CHAPTER III

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EFFECT OF GA_3, SUCCINIC ACID AND CYCOCEL ON THE GERMINATION, GROWTH OF SEEDLINGS, UPTAKE OF NITRATE AND ACTIVITY OF NITRATE REDUCTASE OF PADDY UNDER SALINE CONDITIONS

INTRODUCTION

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. The major salt content 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 crops which are tolerant and adaptable to saline conditions. Salinity has been shown to affect adversely the time and rate of germination of seeds, growth and productivity of plants. It also results in changes in the overall plant anatomy and the metabolic and biochemical aspects (Poljakoff-Mayber, 1975). According to Klotz (1958) high concentrations of ions accumulating in the cell as a result of osmotic adjustment may exert an allosteric effect on the enzyme protein and may effect the structure of membranes and enzymes. This and other modifications led Gale and Poljakoff-Mayber (1970) to suggest that growth hormones may be involved in the response of plants to salinity. One of the hormones to have been 1st studied from this point of view was gibberellic acid (Nieman and Bernstein, 1959).

Since 1950 the research on plant hormones has have increased tremendously and the use of plant growth substances for enhancing the production of crops has now been established. These bioregulants enhance or check the 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). Varner and Chandra (1964) have also shown that GA_3 induces the de novo synthesis of α-amylase and protease.

Succinic acid has also been shown to be one of the important growth regulating substances by Russian scientists. It exhibits its merit by increasing the rate of germination and the growth of crop plants. It has also the capacity to promote flowering and yield of crop plants. Mansingh and Mathur (1965) reported that initiation of inflorescence, number of inflorescence, height of plant and number of tillers in *Pennisetum typhoides* increased when seeds were soaked with succinic acid. Succinic acid, therefore, showed beneficial effects similar to those of other growth substances which are usually expensive.

Plants treated with 2-chloroethyl trimethyl ammonium chloride (cycocel) developed thicker stems, shorter internodes and intensely dark green leaves (Lindstrom and Tolbert, 1960). In most respects the growth alterations brought about by treatment with cycocel were opposite to those of gibberellins. In addition, these effects were reversed by gibberellins in wheat (Tolbert, 1960). However, Shrivastava et al. (1965) have found that cycocel failed to reduce plant height in wheat and Haleem (1978) reported that seeds of *Solanum khasianum* treated with cycocel plus foliar application of nitrogen resulted in the greatest plant height, percentage of fruit set, number no. of berries/plant, berry weight/plant and yield/ha of solasodine. He also reported earlier flowering with dressing and foliar feeding with nitrogen and cycocel.

The present studies were, therefore, taken up with a view to finding out whether the toxic effects of salinity on growth of seedlings, uptake of nitrate and activity of nitrate reductase could be reversed by GA_3, succinic acid or cycocel and the results are presented in this chapter.

MATERIALS AND METHODS

Seeds were germinated as described in Chapter I. The germination medium contained 0.5, 1 and 1.5% sodium chloride with or without 25 mM KNO_3. Salt solutions

(0.5 and 1%) were incorporated with GA_3 (5, 10, 25 mg/l) or succinic acid (5, 10, 20 mg/l) or cycocel (500, 1000, 1500 mg/l) and 25 mM KNO_3 to study the effects of these bioregulants on germination of seeds, growth of seedlings, uptake of nitrate and activity of nitrate reductase.

Appropriate controls were maintained in all experiments.

The seeds 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 endosperms were separated from the seedlings. The length of the shoot and root systems were recorded. Tissues, viz., the axis and the endosperm (dehusked) were dried at 80°C to a constant weight. The total nitrogen of these tissues was estimated as described in Chapter I.

The assay of enzyme activity, and determination of protein and nitrite contents of first leaves ~~was~~ were carried out as described in Chapter II.

RESULTS

Under the influence of 0.5% salt the percentage of germination was as high as 87% at 48 hours (Table 3.1). At 96 hours it almost equalled that of control. At 1% concentration of the salt, the percentage of germination observed at 48 hours was only 60% as compared to that of the control. However, at 96 hours 89% germination was recorded. Seeds treated with 1.5%

Table 3.1. Effect of varying concentrations of NaCl with or without KNO_3* on the germination of paddy.

Treatment		Duration in hours		
		48	72	96
1.	Control	95	100	100
2.	KNO_3	94	100	100
3.	NaCl 0.5%	83	94	98
4.	NaCl 0.5% + KNO_3	80	93	97
5.	NaCl 1%	57	78	89
6.	NaCl 1% + KNO_3	50	77	89
7.	NaCl 1.5%	-	-	11
8.	NaCl 1.5% + KNO_3	-	-	7

* 25 mM

sodium chloride showed deleterious effects on germination (Table 3.1). It can be observed that seeds treated with 0.5, 1 and 1.5% concentrations of sodium chloride along with 25 mM KNO_3 showed no marked difference in the percentage of germination when compared to those subjected to salt alone (Table 3.1).

Salt at a concentration of 0.5% showed a 10% decrease in the growth of the shoot system and it did not show any inhibitory effect on the growth of the root system (Fig. 3.0). However, when the concentration of salt was increased to 1 and 1.5% levels, the growth of the shoot and root systems was markedly inhibited. Seeds subjected to salt at a concentration of 0.5% along with KNO_3 at 25 mM showed greatly enhanced growth of the shoot and root systems.

Parallel to the increase in the growth of shoot and systems with 0.5% NaCl and 25 mM KNO_3 there was an increase in dry weight of the axis (Fig. 3.0), and a rapid decrease in the dry weight of the endosperm (Fig. 3.0).

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 (Fig. 3.A).

Growth of seedlings was greatly enhanced by GA_3 at all concentrations tried(Fig.3.1). Enhancement of growth was also observed at all concentrations of GA_3 under the influence of 0.5 % of salt concentration (Fig. 3.1). Growth

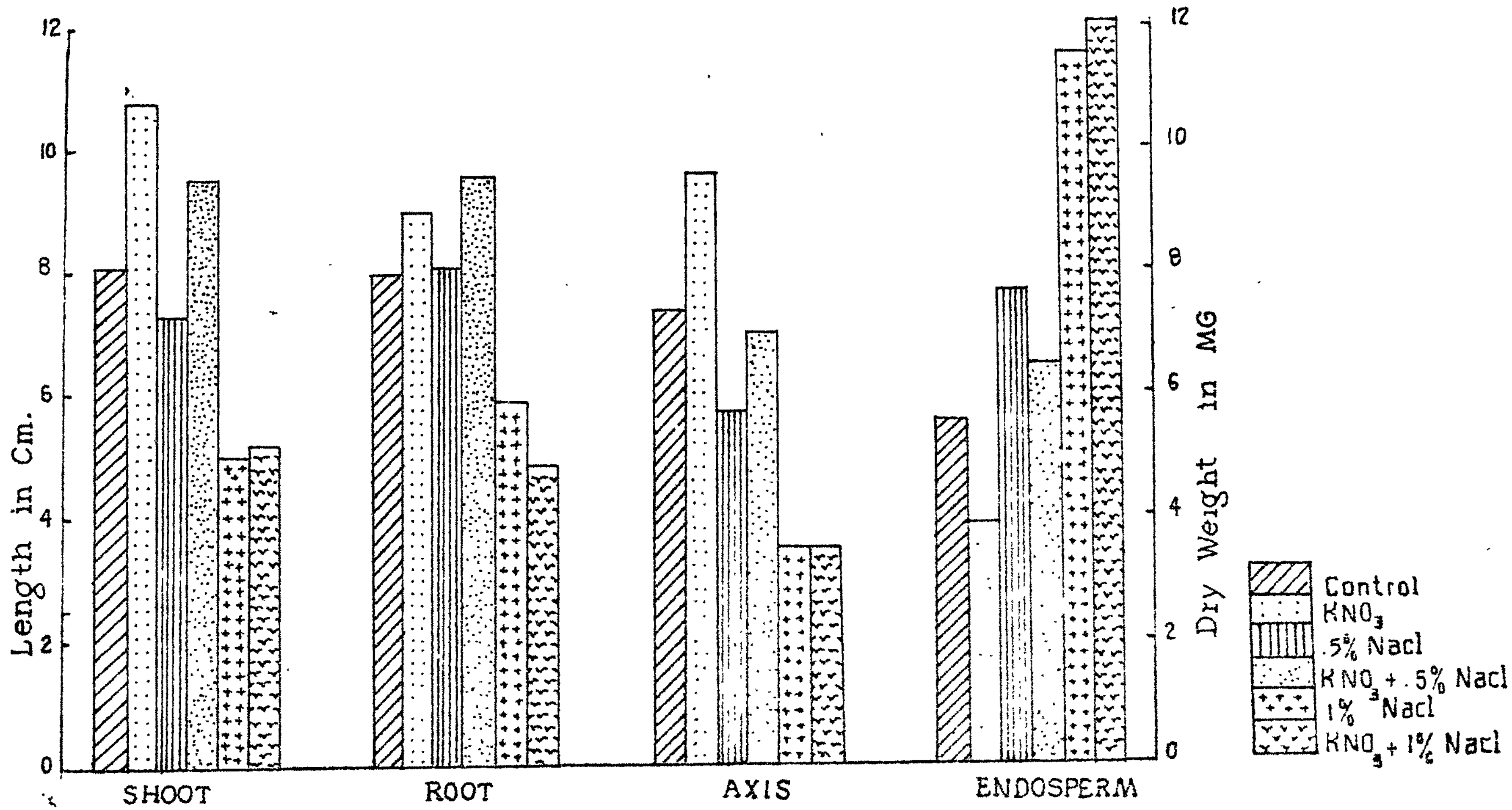

FIG NO 3.0 EFFECT OF Nacl ON THE LENGTH OF SHOOT, ROOT AND DRY WEIGHT OF AXIS AND ENDOSPERM OF PADDY

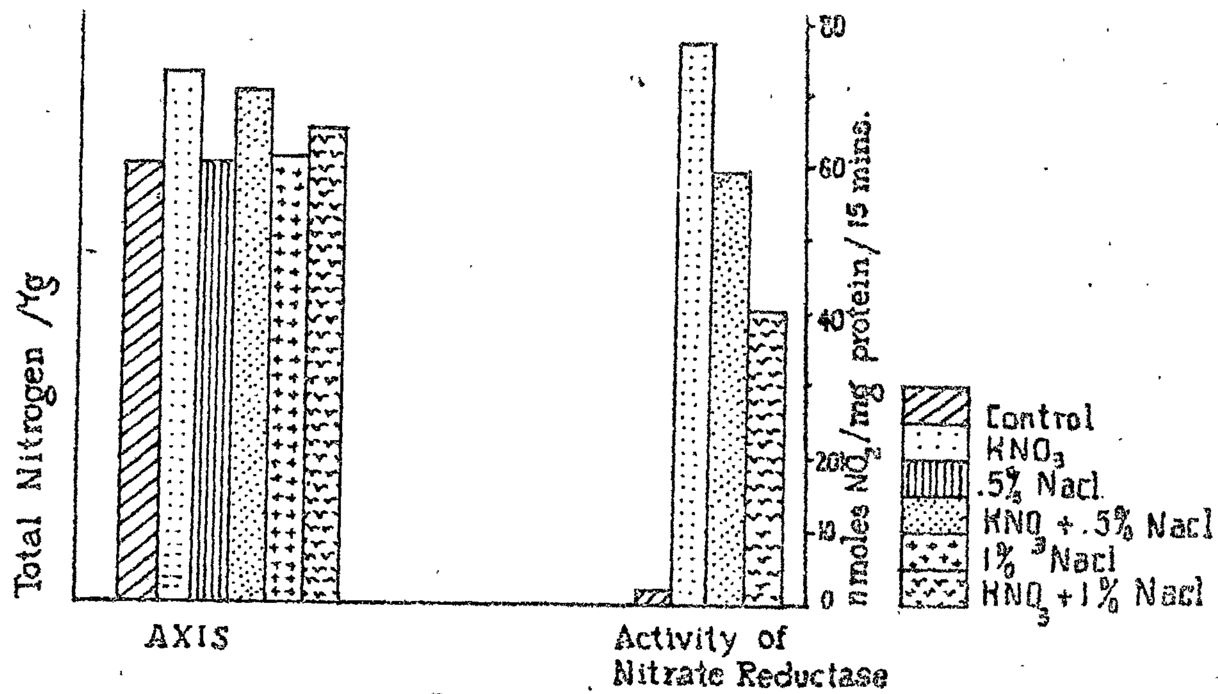

FIG. 3A-EFFECT OF Nacl ON THE UPTAKE OF NITRATE AND ACTIVITY OF NITRATE REDUCTASE OF SEEDLINGS OF PADDY.

NaCl

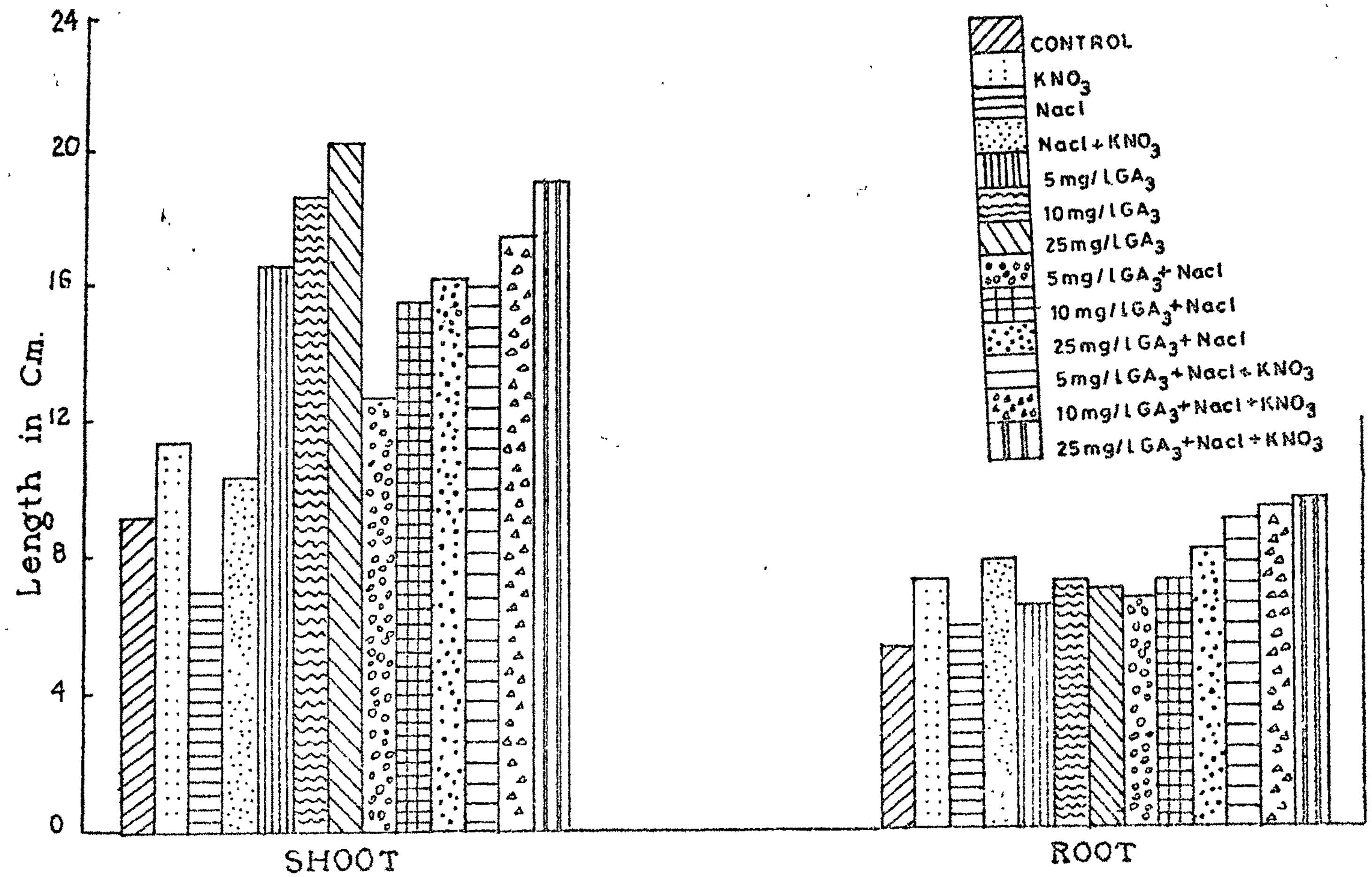

FIG NO 3.1 EFFECT OF GA_3 ON THE GROWTH OF PADDY UNDER THE INFLUENCE OF 0.5% OF Nacl.

was also stimulated with GA_3 (at all concentrations), KNO_3 (25 mM) and 0.5 per cent of salt concentration (Fig. 3.1). The dry weight of seedlings registered a marginal increase at all concentrations of GA_3, GA_3 plus sodium chloride (0.5%) and KNO_3 (25 mM) (Fig. 3.2).

The uptake of nitrogen 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 GA_3 (Fig. 3.3).

The percentage of germination of seed, the growth of seedlings and their dry weight under the influence of 1% salt was enhanced by the application of GA_3 tried at all concentrations (Figs. 3.4, 3.5, 3.6).However, treatment with GA_3 failed to bring about stimulation of uptake of nitrate and activity of nitrate reductase of paddy under a salinity level of 1% (Fig. 3.7).

SA at all concentrations tried brought about a marginal increase in the rate of germination and the % of germination. Growth of seedlings was also slightly influenced by SA.

The growth of the shoot and root systems under the influence of 0.5% of salt was stimulated by succinic acid (Fig. 3.8). Enhanced growth of shoot and root systems by succinic acid (5, 10, 20 mg/l) was observed under the influence of 0.5% salt along with 25 mM concentration of KNO_3 (Fig. 3.8).

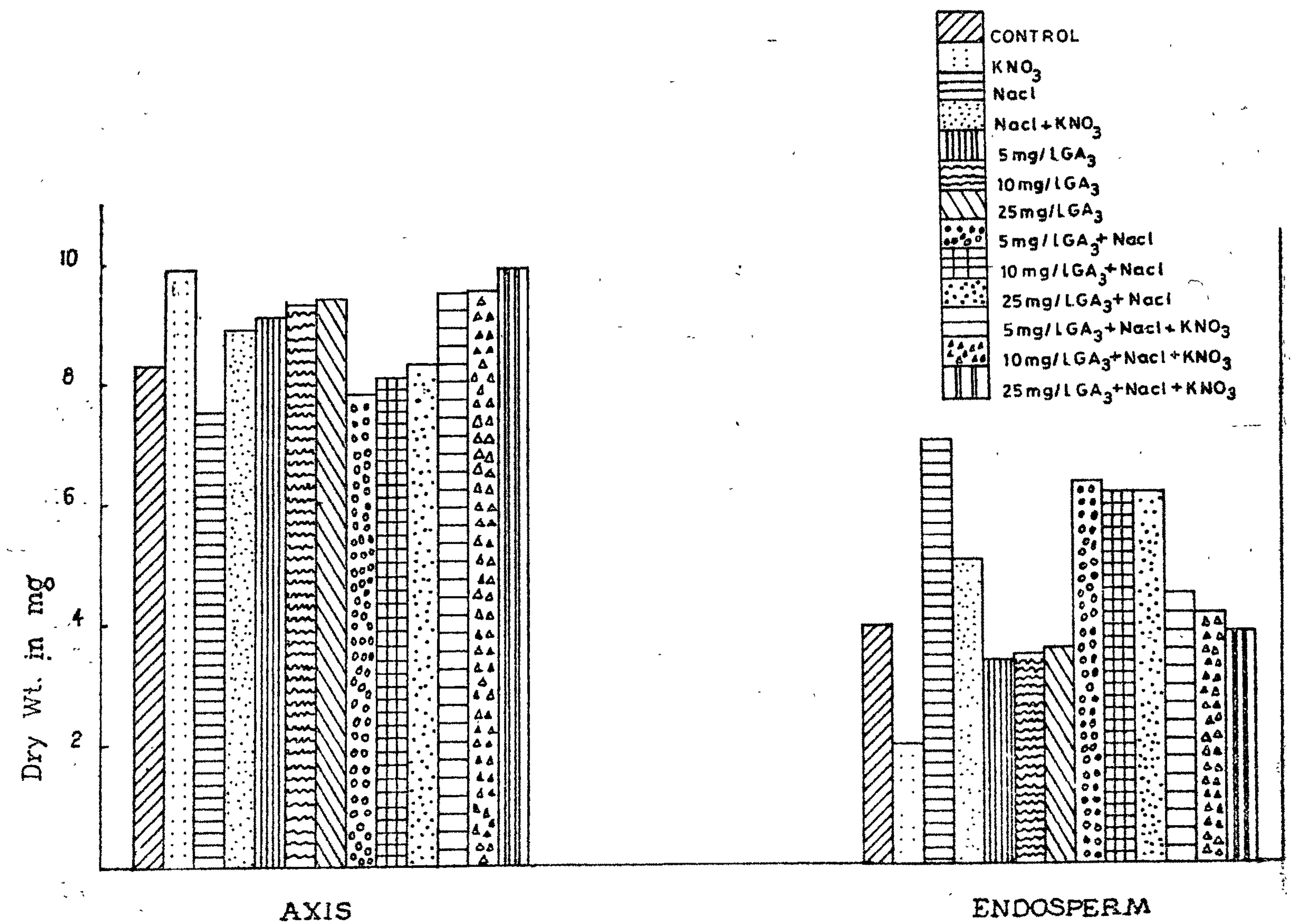

FIG 3.2 EFFECT OF GA_3 ON THE DRY WEIGHT OF AXIS AND ENDOSPERM OF PADDY UNDER THE INFLUENCE OF 0.5% OF Nacl.

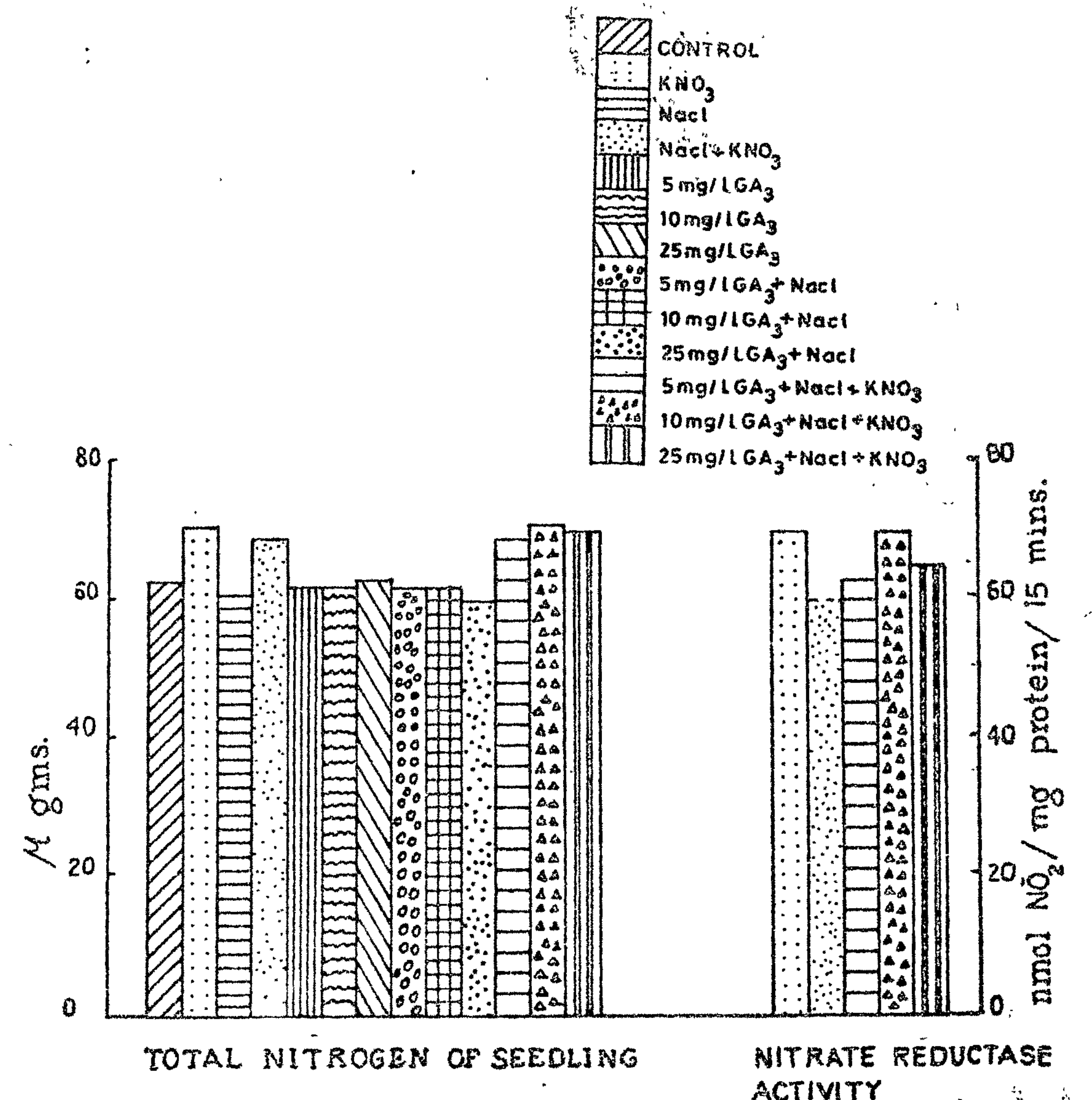

FIG NO 3.3 EFFECT OF GA_3 ON THE UPTAKE OF NITRATE AND ACTIVITY OF NITRATE REDUCTASE OF SEEDLINGS OF PADDY UNDER THE INFLUENCE OF 0.5% OF Nacl.

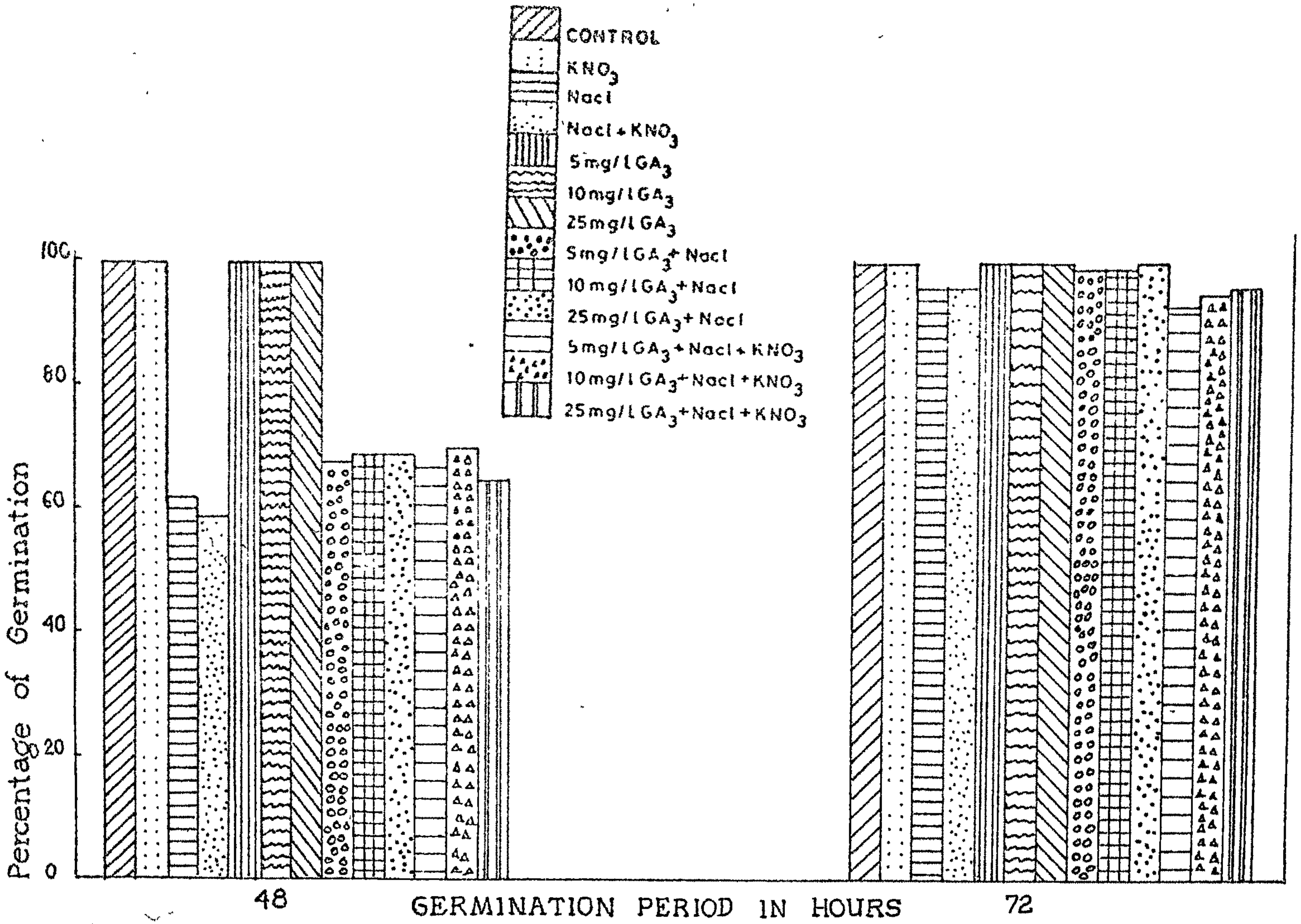

FIG 3.4 EFFECT OF GA_3 ON THE GERMINATION OF PADDY UNDER THE INFLUENCE OF 1% Nacl.

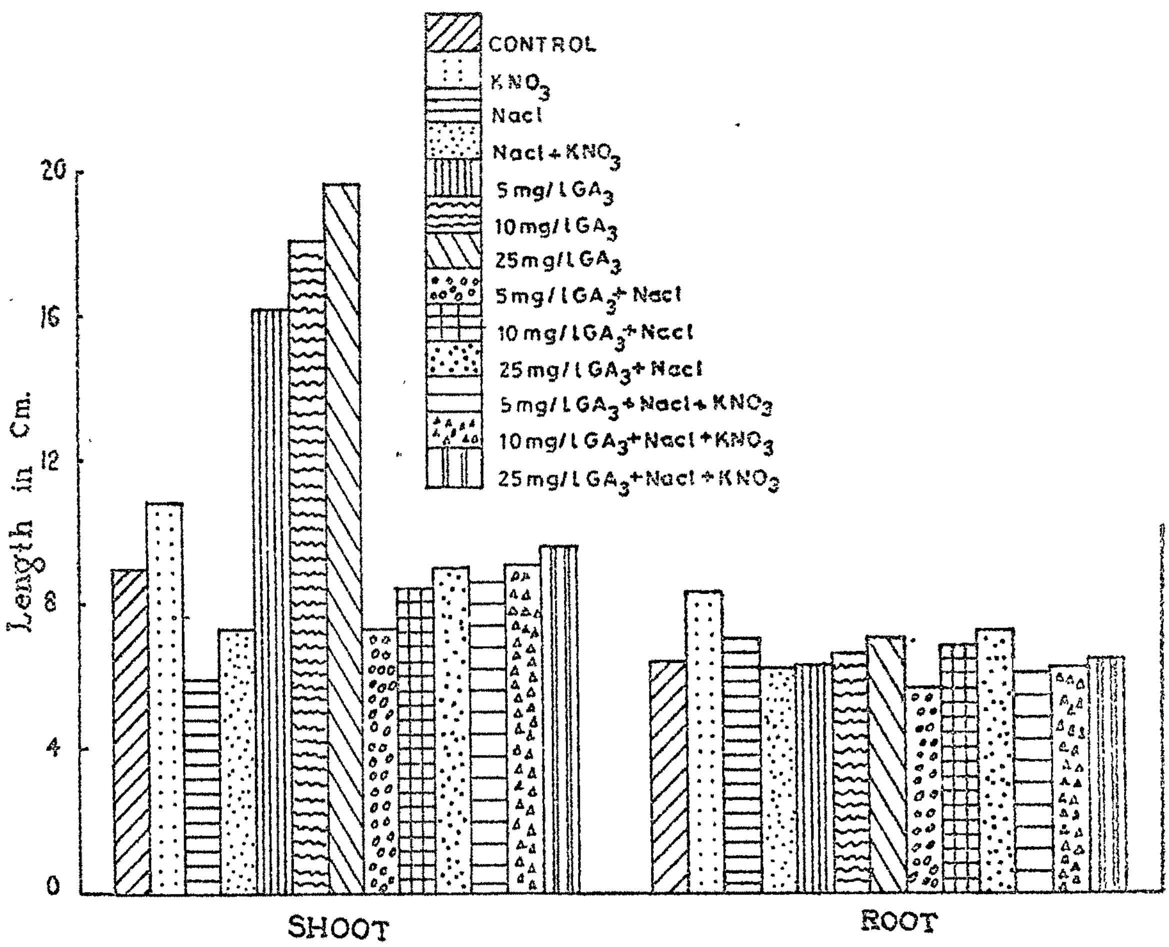

FIG NO 3.5 EFFECT OF GA_3 ON GROWTH OF SEEDLINGS OF PADDY UNDER THE INFLUENCE OF 1% Nacl.

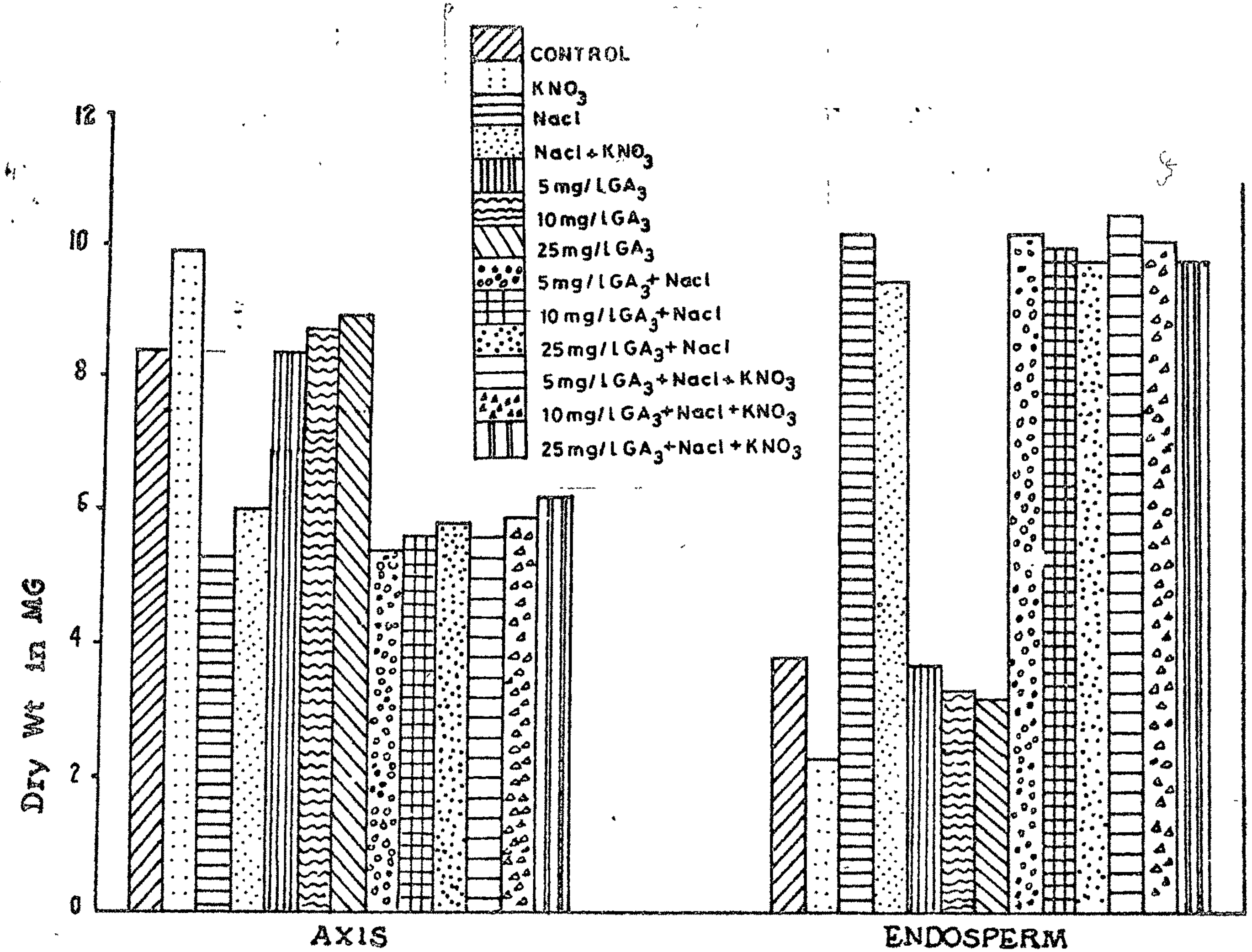

FIG NO 3.6 EFFECT OF GA_3 ON THE DRY WEIGHT OF AXIS AND ENDOSPERM OF PADDY UNDER THE INFLUENCE OF 1% OF Nacl.

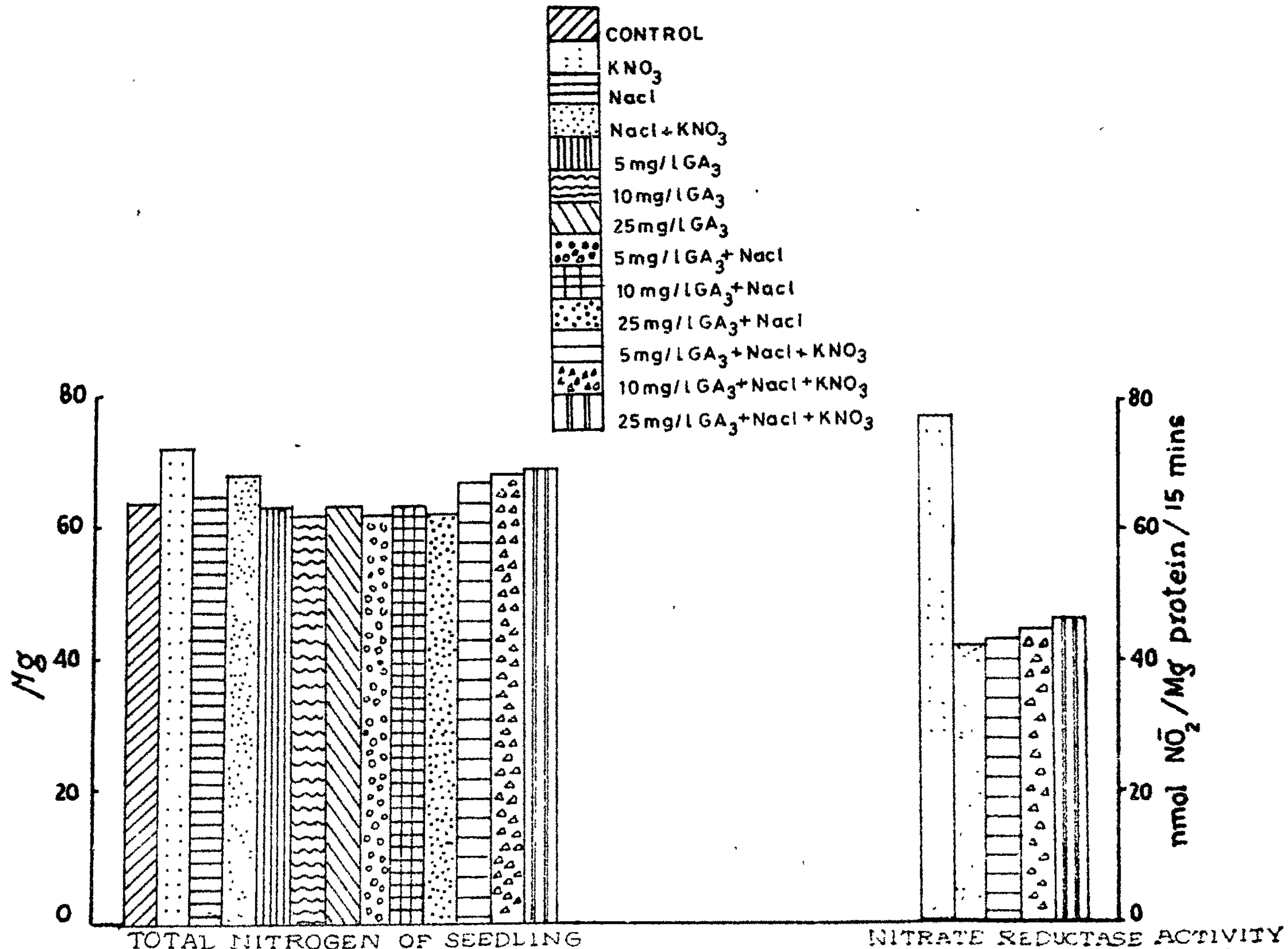

FIG NO 3.7 EFFECT OF GA_3 ON THE UPTAKE OF NITRATE AND ACTIVITY OF NITRATE REDUCTASE OF SEEDLINGS OF PADDY UNDER THE INFLUENCE OF 1% Nacl.

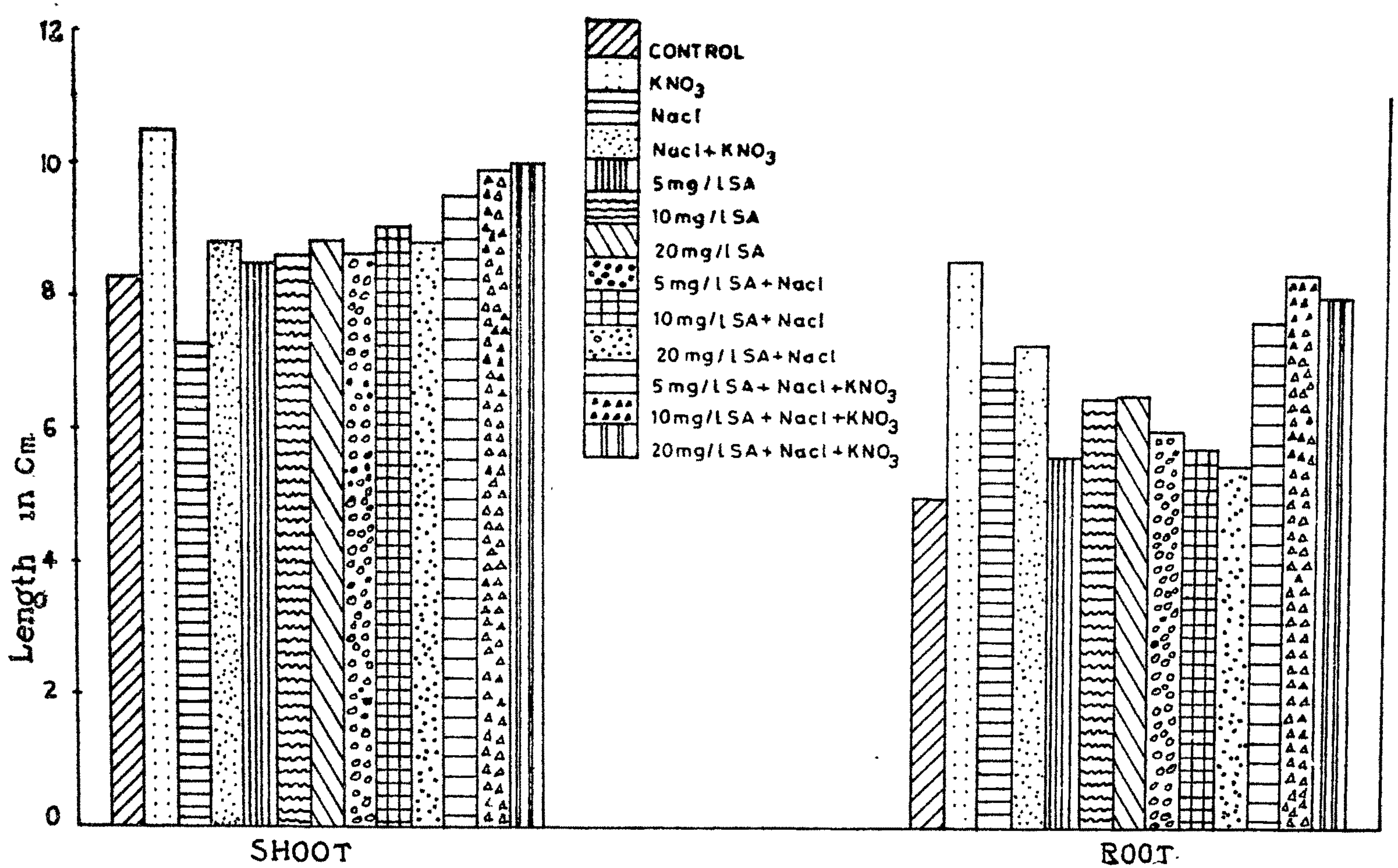

FIG 3.8 EFFECT OF SUCCINIC ACID (SA) ON GROWTH OF SEEDLINGS OF PADDY UNDER THE INFLUENCE OF 0.5% Nacl.

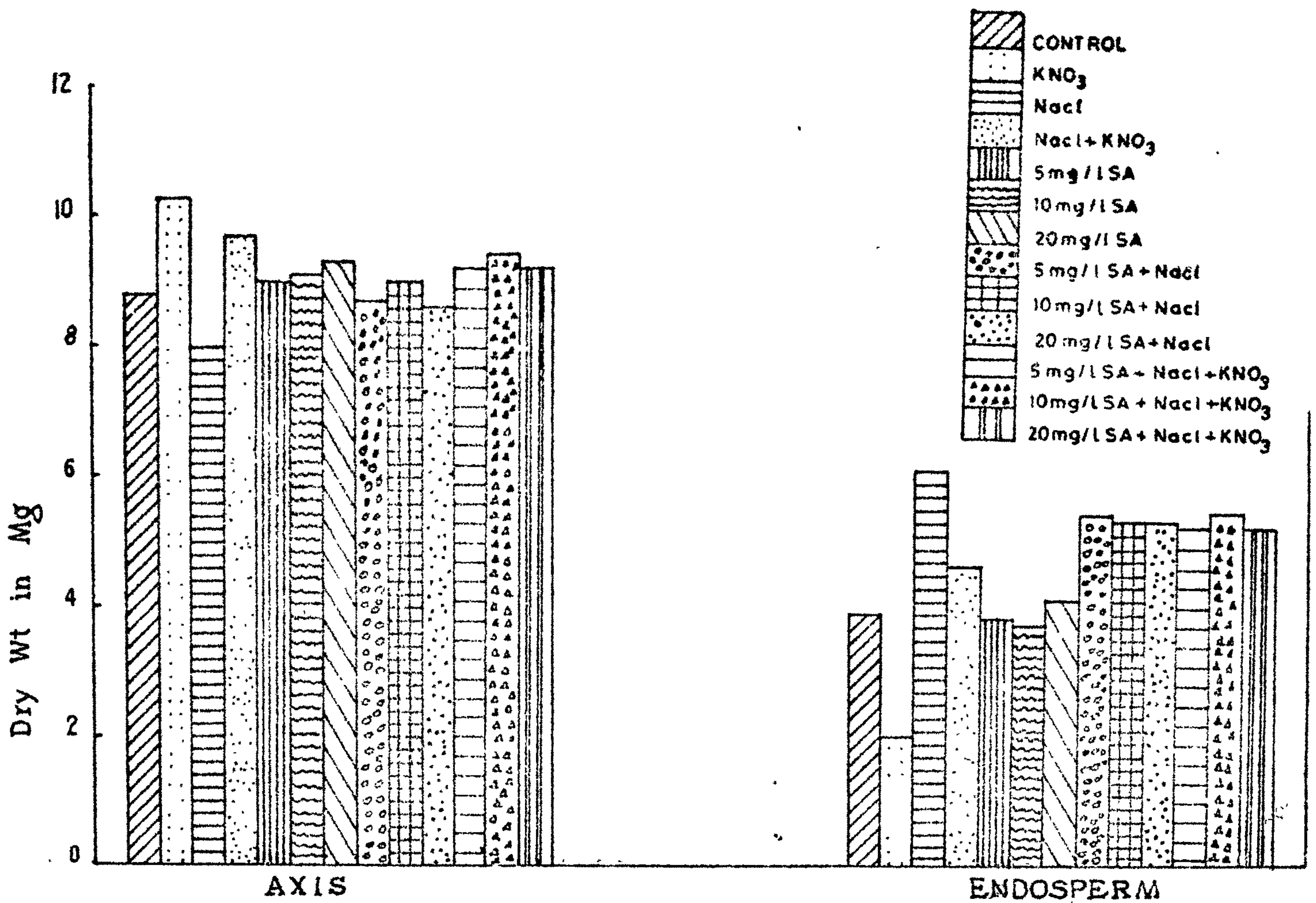

FIG NO 3.9 EFFECT OF SUCCINIC ACID (SA) ON THE DRY Wt. OF AXIS AND ENDOSPERM UNDER THE INFLUENCE OF 0.5% OF Nacl.

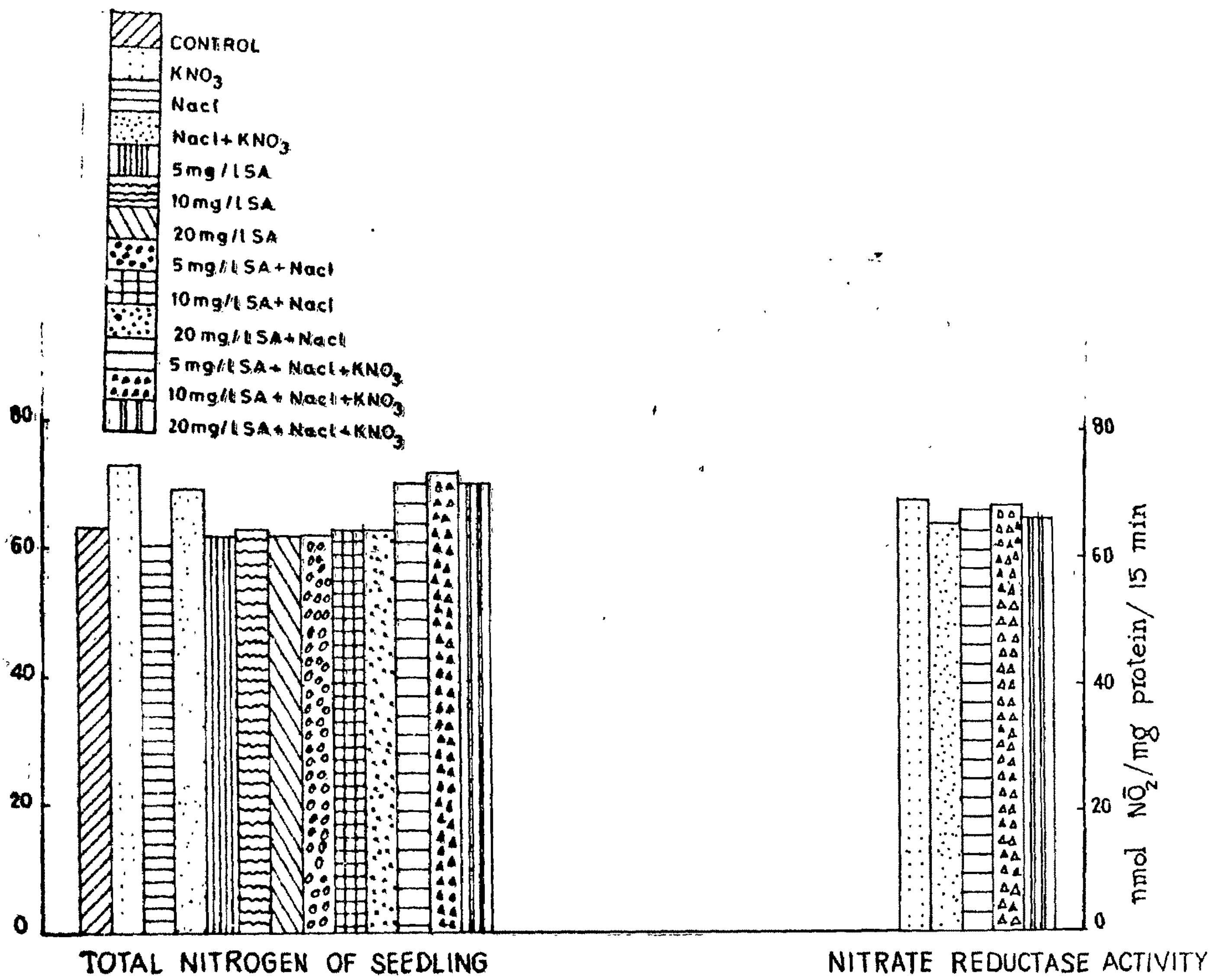

FIG NO 3.10 EFFECT OF SUCCINIC ACID (SA) ON THE UPTAKE OF NITRATE AND ACTIVITY OF NITRATE REDUCTASE OF PADDY UNDER THE INFLUENCE OF .5% OF Nacl.

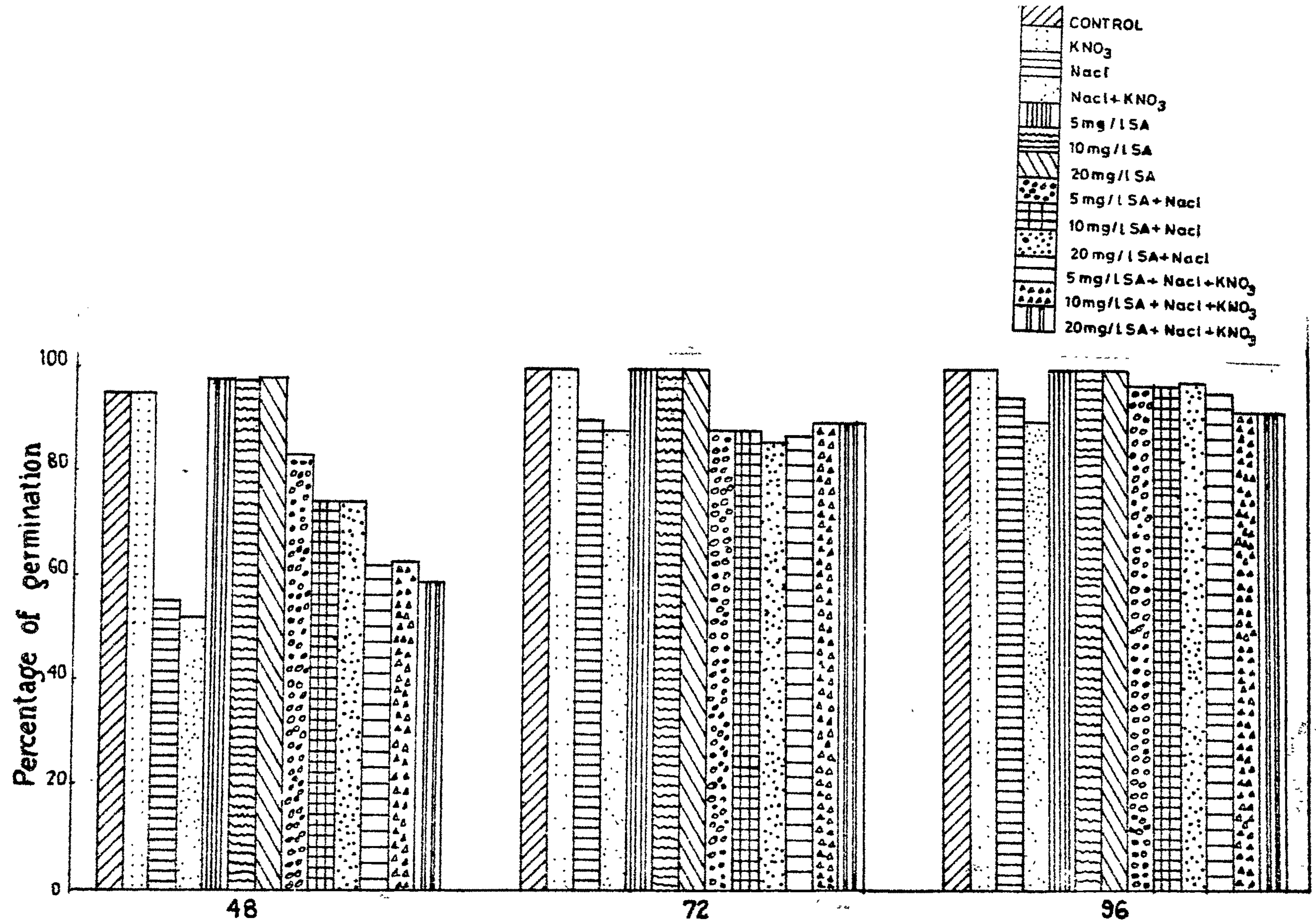

FIG NO 3.11 EFFECT OF SUCCINIC ACID ON GERMINATION OF PADDY UNDER THE INFLUENCE OF 1% Nacl.

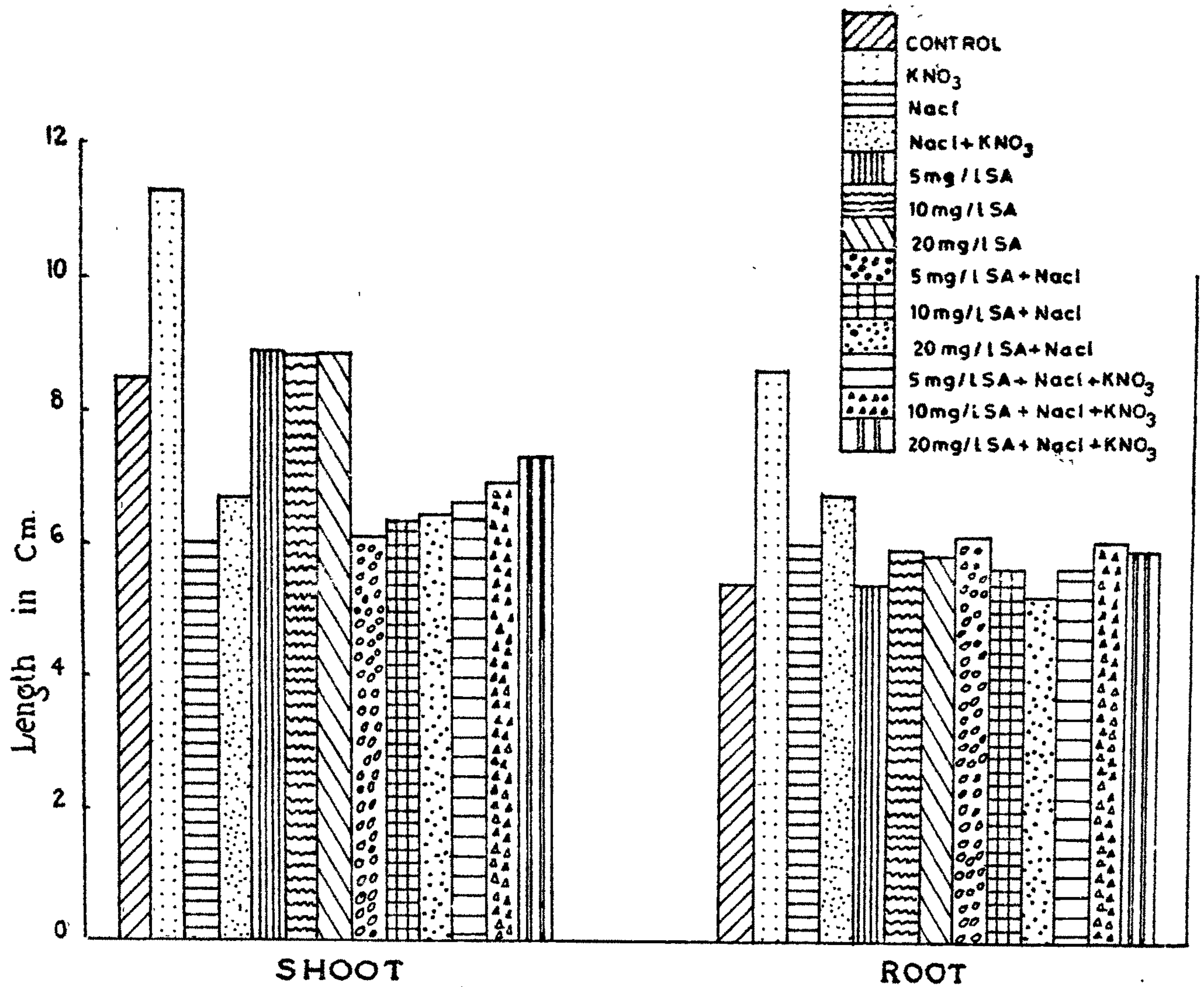

FIG NO 3.12 EFFECT OF SUCCINIC ACID (SA) ON GROWTH OF SEEDLINGS OF PADDY UNDER THE INFLUENCE OF 1% OF Nacl.

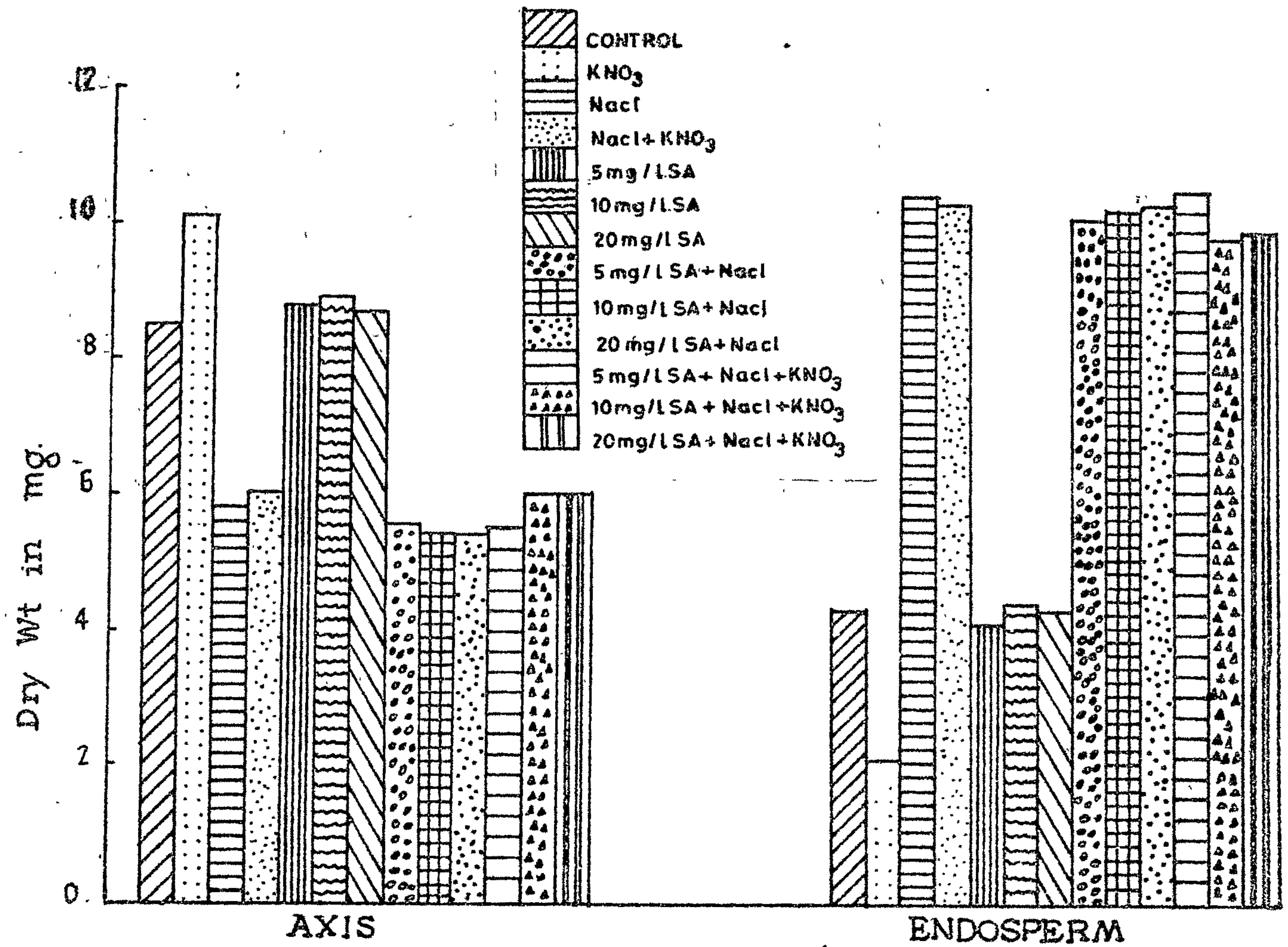

FIG NO-3.13 EFFECT OF SUCCINIC ACID (SA) ON THE DRY Wt. OF AXIS AND ENDOSPERM UNDER THE INFLUENCE OF 1% Nacl.

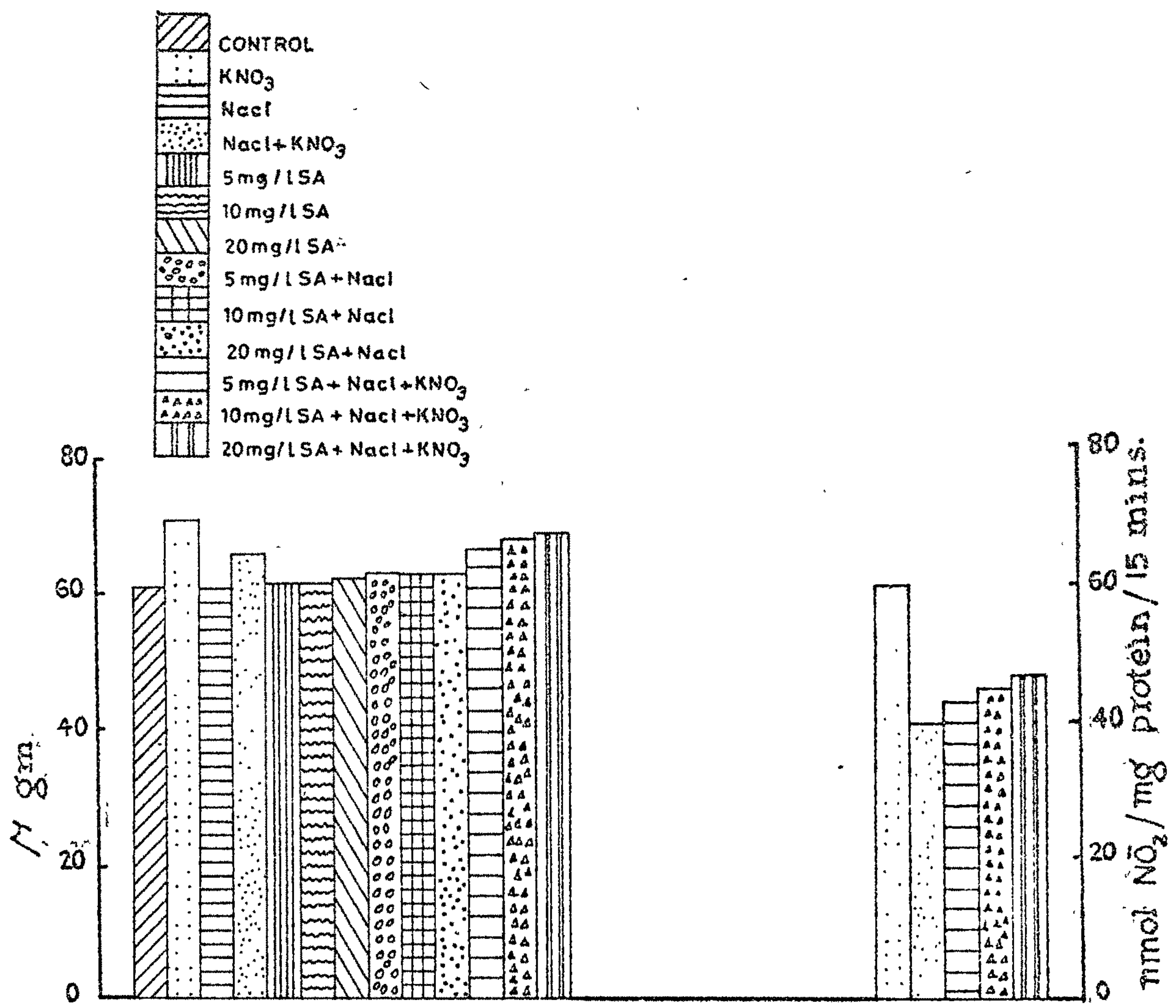

FIG NO 3.14 EFFECT OF SUCCINIC ACID (SA) ON THE UPTAKE OF NITRATE AND ACTIVITY OF NITRATE REDUCTASE OF PADDY UNDER THE INFLUENCE OF 1% OF Nacl.

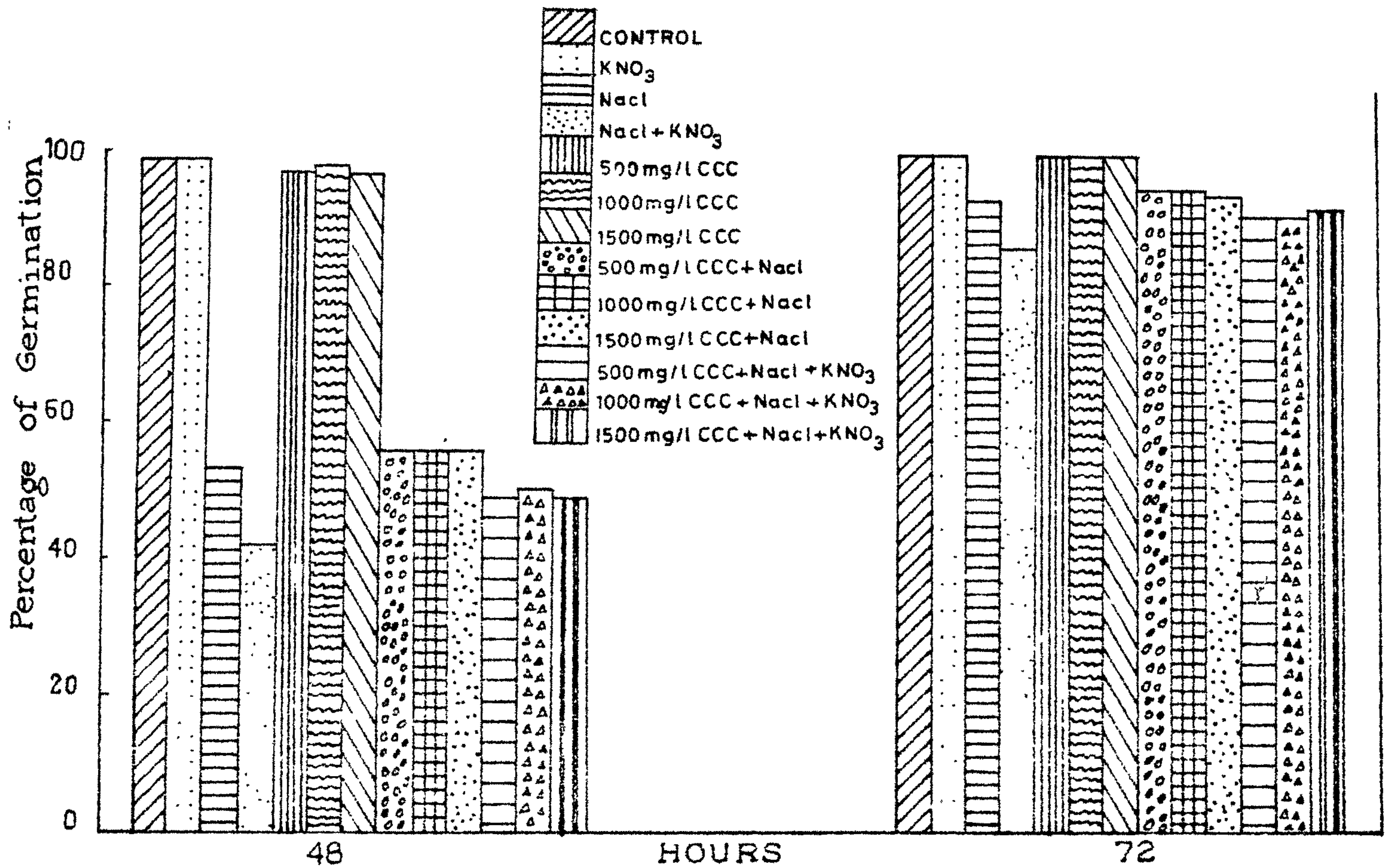

FIG NO 3.15 EFFECT OF CYCOCEL ON GERMINATION OF PADDY UNDER THE INFLUENCE OF 1% OF Nacl.

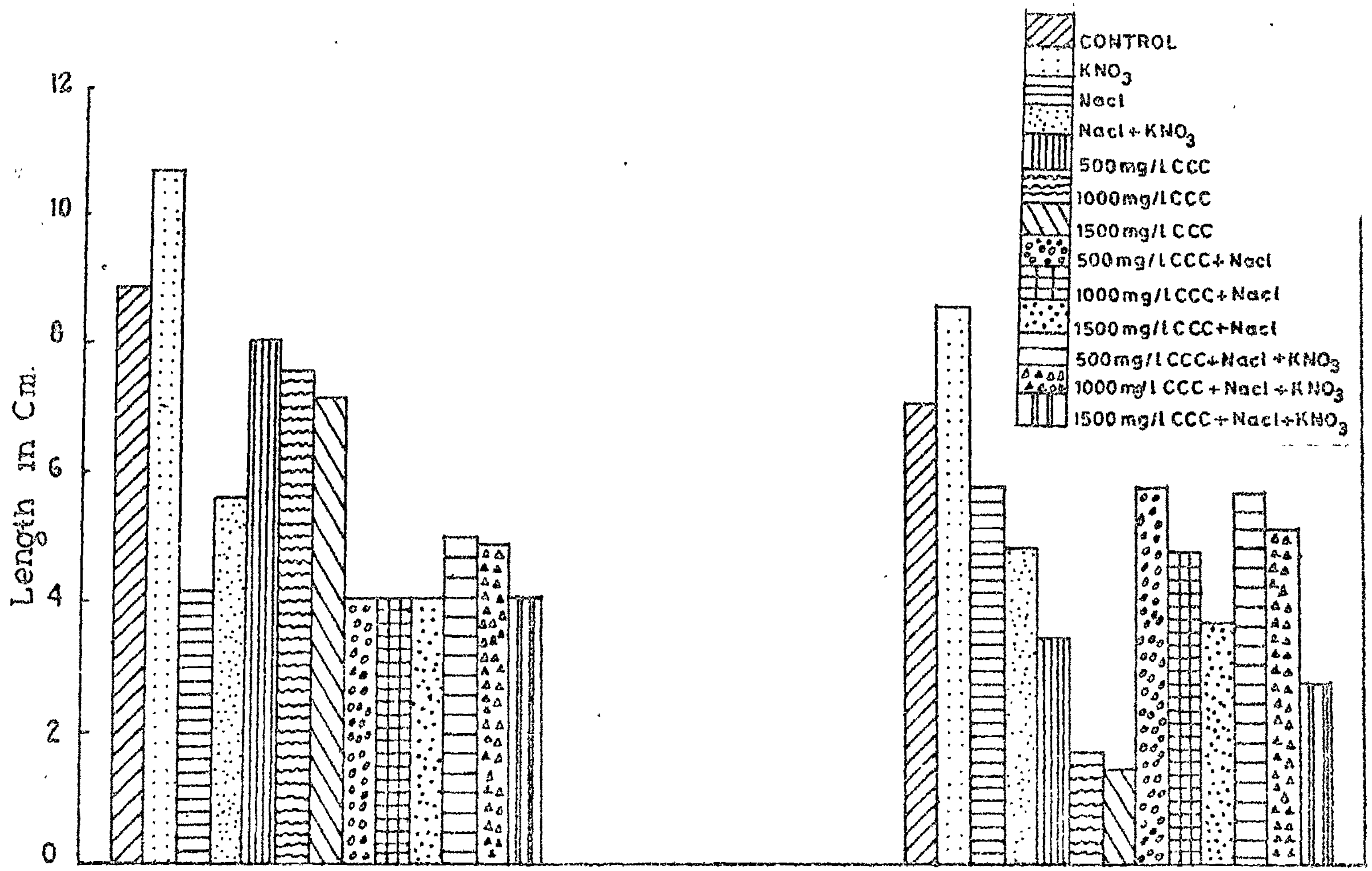

FIG NO 3.16 EFFECT OF CYCOCEL ON GROWTH OF SEEDLINGS OF PADDY UNDER THE INFLUENCE OF 1% OF Nacl.

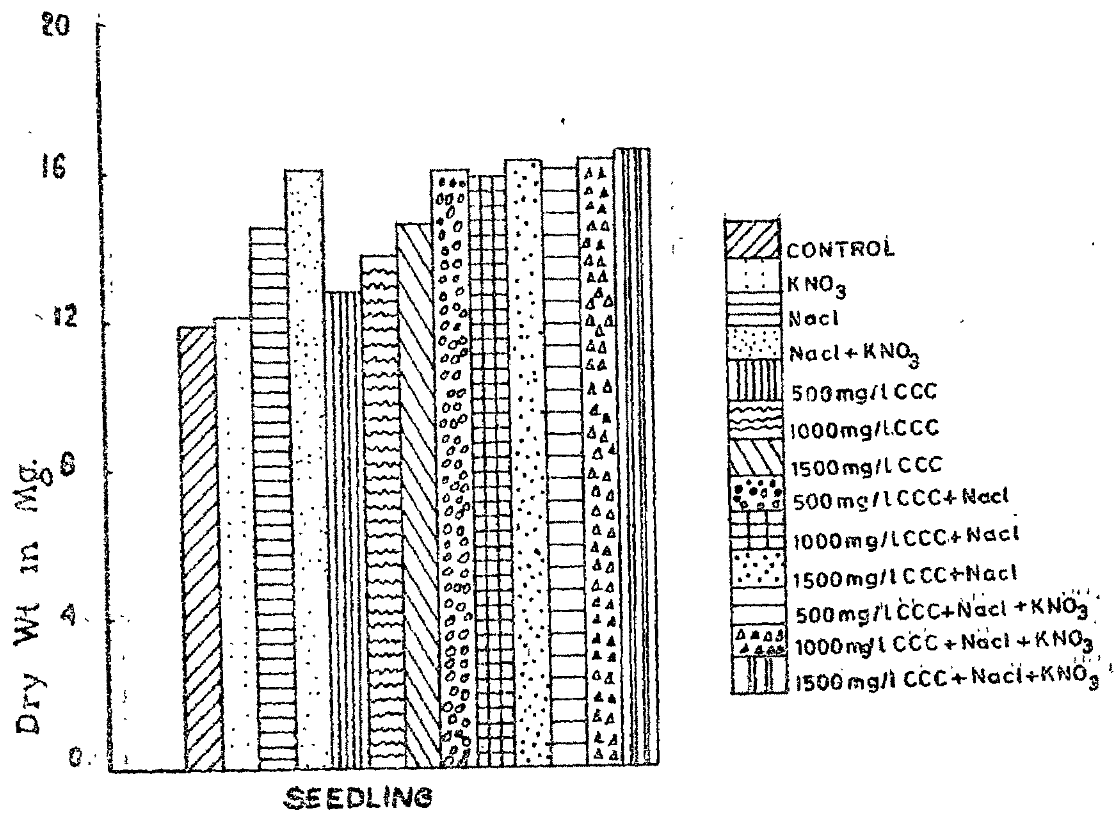

FIG NO 3.17 EFFECT OF CYCOCEL ON THE DRY Wt. OF SEEDLING UNDER THE INFLUENCE OF 1% Nacl.

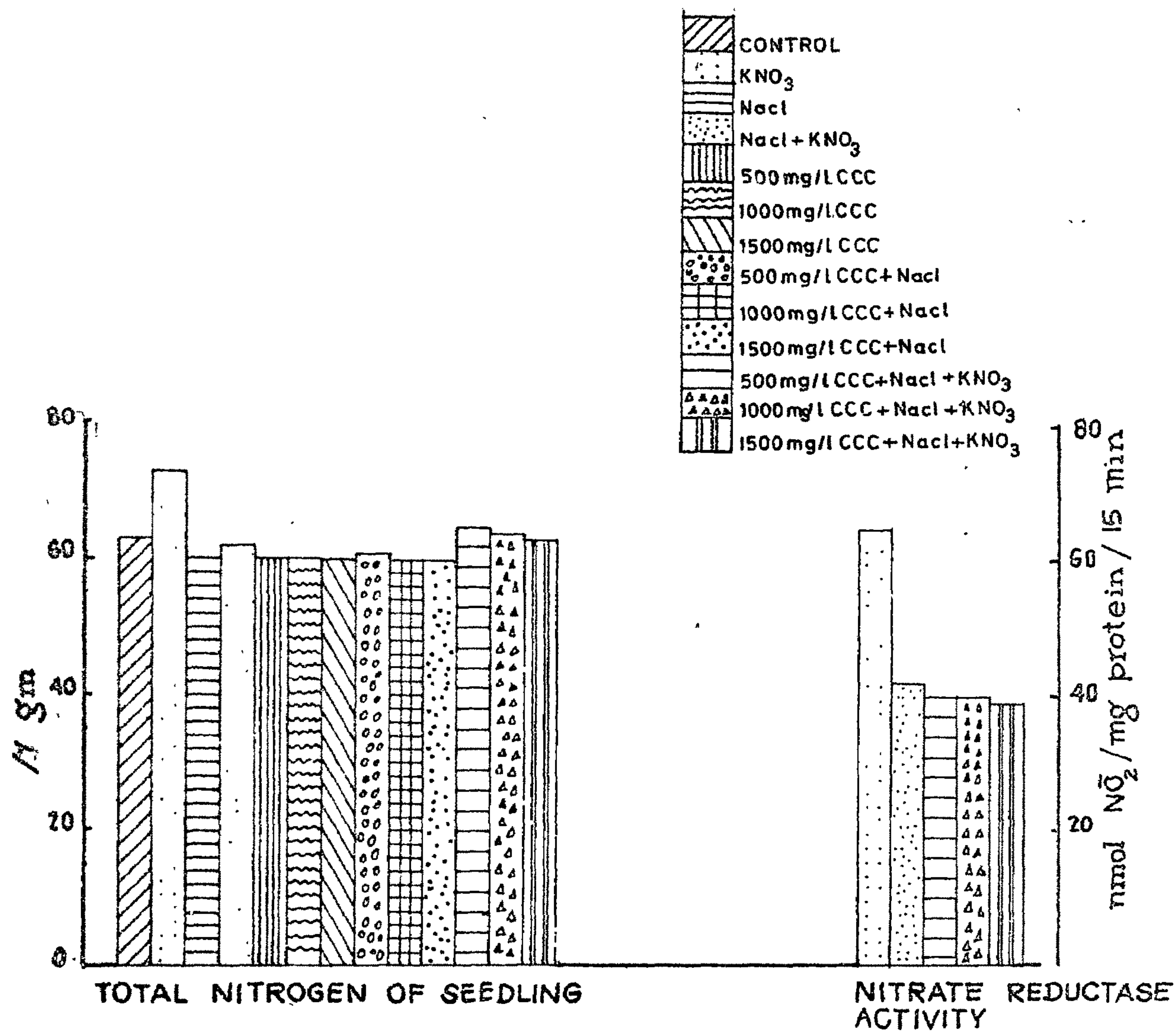

FIG NO 3.18 EFFECT OF CYCOCEL ON THE UPTAKE AND ACTIVITY OF NITRATE REDUCTASE OF PADDY UNDER THE INFLUENCE OF 1% OF Nacl.

Succinic acid tried at all concentrations slightly increased the dry weight of axes under 0.5% of salt alone and salt with 25 mM KNO_3 (Fig. 3.9).

The uptake of nitrogen and the 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 salt (Fig. 3.10).

At 1% concentration of sodium chloride, succinic acid at all concentrations tried increased the percentage of germination (Fig. 3.11) but failed to increase the growth of seedling (Fig. 3.12) and their dry weight (Fig. 3.13). The uptake of nitrate and activity of nitrate reductase, however, showed a marginal increase (Fig. 3.14).

Cycocel at all concentrations tried (500, 1000, 1500 mg/l) increased the percentage of germination (Fig. 3.15), but failed to stimulate growth (Fig. 3.16). However, an increase in the dry weight of seedling was observed (Fig. 3.17). Cycocel failed to stimulate the uptake of nitrate and activity of nitrate reductase under saline conditions (Fig. 3.18).

DISCUSSION

The considerably high percentage of germination recorded with 0.5% salt at 48 hours and 100% germination observed at 96 hours may possibly be due to the tolerance of Bhura Rata to this level of salt. The marked inhibition of the rate and percentage of germination at 1 and 1.5% of salt indicates that higher concentrations of NaCl are toxic to the germination of seeds as reported by Poljakoff-Mayber (1975).

The marginal reduction in the growth of the shoot system and the lack of inhibition noticed in the root system at 0.5% concentration of NaCl is a clear indication that salt at a level of 0.5% is not highly toxic to the shoot system and it has no effect on the root system. The inhibition of the root and shoot system with NaCl at 1 and 1.5% levels is due to the toxic effect of high concentrations of salt on growth. Stress in plant tissues resulting from salinity causes an increase in the content of abscissic acid and a decrease in the endogenous level of cytokinins (Mizrahi et al.,1972). This therefore, results in inhibition of growth. According to Joshi (1976), 0.1 to 0.15 M concentrations of NaCl had stimulating effect, while concentrations above 0.15 M caused toxicity in the rice variety Kala Rata. Poljakoff-Mayber (1975) reported that salinity induces stunting of growth and structural changes at various levels of organization. Strogonov (1962) studied the mechanism of growth inhibition caused by salinity and reported that in leaf cells of barley, tomato and cotton plants exposed to salinity, the protoplast retreated from the cell walls and cells appeared to be plasmolyzed. He also reported that breakage of plasmodesmata occurred from these cells. The dark green colour observed in the case of leaves of seedlings treated with the three levels of salt is probably due to increased chlorophyll synthesis. According to Strogonov et al. (1970) the increase in the chlorophyll content in more tolerant plants may be due to accumulation of either chlorophyll-a or chlorophyll-b. However, as reported by Joshi (1976) increased chlorophyll synthesis does not result in increased photosynthetic efficiency.

The enhanced growth of shoot and root systems and the increase in the dry weight observed in the case of seeds subjected to 0.5% NaCl along with KNO_3 at 25 mM is probably due to the influence of KNO_3 on growth as discussed in Chapter I.

The adverse effect of salt at 0.5% and 1% levels on the uptake of nitrate may be due to the physiological dryness which may be limiting the uptake of water and salt. According to Klotz (1958) high concentrations of ions accumulating in the cell as a result of osmotic adjustment may exert an allosteric effect on the enzyme protein and may effect the structure of membranes and enzymes. The adverse effect of salt on the activity of nitrate reductase, therefore, may possibly be due to the alteration in the structure of enzymes.

It has been observed that GA_3 increases the rate of germination. Hastening of germination by GA_3 has been reported in the case of barley and rice (Hayashi, 1940), peas and beans (Wittwer and Bukovac, 1957) and cotton (Prathapasenan, 1970). A marked loss in the dry weight of the endosperm has been recorded. This dramatic disappearance of the reserve food materials of cotyledons or endosperm of germinating seeds is one of the most striking effects of GA_3. Similar results have been reported by Hayashi in barley and rice (1940), Halevy et al. (1964), in several plant species, Prathapasenan (1970) in cotton and Kamalavalli (1969) in sorghum.

The stimulation of growth obtained under the influence of GA_3 alone and GA_3 and salt (0.5 and 1%) may be due to its effect in lowering or reversing the abscissic acid content and also enhancing the level of cytokinins. It may also be due to its auxin sparing action. The enhanced growth observed with GA_3, salt and KNO_3 may be due to the additive effect of GA_3 and nitrate. They are both known to stimulate not only germination and growth but also respiration. Increase in the rate of respiration as a result of GA_3 treatment has been reported in tomato, corn, bean, wheat, barley, timothy, rape and peas (Coulombe and Paquin, 1959; Nielsen and Bergquist, 1958; Runzha, 1963; Weller et al., 1957). Weissman (1972) reported an increase proportion of total oxidized plus reduced NADP (NADPH) in the case of soybean and sunflower root tissue and therefore, he is of the opinion that nitrate stimulated glycolysis in the roots of these plants. As suggested by Jones (1973) the significant increase in growth observed in the present studies under the influence of GA_3 may be the result of cell elongation, cell division or both. Phinney (1956) found that the most dramatic effect of GA_3 is in the transformation of dwarf plants into tall ones by greatly increased stem elongation. GA_3 has also been found to induce stem elongation in normal plants such as cotton (Prathapasenan et al., 1969), and sorghum (Kamalavalli, 1969). However, Nieman and Bernstein (1959) were unable to find a relationship between GA_3 and salinity. Studies on the active mechanism of plant hormones suggested that the primary reaction induced by the hormones in the cell are at the level of nucleic acids - protein metabolism (Key, 1969). This implies that hormones may act in such a way that it consequently affects the production of a specific enzyme - the enzyme may be induced, promoted or inhibited. In the present studies it can be observed that GA_3 promoted the uptake of nitrate and activity of nitrate reductase in the presence of salt at a concentration of 0.5% NaCl. Increased glycolytic activity in the roots of nitrate treated seedlings may perhaps be the reason for marked absorption and activity of nitrate reductase (Wiesman, 1972). GA_3 failed to stimulate the activity of nitrate reductase at 1% level of NaCl. This may partly be due to the decreased absorption of nitrate or, as reported by Nir et al. (1970) due to a break-down of polysomes during stress.

Treatment of seeds with succinic acid brought about a marginal increase in the rate and percentage of germination. This is in conformity with the reports of Gertsuskie (1959) and Maurina et al. (1969) in corn, Drozdov and Babuk in cereal seeds (1968), Lagutina (1966) in siberian larch seeds, Siuliauskas (1967) in barley and lupine and Kudinov (1967) in a variety of species.

The slight increase observed in the growth of seedlings observed under the influence of succinic acid confirms the reports of Kotyashkina (1968) who observed promotion of growth in corn and cabbage, Maurina et al. (1969) in corn and Artemova (1968) in cucumber. Succinic acid has been found to increase dry matter production. Similar increase in dry weight has been reported by Koeoleva (1964).

The slight increase in the uptake of nitrate and the activity of nitrate reductase observed in the case of treatment of seeds and seedlings with succinic acid may presumably be due to the increased supply of energy and availability of the cofactor NADH since succinic acid is a compound of the tricarboxylic acid cyclic.

It is interesting to note that the increase in the percentage of germination in the case of paddy seeds treated with cycocel is in accordance with the results of Kretschman and Begar (1971) who reported an increase in the percentage of germination from 77-95 and 91.5-97 in the seeds of wheat variety Tanal and Remo respectively.

Seedlings treated with cycocel developed thicker stems and shorter internodes and intensely dark green leaves. Tolbert (1960) has also reported reduction in height of plants accompanied by an increase in stem diameter. The changes observed due to the effect of cycocel simulated those caused by exposure of plants to high light intensities, a short photoperiod and low temperatures. They were opposite to and more persistent than those induced by gibberellin. Slight but significant increases in dry matter accumulation occurred with tomato plants under the influence of cycocel (Wittwer and Tolbert, 1960). Increase in the dry weight was also observed in the present studies. However, treatment with cycocel failed to influence the absorption of nitrate from the medium and thus it inhibited the activity of nitrate reductase.

*****

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