were surface sterilized with 0.1% mercuric chloride for three minutes and washed thoroughly with glass distilled water. 1. These seeds were placed in Petri dishes containing Whatman no. 1 filter paper moistened with distilled water, or with concentrations of KNO_3 ranging from 10 to 100 mM and were incubated at 30±1°C under dark conditions. From day 6 onwards the seedlings were exposed to light (1500 Lux) daily for a period of 10 hours. On day 9 the seedlings were harvested and the length of the shoot and root systems were recorded.2.The sterilized seeds were placed in between two layers of sterile neutral sand moistened with distilled water or 25 mM KNO_3 or 12.5 mM $(NH_4)_2SO_4$, in Petri dishes and were incubated at 30±1°C under dark conditions. From day 3 onwards seedlings were exposed to light (1500 Lux) daily for a period of 10 hours. Germination counts were made at every 24 hours. Seedlings were harvested at an interval of 3 days upto a period of 21 days. The seedlings were washed with distilled water and blotted. The length of the root and shoot systems were also recorded. The seedlings were then transferred to an oven maintained at 80°C for drying. After 48 hours the dry weight of the seedlings and the endosperm (dehusked) were noted and the total nitrogen of these was estimated separately.

Seedling or axis refers to shoot and root systems minus endosperm.

Estimation of total Nitrogen

Fifty milligrams of dry powder (of endosperm or seedling) was digested using a micro kjeldahl unit with 10 ml of $2NH_2SO_4$ containing $CuSeO_3$ (0.20 mg/100 ml of $2NH_2SO_4$) till the samples became colourless. The volume of the digested sample was made up to 25 ml with distilled water. An aliquot of the digested sample was taken for the determination of its nitrogen content using double iodide reagent (Umbreit et al., 1959).

RESULTS

The seeds treated with low concentrations of KNO_3 namely 10 and 12.5 mM showed a gradual increase in the shoot and root system. KNO_3 at a concentration of 25 mM was found to be the optimum for vegetative growth. The growth of the shoot and root was on the decline in the case of seeds treated with KNO_3 at a concentration of 50 mM. However, it was significant when compared to that of control. Marked inhibition of growth of seedlings was observed under the influence of KNO_3 at a concentration of 100 mM (Fig. 1.1).

The treatment of seeds with KNO_3 or $(NH_4)_2SO_4$ did not bring about any significant difference in their rate of emergence especially during the early stages of germination (Table 1.1). However, at the end of 96 hours, the rate of emergence of seeds treated with $(NH_4)_2SO_4$ showed a marked decline. The decrease in the rate of emergence observed at 120 and 144 hours was also significant (Table 1.1). The growth of the shoot system was highly enhanced by KNO_3 whereas it was significantly inhibited by $(NH_4)_2SO_4$ (Table 1.2). The root system showed a better growth under

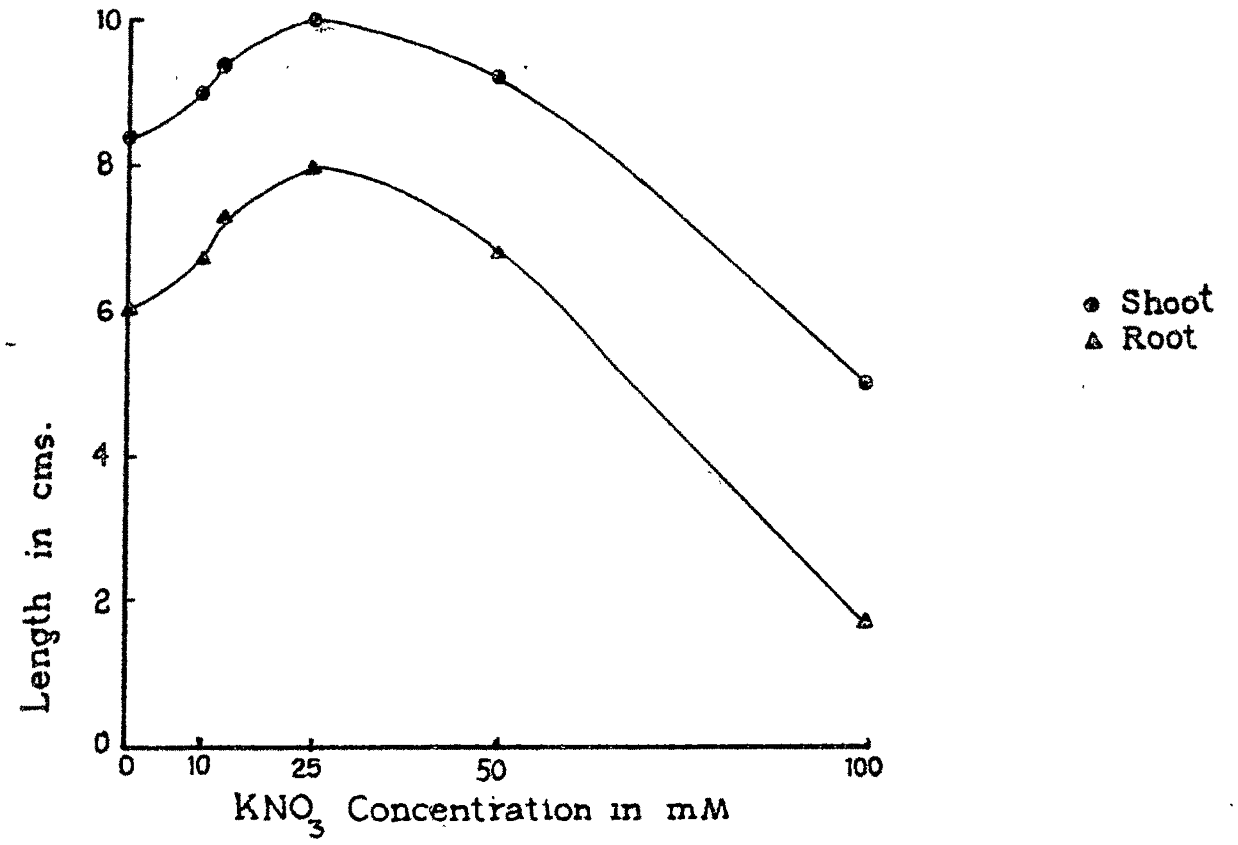

FIG : 1.1 EFFECT OF VARYING CONCENTRATION OF KNO$_3$ ON THE LINEAR GROWTH OF SHOOT AND ROOT SYSTEMS OF NINE-DAY-OLD SEEDLING OF RICE.

Table 1.1. Effect of potassium nitrate and ammonium sulphate on germination of paddy.

Treatment	Percentage of germination in hours				
	48	72	96	120	144
Control (water)	50	62	91	97	98
KNO_3 (25 mM)	52	62.5	89	95	98
$(NH_4)_2SO_4$ (12.5 mM)	50	58	76	89	95
L.S.D. at 5%	5.3	5.9	4.8	5.7	2.7
L.S.D. at 1%	8.0	9.1	7.4	8.6	4.1

Table 1.2. Effect of potassium nitrate and ammonium sulphate on the growth of shoot system of seedlings of paddy.

Treatment	Duration of growth in days						
	3	6	9	12	15	18	21
Control (water)	0.46	4.5	10.8	17.3	20.5	21	22.2
KNO_3 (25 mM)	0.61	5.6	11.8	21.1	25.5	27.2	27.5
$(NH_4)_2SO_4$ (12.5 mM)	0.45	2.8	7.4	12.1	15.1	15.9	16.4
L.S.D. at 5%	0.05	1.00	0.9	1.50	0.84	1.80	2.60
L.S.D. at 1%	0.07	1.50	1.3	2.30	1.30	2.80	4.00

Length of the shoot system expressed in cm.

the influence of KNO_3 than that of control (Table 1.3). $(NH_4)_2SO_4$ significantly inhibited the growth of the root system (Table 1.3). The salt appears to be highly toxic for the growth of roots right from its emergence upto day 21. KNO_3 brought about a marked increase in the dry weight of seedlings right from day 3 to day 21. This has been found to be highly significant when compared to that of control (Table 1.4). The $(NH_4)_2SO_4$ treated seedlings showed a considerable decline in the dry weight (Table 1.4). During germination a rapid decrease in the dry weight of the endosperms was observed in the case of seeds treated with KNO_3 in contrast to a slow reduction in the dry weight of those treated with $(NH_4)_2SO_4$ (Table 1.5). The seedlings under the influence of KNO_3 showed a higher level of nitrogen content as compared to that of control. In the case of seedlings treated with $(NH_4)_2SO_4$ an increase in nitrogen content was observed only from day 12 onwards (Fig. 1.2). The total nitrogen in the axis showed a rapid increase in all the three treatments from days 6-12. However, upto day 9 the nitrogen content was highly significant in the axis of the KNO_3 treated seedlings (Table 1.6). In the KNO_3 and $(NH_4)_2SO_4$ treated seeds the increase in the total nitrogen continued upto day 15 (Table 1.6).

A rapid depletion of the nitrogen content of the endosperm was observed. This was found to be highly

Table 1.3. Effect of potassium nitrate and ammonium sulphate on the length of root system of paddy.

Treatment	Duration of growth in days						
	3	6	9	12	15	18	21
Control (water)	1.50	5.0	6.8	7.7	8.3	9.5	10.1
KNO_3 (25 mM)	1.70	5.3	8.1	8.6	9.1	10	10.5
$(NH_4)_2SO_4$ (12.5 mM)	0.90	2.6	3.1	3.1	3.2	3.4	3.5
L.S.D. at 5%	0.087	0.17	0.15	0.10	0.19	1.10	0.30
L.S.D. at 1%	0.132	0.26	0.22	0.16	0.29	1.70	0.50

Linear growth of root system expressed in cm.

Table 1.4. Effect of potassium nitrate and ammonium sulphate on dry weight of seedlings of paddy.

Treatment	Duration in days						
	3	6	9	12	15	18	21
Control (water)	0.011	0.039	0.09	0.13	0.132	0.140	0.141
KNO_3 (25 mM)	0.02	0.044	0.10	0.14	0.146	0.153	0.159
$(NH_4)_2SO_4$ (12.5 mM)	0.01	0.026	0.05	0.09	0.100	0.105	0.113
L.S.D. at 5%	0.002	0.007	0.010	0.009	0.005	0.008	0.005
L.S.D. at 1%	0.003	0.010	0.016	0.013	0.007	0.013	0.008

Weight expressed in gm.

Table 1.5. Effect of potassium nitrate and ammonium sulphate on dry weight of endosperm of paddy.

Treatment	Duration in days						
	3	6	9	12	15	18	21
Control (water)	0.41	0.150	0.09	0.009	-	-	-
KNO_3 (25 mM)	0.38	0.145	0.09	0.009	-	-	-
$(NH_4)_2SO_4$ (12.5 mM)	0.39	0.160	0.129	0.012	-	-	-
L.S.D. at 5%	0.017	0.009	0.009	0.006			
L.S.D. at 1%	0.025	0.014	0.014	0.009			

Weight expressed in gm.

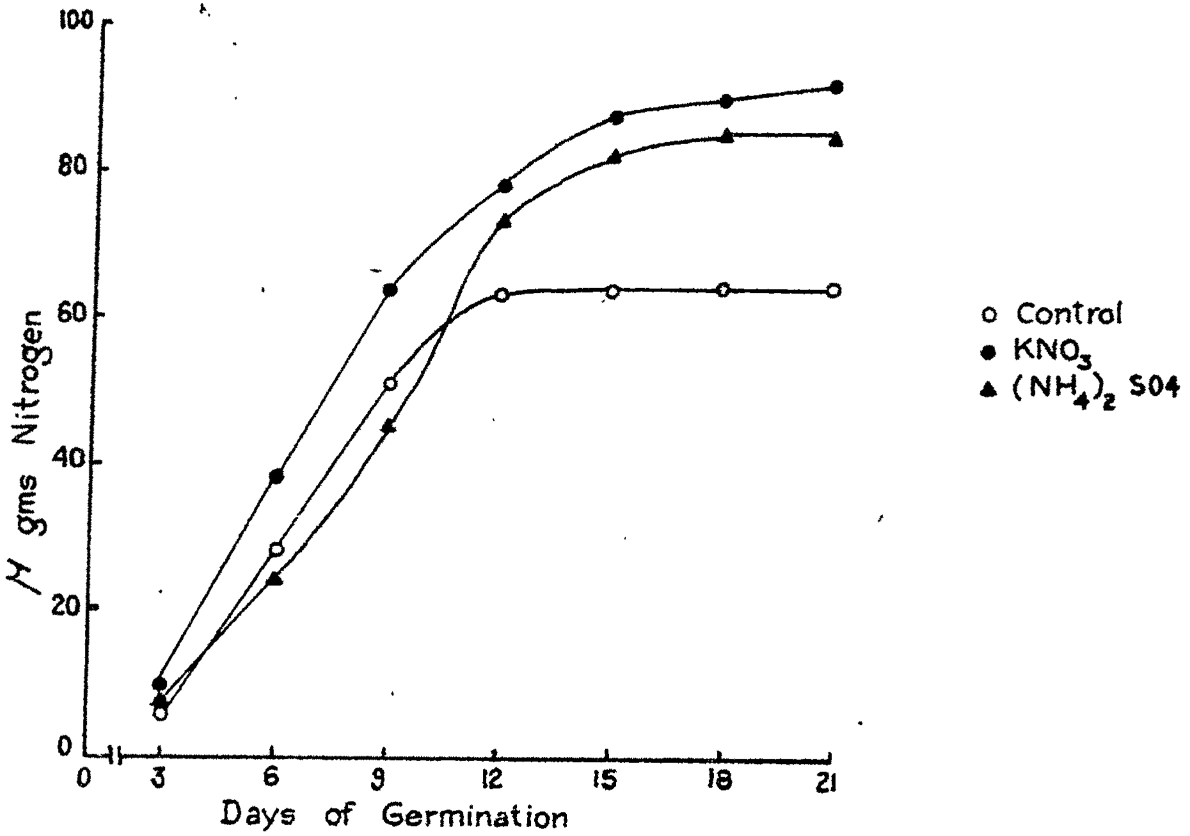

FIG : 1.2 EFFECT OF KNO_3 & $(NH_4)_2$ S04 ON THE NITROGEN CONTENT OF SEEDLING OF PADDY (MINUS ENDOSPERM)

Table. 1.6. Effect of potassium nitrate and ammonium sulphate on the total nitrogen of axis of paddy.

Treatment	Duration in days						
	3	6	9	12	15	18	21
Control (water)	6.0	28.5	51.1	63	64	62.5	64
KNO_3 (25 mM)	9.0	38.1	64	78	88	90.5	92
$(NH_4)_2SO_4$ (12.5 mM)	7.4	24	45	73	82.6	82	85
L. S. D. at 5%	0.93	4.1	5.8	5.6	5.3	5.7	5.8
L. S. D. at 1%	1.4	6.3	8.8	8.5	7.3	8.8	8.9

Total nitrogen per seedling in µg.

Table 1.7. Effect of potassium nitrate and ammonium sulphate on the total nitrogen of endosperm of paddy.

Treatment	Duration in days						
	3	6	9	12	15	18	21
Control (water)	59.5	36.6	18	1.3	-	-	-
KNO_3 (25 mM)	67	44.5	20	6.5	-	-	-
$(NH_4)_2SO_4$ (12.5 mM)	64	47	32	10.3	-	-	-
L. S. D. at 5%	4.8	3.3	2.6	0.58			
L. S. D. at 1%	7.3	5.1	4.0	0.87			

Total nitrogen per endosperm in µg.

significant in the control and KNO_3 treated seeds (Table 1.7).

DISCUSSION

The marked increase in the rate of emergence, growth, dry weight and uptake of nitrogen in the case of KNO_3 treated seedlings may possibly be due to the fact that plants utilize nitrate over a much wider pH range than ammonium (Weissman, 1950). Naftel (1931) found that the absorption of ammonium by the plant increased as the acidity of the culture solution decreased, while the absorption of nitrate was only slightly affected by the reaction of the medium. However, Clark and Shive (1934) found a greater absorption of ammonium from solutions of high pH and a greater absorption of nitrate from solutions of low pH. Metivier and Dale (1977) reported that when barley cultivars Julia and Forma with low grain nitrogen were supplied with nitrate at planting or on day 2, the rate of depletion of endosperm was found to increase. This according to them resulted in an increase in the dry weight of the axis. Similar results were obtained in the present study when the seedlings were treated with KNO_3. However, Weissman (1959) reported that when wheat seedlings were treated with ammonium or nitrate, the total nitrogen was greatest in shoots supplied with ammonium. This in contrast to the present study where the total nitrogen in the axis has been found to be greater in the nitrate treated seeds. Weissman (1972) reported an increased proportion of total oxidized plus reduced NADP (NADP[H]) in the case of soybean and sunflower root tissue. He is of the opinion that nitrate stimulates glycolysis in the roots of these plants. This may perhaps be the reason for the marked absorption and utilization of nitrogen in the case of nitrate treated seedlings. Investigations conducted by Ivanko and Ingversen (1971) on the uptake and assimilation of nitrate and ammonia in Zea mays reveal that nitrogen starved roots of maize are capable of accumulating a potential capacity for nitrogen uptake and assimilation. They reported that reestablishment of nitrogen supply leads to intense uptake reaching to 154% of the reference variant level after 24 hours when nitrate is supplied and 121% when ammonium is supplied. This is confirmed by the present studies also. However, the conditions were dissimilar, for in the present study the seedlings were not subjected to stress. Yamasaki (1964) reported higher oxidation power and stronger respiratory ability of seedlings treated with nitrate nitrogen. He also observed better establishment of seedlings when treated with nitrate nitrogen than ammonium when the flooded water was completely drained off. This is comparable with the present experimental conditions. Ishizuka (1964) suggested that the conditions of the soil which allows the existence of nitrate nitrogen will contribute towards achieving a high yield. Again, the healthy growth of nitrate treated plants is in conformity with the results obtained by Malvolta (1954) who reported that a medium containing nitrate to be more favourable for good growth of rice plants. Tanaka and others (1959a, 1959b, 1959c), show that at 20 ppm of nitrogen in culture solution, nitrate is as good a source of nitrogen as ammonia, while 100 ppm of nitrate is even superior to ammonium. In the present experiments seedlings subjected to 25 mM KNO_3 were found to be far superior to those treated with $(NH_4)_2SO_4$ containing equimolar concentrations of nitrogen. Atanasiu *et al.* (1978) reported that rice elongation was promoted more by nitrate than ammonium nitrogen if applied in one dose at the beginning of the vegetative period. The results obtained in the present study indicate that the rice plant has no difficulty in using nitrogen in the nitrate form. Much depends on the proper timing and placement of nitrogenous fertilizer application. As reported by Atanasiu *et al.* (1978) ammonia containing and ammonia producing fertilizers are commonly used in the cultivation of rice. However, the above workers observed that in the cultivation of high yielding varieties split application of complex fertilizers differing with different nitrate and ammonium content are used to a large extent.

The extremely favourable vegetative growth, dry weight and total uptake of nitrogen obtained in the case of potassium nitrate treated seedlings upto day 21 may presumably be favouring further growth, available tillers and increase yield.

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