PHYSIOLOGICAL BASIS OF YIELD VARIATION IN *BRASSICA JUNCEA* L. UNDER THE INFLUENCE OF NITROGEN SOURCE AND SALINE WATER IRRIGATION

By

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in

PLANT PHYSIOLOGY

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Dedicated
To My
Beloved Parents

CERTIFICATE - I

This is to certify that this dissertation entitled, "**Physiological basis of yield variation in *Brassica juncea* L. under the influence of nitrogen source and saline water irrigation**", submitted for the degree of Ph.D. in the subject of **Plant Physiology** of the Chaudhary Charan Singh Haryana Agricultural University, is a bonafide research work carried out by **Mr. Narayan Singh Nathawat** under my supervision and that no part of this dissertation has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE - II

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Singh.

(NARAYAN SINGH NATHAWAT)

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LIST OF ABBREVIATIONS

ADP	-	Adenosine-5'-diphosphate
$Ca(NO_3)_2$	-	Calcium nitrate
$Ca(NO_3)_2 + (NH_4)_2SO_4$	-	Calcium nitrate + Ammonium sulphate (combined)
DTT	-	Dithiothreitol
EDTA	-	Ethylene diamine tetraamino acetic acid
FAD	-	Flavin adenine dinucleotide
GDH	-	Glutamate dehydrogenase
GS	-	Glutamine synthetase
GOGAT	-	Glutamate 2-oxoglutarate amino transferase (Glutamate synthase)
NADH	-	Nicotinamide adenine dinuclotide reduced
NADP	-	Nicotinamide adenine dinucleotide phosphate (oxidized form)
$(NH_4)_2\ SO_4$	-	Ammonium sulphate
NR	-	Nitrate reductase
NiR	-	Nitrite reductase
NUE	-	Nutrient use efficiency
TCA	-	Trichloro acetic acid
Tris	-	Tris (hydroxymethyl) amino methane

CHAPTER - 1

INTRODUCTION

Salinization of land is progressively increasing throughout the world (Kozlowski, 1997). Excessive salt concentration can transform fertile and productive lands to barren lands and often leads to loss of habitat and reduction of biodiversity (Ghassemi *et al.*, 1995). It is estimated that about a third of the world's irrigated lands and half the lands in semi-arid and coastal regions are affected by salinization and 10 million hectare irrigated lands are abandoned annually because of excessive salinity (Abrol *et al.*, 1988; Rhodes and Loveday, 1990).

In India, soil deterioration through accumulation of excess salts has attained a serious dimension, especially in the regions where irrigation water has a high salt concentration. A total of 10.1 million hectare of land in the country is salt affected, of which about 2.5 million ha occurs in Indo-Gangetic plain alone (Sehgal and Abrol, 1994).

India is one of the largest oilseed growing country in the world but its productivity per unit area is low. One of the main reasons for low productivity is the poor return from the vast semi-arid regions of north-west and south part of country. In India, Brassica ranks second after groundnut and is grown in area of about 68.57 lakh ha with production of

69.74 lakh tonnes (Anon., 2000), whereas in Haryana, it is grown in 645 thousand ha of land with production and productivity of 900 thousand tonnes and 1395 kg/ha, respectively. In Haryana, rapeseed and mustard accounts for more than 92 per cent of total oilseeds being grown. Salinity is a major problem here particularly in the districts of Jind, Hisar and Rohtak. The salinity affected land in the state is estimated to be 0.45 million hectares, which is about 10.29 per cent of the total geographical area of the state (Rao *et al.*, 1995). Rapeseed and mustard are generally grown in arid and semi-arid regions which are generally affected either by salinity of soil or irrigation water. Hence, crop suffers salt stress right from germination to various stages of crop growth leading to reduced yield or even crop failure.

Salinity poses several problems for plant growth and development, especially for glycophytes, by inducing physiological disfunctioning (Shannon *et al.*, 1994). Soil salinity is an important environmental constraint in crop production. Salts in the rooting medium generally alter a wide array of metabolic processes, culminating in stunted plant growth. Salinity-induced nutritional imbalance often produces specific nutrient-deficiency symptoms and tolerance of plant might be improved by nutritional status (Rao and Yadav, 1997).

Garg *et al.* (1993) observed detrimental effects of salt stress on soluble protein and starch levels in Indian mustard besides increass in free proline and amino-acids. Activities of NR, GS, GOGAT and GDH were also inhibited by increasing level of salinity indicating a disturbed

N-metabolism. Salinity induced changes in the level of leaf metabolites, such as, soluble protein, free proline, total chlorophyll and reducing sugars and enzyme activities (NR, GS, GOGAT and GDH) were consistently less in tolerant genotypes, compared with sensitive genotypes (Garg *et al.*, 1997).

Amino acids are one form of nitrogen containing compounds (NCC) which have been shown to accumulate in glycophytes and in halophytes under salinity (Dubey and Pessarakli, 1995; Amonkar and Karmarkar, 1995). These amino acids include proline, arginine, alanine, glycine, serine, leucine and valine. Among increased amino acids, proline accumulates in larger amounts compared with other amino acids and in many plant species remarkable increase in proline content was observed (Dubey, 1997; Ali *et al.*, 1999). Plants vary greatly in their capacity to accumulate proline or other amino acids under salinity. When plants are subjected to salt stress, salt tolerant genotypes accumulate more free amino acids, particularly proline, than sensitive ones (Dubey and Rani, 1995; Ashraf, 1997; Dubey, 1997). Proline as well as other amino acids accumulated under stress condition have been mainly recognized as osmotic adjustment agents. They lower the cell osmotic potential to allow water absorption despite decreased soil water potential. Proline as well as other amino acids, even at higher concentrations, do not interfere or inhibit the enzyme activities (Wyn Jones *et al.*, 1984; Ashraf, 1997; Dubey, 1997). Proline has been also considered a nitrogen storing agent (Rains, 1989). This is because in stress plants proline can be easily converted to

glutamate which is involved in synthesis of other essential amino acids (Ashraf, 1997).

Nitrogen metabolism is complex and varies with species. In general, the first step in the assimilation of nitrate is its reduction by the enzyme nitrate reductase to nitrite and then by nitrite reductase to NH_4^+. The nitrite and NH_4^+ are toxic. NH_4^+ is rapidly converted to amide group of glutamine, glutamate and sometime asparagine or other amino acids (Mansour, 2000). Salinity altered the activities of NO_3^- assimilation enzymes; nitrate reductase (NR), nitrite reductase (NiR), glutamine synthetase (GS) and glutamate synthase (GOGAT). The activity of nitrite reductase was either inhibited or unaffected in many crop species and halophytes since this enzyme is generally less sensitive to salinity than nitrate reductase. Salinity increased or decreased the activity of glutamine synthetase and glutamate synthase depending on species and their sensitivity to salinity. Higher activities of both enzymes are usually found in salt tolerant species and halophytes. In many plants, salinity increased the activity of glutamate dehydragenase showing that these plant may have the potential to assimilate NH_3 by this enzyme in saline environment (Amonkar and Karmarkar, 1995; Dubey, 1997).

Saline environment is generally deficient in nitrogen (Amonkar and Karmarkar, 1995) and in addition salinity interfers with NO_3^- uptake in many plant species which decreases NO_3^- content (Khan and Srivastava, 1998). The reduction in NO_3^- uptake could be due to high Cl^- content in saline soil. Addition of N to plants subjected to salinity improved their

growth and yield and thus their salt tolerance (Dubey and Pessarakli, 1995). Application of Ca^{2+} also increased NO_3^- uptake under salt stress and this may be attributed to Ca^{2+} involvement in maintaining membrane integrity or increased activity of NO_3^- transporter by Ca^{2+} in saline environment (Dubey, 1997).

Lipids present in other forms such as storage reserves also seems to be affected by salinity. This is significant in rapeseed-mustard where decreased oil content with salinity has been reported (Sharma and Manchanda, 1997) which is highly undesirable from economic point of view. The oil quality is important from the view point of nutritional properties, physiological effects of fatty acids and storage. A high proportion of long chain of polyunsaturated fatty acids (PUFAs) is desirable from human nutrition point of view. Linoleic acid plays an important role in the synthesis of prostaglandins, which regulates various body functions. The PUFAs are known to lower the abnormally high cholesterol level in blood and are recommended for patients with risk of coronary diseases due to their hypocholesteremic nature. Linolenic acid is highly unsaturated and oil rich in it find use in paint and varnish industry. However, information regarding the effect of salinity on the quality aspects of mustard oil is very scarce.

Glucosinolates are the main secondary metabolites of cruciferous plants, and the composition of the glucosinolate profile is species-specific (Louda and Mole, 1991). The profile is further influenced by development stage, tissue specificity and biological or physical stress on the plant. The

importance of glucosinolates in plant interactions with insects and herbivores has also been frequently reported (Blau *et al.*, 1978; Roessing *et al.*, 1992). Furthermore, induction of certain glucosinolates in response to defence related substances such as salicylic acid and jasmonate (Kiddle *et al.*, 1994; Doughty *et al.*, 1995). High levels of glucosinolate hydrolysis products can adversely affect the feeding value of rapeseed meal (Fenwick, 1983). Higher seed glucosinolate contents have been associated with water stress (Mailer and Cornish, 1987), high temperatures during seed growth and disease (Salisbury *et al.*, 1987).

The physiological responses of plants to different forms of nitrogen from its reduced stage (ammonium) to its most oxidized stage nitrate, developed simultaneously in two scientific areas : (a) the molecular and physiological studies of nitrogen uptake, assimilation and their relationship to plant growth and development and (b) the agricultural interest on the practical aspects of nitrogen use application in intensive field production with daily fertigation (Lips *et al.*, 1990).

In the semi-arid parts of India, growth of Indian mustard is often restricted as the ground water used for irrigation are mostly moderate to highly saline. A better understanding of how salinity affects physiological processes is important for such water to be used efficiently. Since nitrogen contributes substantially to plant growth and is directly related to crop yield potential, the influence of saline stress on N-metabolism is of great interest. Nitrate (NO_3^-) and ammonium (NH_4^+) are most abundant N-sources for higher plants and their availability usually constitutes a limiting factor for plant growth (Causin and Barneix, 1993).

Saline condition can influence the different steps of nitrogen metabolism such as uptake, reduction and protein synthesis, that may be responsible, at least in part, for the lower plant growth rate observed under such condition. Moreover, the effect of salinity on nitrogen metabolism and growth may vary with different nitrogen sources. However, under different salinity situations, the application of fertilizer has been reported to significantly alleviate the adverse effects of salt stress on a number of crops. In mustard, N-fertilization has also been found to increase the seed yield when irrigated with saline water. But there is no information on the processes regulating this salinity-fertility interaction for this crop. It may, however, be speculated that an improved soil fertility may increase nutrient uptake, particularly that for N, which may in turn enhance N-metabolism which augments the growth and yield despite of salt stress.

This investigation was conducted to evaluate whether the combined effect of salinity and N-fertilization [$Ca(NO_3)_2$, $(NH_4)_2 SO_4$ and ($Ca(NO_3)_2$ + $(NH_4)_2 SO_4$] on the growth and N-metabolism of Indian mustard in order to determine the best N-source under saline condition, and to ascertain whether the reduction in growth and N-metabolism caused by salinity is related to a decrease of N-uptake.

Scanning of literature reveals very little work on responses of brassica to interactive effects of salinity and fertilizers at various stages of growth. Hence, present study is proposed to know some morpho-physiological and biochemical responses of brassica at various growth stages in relation to its susceptibility/resistance which could be suggested

for improved nitrogen fertilization under salt stress with following objectives:

1. To investigate the influence of nitrogen source on growth and development, nutrients and quality characters in Brassica under saline irrigation.
2. To probe into the biochemical basis of nitrogen metabolism under interactive effects of nitrogen fertilizer and saline irrigation in Brassica.

CHAPTER - 2

REVIEW OF LITERATURE

Salinity is one of the major problems to crop productivity among environmental stresses. The reduction in growth of crop plants by salinity may result from its effects on dry matter production, ion relation, water status, physiological processes, biochemical reactions or a combination of such factors (Zidan, 1991 and Malibari, 1993). Indian mustard is confined mainly to arid and semi-arid region and the use of saline water for irrigation purpose in these regions is a problem in realizing satisfactory seed yield. The plant performance and seed yield might be reduced due to deleterious effects of salinity on almost all the growth and developments traits (Munns and Termaat, 1986; Flowers and Yeo, 1989). A number of biochemical processes viz., protein synthesis, carbohydrate and proline metabolism, photosynthesis and enzyme levels are adversely influenced by salt stress in these crop (Garg and Gupta, 1998). The harmful effects of salinity on the crop performance may be attributable to the following factors: (i) Ionic effect (toxicity due to salt ions), (ii) Osmotic effect (non-availability of water), (iii) Alteration in ionic composition leading to deficiency of nutrient ions and excess of salt-ions, (iv) Diversion of photosynthesis from growth functions to osmotic adjustment and (v) Extra expenditure of energy on ion transport

In rapeseed-mustard, various species differ in response to salinity depending on how far they can tolerate the stress. Tolerance to salt stress is a complex trait, which is greatly modified by cultural, climatic and biological factors and by the degree of heterogeneity in saline crop lands. *Brassicas* exhibits susceptibility to salinity at seedling emergence and early seedling growth but are relatively more tolerant at later growth stages, particularly from flowering to formation of the siliquas (Kumar, 1995).

Only limited success have been achieved so far to control the problem of salinity through soil reclamation and soil amendments. To alleviate the adverse effect of salinity, application of nitrogen fertilizer in different source have been employed. Some encouraging results which have been achieved so far suggest that use of different sources of nitrogen fertilizer may provide some insight to solve the evergrowing and alarming problem of salinity. The literature available on the effects of salinity and its alleviation by the application of different nitrogen sources has been reviewed under the following appropriate heads emphasizing the topics relevant to the present investigation.

2.1 Effect of salinity

2.1.1 Growth and development

The most common and conspicuous effect of salinity is growth retardation. As salt concentration increases beyond a threshold level, both the growth rate and ultimate size of most plant species progressively decrease. With salinity, decreased plant growth in rice (Ashraf and Yousaf, 1998), barley (Cho Jinwoong and Kim Choongsoo, 1998), cowpea (Kurban *et al.*, 1998) has been observed. Top growth is often suppressed more than the root growth (Meiri and Poljakoff-Mayber, 1970; Mass and Hoffman,

1977). Recently, in barley seedling under solution culture, decreased shoot/ root ratio was observed with increasing NaCl concentration (Cho Jinwoong and Kim Choongsoo, 1998). Moreover, saline conditions cause restricted branch number decreased leaf size, dark green leaves, rest ricked root development, reduction in size fruit, fall in fresh and dry weight of various plant parts and decrease in number and size of seeds and consequently, the yield (Ansari *et al.*, 1998; Chon Jinwoong and Kim Choongsoo, 1998) and decreased root growth and seed yield due to water stress in *Brassica juncea* (Singh and Singh, 1991). Salinity levels exceeding electrical conductivity of 4 dsm^{-1} is detrimental to germination of seeds and growth (Mishra and Sharma, 1994).

Several workers have studied the growth under salt stress conditions and observed a decrease in length, fresh and dry weight of seedling (Kuhad *et al.*, 1989; Taneja *et al*, 1992). Saline condition care in general, stunted growth, limited development, restricted branch number, decreased leaf size, dark green leaves, restricted root development, reduction in size of fruit, fall in fresh and dry weight of various plant parts and decrease in number and size of seeds and consequently the yield (Sinha *et al.*, 1986; Chauhan, 1991).

Numerous studies have shown that length, fresh weight and dry weight of seedling decreased under salt stress (Singh and Singh, 1982; Sharma *et al.*, 1996). Delzoppo *et al.* (1999) reported that increasing concentrations of Nacl significantly reduced root and shoot fresh weight in wild wheat.

Leaf area, dry weight accumulation at different crucial growth stages like flowering and grain filling also declined with salinity stress and ultimately resulting in lower yield (Sharma, 1987). Eharet *et al.* (1990)

observed great reduction in leaf area and plant dry weight of salt stressed plants of wheat and barley. A decline in plant height, root length, number of leaves, leaf area, and dry weight of root, stem and leaves in two cultivars of wheat was observed by Sharma *et al.* (1994). Similarly Gorham and Bridges (1995) claimed a significant reduction in fresh and dry weight of cotton under NaCl salinity. While, Grattan and Maas (1988) reported reduction in shoot dry weight in soybean plant under saline environment. Malibari (1993) observed a significant decline in dry matter of shoots and roots of wheat with increasing level of salinity.

It is well known that excessive amounts of salt in the soil or irrigation water, limit plant growth and development. Such salt induced growth reductions are a function of total electrolyte concentration, soil water content and soil matrix potential. However, whole plant response to salinity may also depend on salt concentration in the tissues, exposure time and salt composition (Shannon *et al.*, 1993) and also on the climatic conditions. Salinity has been reported to both stimulate and adversely affect the growth of Indian mustard. At low to moderate salinity of irrigation water (2-8 dSm^{-1}) stimulation of growth resulted from increased availability of nutrients and also due to sparse plant population resulting from decreased germination and early mortality of seedling on light textured soil (Kumar and Singh, 1980; Kumar and Malik, 1983; Dhawan *et al.*, 1987; Chauhan *et al.*, 1988; Sinha, 1991). Available reports indicate that rapeseed and mustard are highly susceptible to saline water during germination and early seedling growth (Kumar, 1984; Kumar and Kumar, 1990; He and Cramer, 1993a). Minhas *et al.* (1990) working on Indian mustard (*Brassica juncea*) and found that increasing level of salinity (EC 4.3, 7.9 and 12.3 dSm^{-1}) significantly decreased plant height as compared to control plants.

He and Cramer (1993b) observed that *Brassicas* were vulnerable at the seedling stage as there was reduction to the extent of 72% in whole plant mean dry weight of 6 species at 24 days after sowing. However, inter specific differences in growth occur under saline conditions. For instance, reduction in growth of tolerant *B. napus* was related to the relative growth rate (RGR), linked with a reduction in leaf area ratio (LAR). On the contrary, growth reduction in sensitive *B. carinata* was primarily due to reduction in net assimilation rate (NAR) during the early stages with saline sea water of 8 dSm^{-1}. Relative growth rate (RGR) is defined as increase in plant material per unit of time. AGR provides more information in comparison to plant relative performances. Watanable *et al.* (1992) studied seedlings of twelve species of wheat subjected to salinity treatment at 5.5-6.5 leaf stage and calculated RGR, net assimilation rate (NAR) and leaf area ratio (LAR) from weight of leaf blades, leaf sheath and roots , leaf area and observed reduction in all these parameters with salinity. NAR, RGR, CGR were calculated by Joshi and Nimbalkar (1983) and reported that in *Cajanus cajan*, NaCl treatment caused great decrease in RGR than in Na_2SO_4 and same was observed for NAR also. At higher concentrations (15 ECe) under NaCl stress dry matter production was lowered by 83% while only by 37.12% under Na_2SO_4 stress.

He and Cramer (1996) studied on *B.carinata* and *B. napus*, reported that the greater sensitivity of *B. napus* has been linked to a greater reduction in net assimilation rate. They also found that increase in abscisic acid (ABA) are involved in the reduction of growth by salinity. Salinity (EC 8.0 dSm^{-1}) caused increase of ABA concentrations in shoot, root and callus of both species. ABA concentration were lower in the salt tolerant species, *B.*

napus, than the salt sensitive species, *B. carinata*, both the whole plant and callus.

The reduction in leaf area by increasing salinity is a common feature which affects plant productivity by reducing the rates of total photosynthesis by the crop canopy (McCree, 1986). The salinity induced decrease in leaf area is particularly severe in salt sensitive crops like most of legumes (Garg and Lahiri, 1986). In contrast, Terry and Waldron (1984) claimed Na_2SO_4 more deleterious than NaCl to growth and development of plant. Similary chandler and Thorpe (1987) and Paek *et al.* (1988) found that growth of *Brassica napus* was affected more in sulphate salinity than under chloride salinity.

2.1.2 Water relations and gas exchange

Salinity exerts complex effects on the plant as a result of ionic, osmotic and nutritional interactions, although the exact physiological mechanism of salt stress is unknown. Salt tolerance often depends on the anatomical and physiological complexity of the plant. Salinity stress causes non-availability of water to plants causing 'physiological drought'. Thus, plant faces inhibition of water absorption due to lowering of external water potential. The plant growth under salinity is suppressed due to osmotic effect (Bernstein and Hayward, 1958) or due to effect of specific ions on metabolic processes (Kuhad and Garg, 1984) or due to ionic effects (Epstein, 1983). Many important physiological processes as stomatal opening, photosynthesis, transpiration are directly affected by the reduction of leaf turgor potential which accompanies the loss of water from leaf tissue. The plant ability to maintain leaf turgor is an important adaptation to water deficits. Leaf water potential is lowered by salinity (Kumar *et al.*, 1984).

Jain and Nainawatee (1993) analysed water relations and osmoregulation under salt stress in two genetically stable *in vitro* selected salt tolerant variants (SR-2 and SR-3), a non-selected somaclone (CP-5) and parent cv. Prakash of Indian mustard. Relative water content (RWC) declined to a great extent in cv. Prakash and CP-5 as compared to SR lines under salinity. A linear regression between leaf osmotic potential and RWC indicated a variable degree of osmotic adjustment in SR lines and non-selected lines. The selected SR lines had two to three times greater degree of osmotic adjustment than non selected lines. Differences in osmoregulation also correlated well with seed yield (r=0.92). High degree of osmoregulation is known to be associated with higher seed yield in oilseed brassicas and, therefore, genotypic responses in this regard assume significance in salt tolerance studies. Among water, salt and freezing stresses, salt stress was found to be the most injurious to the tissues regarding relative water content and viability as well as banding pattern of some isoenzymes in Indian mustard callus (Gangopadhayaya *et al.*, 1995). Other reports also indicate such responses in RWC and tissue viability under different stresses (Mukherjee *et al.*, 1991).

Delzoppo *et al.* (1999) observed that increasing concentration of NaCl significantly reduced leaf water potential and osmotic potential. Similar results also reported by Carvajal *et al.* (1998) that reduced stomatal conductance, water potential and osmotic potential was observed under different salinity treatment (20, 40 and 60 mm NaCl).

Water relation in *Brassica* species were studied by Madan *et al.* (1994). It was reported that lower values of leaf osmotic potential with increasing salt concentrations in all species and observed that first leaf

showed lower osmotic potential than others. The relative water content (RWC) of leaves was also adversely affected by salt in all the genotypes and RWC of first leaf of plant irrigated with 100 mM NaCl containing nutrient solution was even higher than the fourth leaf of untreated plants. Water content in root and shoot of chick pea genotype under salinity was studied by Dua (1998) and observed that water content decreased with increasing salinity levels in sensitive genotype but tolerant genotype maintained its water content.

Kumar (1984) reported that leaf water potential decreased with increase in salinity both in tolerant and sensitive varieties but the magnitude of decrease was markedly higher in the latter. Leaf osmotic pressure observed by Datta *et al.* (1993) and showed decline under sulphate salinity though it was also much declined in predominant chloride treatment. Paek *et al.* (1988) observed osmotic changes under Na_2SO_4 and NaCl, which were correlative to changes in growth of callus in *Brassica campestris*, which clearly attained its most negative value in NaCl and Na_2SO_4 alike. Tissue water relations analysis were made using RWC (relative water content) and turgor potential have been used in the analysis of tissue water relation (Kumar and Elston, 1992). RWC and osmotic potential were used in calculations of osmotic adjustment by Singh (1992) and Madan *et al.* (1994) in *Brassica* under salinity.

There are numerous reports of a general reduction in photosynthesis in crop plants by various types of salinity i.e. cotton, onion, beans, tomato, wheat, barley, pearl millet, maize, pea, sugarbeet, rice and many others, (Downton, 1977; McCree, 1974). The concentration at which salinity cause severe reduction in photosynthesis varies greatly in different plant species

and varities. The step of photosynthesis affected may also vary depending upon the type and level of salinity and plant species. The reduction in photosynthesis in glycophytes may occur in the following ways : (i) Low diffusion of CO_2 in the chloroplasts (ii) Alteration in structure and function of organelles responsible for photosynthesis (iii) Changes in photosynthetic reaction i.e. light and dark reactions (iv) Effects on transport of assimilated products and intermediary compounds in several investigations the primary effect of salinity is increased stomatal resistance to CO_2 difference as known under water stress (Walker *et al.*, 1979). This causes a reduction of photosynthesis whenever CO_2 is limiting factor e.g. under high light intensity or due to the failure of guard cell to adjust osmotically, ionic interferences with stomatal function and increased indigenous content of abscisic acid. Boari *et al.* (1997) studied on Broccoli at different salinity levels and reported that leaf net CO_2 assimilation rate (A), transpiration rate (T) and stomatal conductance (g_S) were decreased significantly at ECe higher than 12 dSm^{-1}. Leaf diffusive conductance, photosynthesis and gaseous exchange under salinity have been studied by many workers (Mc Cree, 1974; Greenway and Munns, 1980; Pasternak, 1987). Different species have been studied using salt levels and under different environmental conditions. Limitations to photosynthesis by salinity may be attributed to two major factors viz. (i) limitation due to decreased stomatal conductance, thus decreased carbon dioxide for reduction to leaf cells (Gale *et al.*, 1967; Walker *et al.*, 1982; West *et al.*, 1986). (ii) disturbance in biochemical processes of photosynthesis (Downton and Loveys, 1981; Longstreth *et al.*, 1984; Seemann and Critcheley, 1985). Salinity decreased stomatal conductance of spinach

leaves much more than photosynthesis (Robinson *et al.*, 1983). Parallel response of photosynthesis and transpiration to salinity associated with unchanged intercellular CO_2 pressure (Yeo *et al.*, 1995). Thus suggest a similar quantitative response of stomata and biochemical capacity of chloroplasts although the two are unrelated. When turgor is maintained, photosynthesis was found more sensitive than stomatal conductance (Lloyd *et al.*, 1987).

He and Cramer (1996) studied on *B. carinata* and *B. napus* and reported that more reduction in photosynthesis of salt sensitive species *B. carinata* at 8.0 dSm^{-1} salinity level, corresponding to the reduction in growth as compared to salt tolerant species i.e. *B. napus*. It is possible that in sensitive species, *Citrus sinensis* and *Phaseolus vulgaris*, biochemical process may be inhibited under salinity due to direct affect on CO_2 assimilation and transpiration. Plaut *et al.* (1989) observed that decrease in transpiration was more than the decrease in net assimilation and the inhibition of net CO_2 assimilation at this stage was attributed partly to a specific sodium ion effect and partly to low plant water status. A linear relationship between leaf sodium content and net photosynthesis was also evident at this stage. Brugnoli and Bjorkmann (1992) reported that salinity affect stomatal conductance more than the carbon dioxide uptake. Seeman and Critcheley (1985) observed that *Phaseolus vulgaris* the photosynthetic rate was reduce with increasing leaf chloride concentration which may be a consequence of the reduction in photosynthetic apparatus. Salinity can affect photosynthesis at the stomatal/mesophyll level, depending on types of salinity, duration of treatment species and plant age (Kleinkof *et al.*, 1976). However, there are many other factors which may also contribute to

this difference in relation to growth stage. Photosynthetic tolerance to salinity may be due to the adjustment of the photosynthetic machinery to salinity at the early growth stage (Plaut *et al.*, 1989). Photosynthetic rate of the plants treated with sodium salts decreased with time, a response also reported by Munns *et al.* (1988). Salinity generally reduces stomatal aperture, which interferes with CO_2 diffusion, thus reducing photosynthesis whenever CO_2 is a limiting fact, i.e. under high light intensity and unimpaired light reactions and biochemical pathway of photosynthesis. Stomata often partially close in plants exposed to salinity even when they have fully adjusted the internal osmotic potential and turgor is high. When a sensitive plant species is exposed to high salinity then the photosynthesis is disrupted even more. This disruption is initially manifested by leaf chlorosis and then by increases and reduction in photosynthesis per unit leaf area (Awada *et al.*, 1995).

Plants subjected to salt stress have reduced water availability (osmotic effects) and respond to changes in the processes related to maintenance of favourable water balance. Increased salinity resulted in decreased transpiration rates and increased leaf differences resistance (LDR) in mustard plant (Qadar, 1994). Higher LDR coupled with low transpiration rate might be contributing to moisture conservation in plants. It was further found that LDR was higher in salt tolerant varities Kranti and Varuna than salt sensitive CS 40 and CS 60 but reverse was true for transpiration rate.

Stomatal frequency decreased in the tolerant genotypes of mustard but did not under go much change in sensitive genotypes with increasing salinity levels (Kumar, 1984). Stomatal pore width decreased slightly with

rise in salinity but no marked differences were discernible in tolerant and sensitive genotypes. On the contrary, leaf succulence increased in response to salinity and the increase was twice at higher salinity levels in tolerant varities as compared to sensitive ones.

2.1.3 Biochemical parameters

2.1.3.1 Chlorophyll and carotenoid content: Increases as well as decreases in content of chlorophyll in different species under salinity have been reported by various investigators. According to several workers, salinity decreases the chlorophyll content of plants (Garg and Garg 1980; Garg and Lahiri, 1986) whereas several other studies reveal that salinization increases the chlorophyll content of plants (Strogonov, 1974). Mishra *et al.*(1995) studied on *B. juncea* cv. Pusa bold in different concentrations of Nacl (0-3%) and reported that chlorophyll and corotenoid content increased at 0.5 and 1.0% NaCl levels, but decreased significantly over control at 3% NaCl. Garg and Lahiri (1986) observed that salinity generally reduced chlorophyll content in number of arid zone crops such as pearl millet, cluster bean, mungbean, wheat and Indian mustard. Recently Sahu *et al.* (1998) working on *Brassica juncea* and *Vigna radiata* in different salinity treatment (0, 0.5 and 1.0% NaCl), reported that chlorophyll content of both plants decreased with 1% NaCl. Delzoppo *et al.* (1999) reported that increasing NaCl concentrations significantly reduced chlorophyll$_a$ and chlorophyll$_a$ and chlorophyll $_b$ in wild wheat.

Cho Jinwoong and Kim Choongsoo (1998) observed decline in chlorophyll content (% of dry weight) with increasing NaCl concentration in barley seedling grown under solution culture, similar decrease in

chlorophyll content with salinity was observed in different genotypes of *Oryza sativa* L. (Ashraf and Yousaf, 1998) and pea plants (Hernandez *et al.*, 1998). Experiments done on leaves of barley seedlings exposed to salt stress revealed that chloroplast degradation and tonoplast functional damage induced by salt stress may be mainly due to the accumulation of active oxygen (Chen Quin *et al.*, 1998).

Zaidi and Singh (1995) found inhibition in the total chlorophyll, chlorophyll$_{a/b}$ ratios with increase in the soil salinity. However, Strogonov *et al.* (1970) reported that amount of xanthophylls increased in white and red cabbage under saline conditions. Chavan and Karadge (1980) found that chlorophyll content decreased under saline conditions in peanut plants. Fadl and El- Deen (1980) found that , Chl$_a$, Chl$_b$ and carotene content were decreased in olive plants grown under saline conditions. Munjal and Goswami (1995) observed that salinity decreased total chlorophyll and carotenoid content in cotyledonary leaves, carotenoid content decreased more slowly than chlorophyll in cotton. Total chlorophyll, Chl$_a$ and Chl $_b$ content decreased under salt stress in *Vigna radiata* L. (Singh *et al.*, 1994) and *Vigna mungo* L. (Ashraf, 1989). Rao and Rao (1981) ascribed the reduction in chlorophyll content to the increased activity of chlorophyllase in pigeon pea leading to the enhanced breakdown of chlorophyll molecules under saline conditions. However, Sudhakar *et al.* (1991) suggested that decrease in chlorophyll content might be partially due to the interference of salt ions with the *de novo* synthesis of proteins, the structural components of chloroplast rather than the breakdown of chlorophyll molecules. Recently, Gadallah (1999) reported reduction in chlorophyll contents with increased soil salinity.

2.1.3.2 Carbohydrate metabolism: Generally carbohydrate metabolism in plants is found to be affected by salinity as well as by type of salt ions contributing to the salinity (Polonenko *et al.*, 1983). Salinity affects the carbohydrate metabolism either by decreasing or increasing the level of soluble sugars. An increase in the soluble sugar contents were observed in *Phaseolus vulgaris* (Petotino and Leone, 1980), *Hordeum vulgare* (Delane *et al.*, 1982) and cotton (Hendrix and Huber, 1986) under salt stress conditions. Sucrose, a product of photosynthesis and the main form in which carbon is translocated out of the leaves, is known to be instrumental in regulating photosynthesis by feed back inhibition in event of its accumulation in leaves. A marked increase in the sucrose content and decrease in reducing sugar were observed in wheat leaves under NaCl salinity (Weimberg, 1987).

An increase in soluble sugars has been correlated with the salt tolerance in many plants (Rathert, 1983). Mishra and Dwivedi (1995) observed an increase in soluble sugars with increase in salinity in chickpea. Binzel *et al.* (1987) also observed an increase in total soluble sugars in tobacco cells adopted to NaCl salinity and inferred that organic solutes may also take contribution significantly in osmotic adjustment. Katsuhara *et al.* (1997) observed that within one hour of initiation of salt stress level of glucose -6- phosphate and UDP glucose decreased markedly and suggested that such decrease might lead directly or indirectly to death of barley root cells under salinity. Rao *et al.* (1980) observed that NaCl salinization caused a decrease in reducing sugars and starch content in seedling axis of *Phaseolus radiata*. Similarly, Sharma *et al.* (1990) found that content of starch and total soluble carbohydrates decreased with increasing salinity in the leaves of chickpea. Cayuela *et al.* (1996) reported

an increase in sugars content in tomato leaves under salinity. Recently, Kuznetsov and Shevyakova (1997) observed increased level of soluble sugars and decreased level of starch in tobacco cells under saline conditions. Handia and EL-Komy (1997) claimed increase in soluble carbohydrates in *Triticum aestivum* L. under varying salinity. Gadallah (1999) claimed reduction in soluble sugars content with increasing soil salinity in wheat.

2.1.3.3Proline and other free amino acids: Metabolic alterations in Indian mustard and related species have been studied with view to under stand physiological and biochemical basis of salt tolerance (Garg *et al.*, 1997). In mustard also accumulation of free proline under salinity has been proposed as a selection criterion in studies conducted on the whole plant (Kumar, 1984; Alia *et al;* 1993; Das *et al.*, 1994) and in somaclones of *B. juncea* (Chandler and Thorpe, 1987; Jain *et al.*, 1991; Madan *et al.* 1994). However, an inverse relationship between salt tolerance and proline accumulation in the salt tolerant *B. napus* strain has also been reported (Borodina, 1991) The most commonly observed effect of salinity on amino acids metabolism is free proline accumulation both in glycoplytes and halophytes (Greenway and Munns, 1980, Levitt, 1980; Heuer, 1994). Under stress conditions proline accumulation is common. Garg *et al.* (1993) observed detrimental effects of salt stress on soluble protein and starch levels in Indian mustard besides increases in free proline and amino acids. Salinity induced changes in the levels of leaf metabolites, such as soluble protein, free proline, total chlorophyll and reducing sugars were consistently less in to learnt genotypes, compared with sensitive genotype (Garg *et al.*, 1997). Salinity induced proline accumulation in *Vigna unguiculata* (Somal and Yapa, 1998). Proline increased with increasing NaCl concentration in

Brassica campestris cv. B-9 and B-54 seeds when exposed to 0-100 mM NaCl (Das *et al.*, 1994). In *Brassica juncea* cv. DIRA 367, increased proline content in cotyledons with increasing level of NaCl in the medium had been reported (Alia *et al.*, 1993). It is suggested that proline accumulation has adaptive significance as its lowers the generation of free radicals and thus, reduces lipid peroxidation linked membrane deterioration under salt stress. Levels of sugars, free amino acids, and proline increased with the level of adaptation. Proline accumulation was correlated with osmotic potential both in the presence or absence of osmotic stress, suggesting that proline accumulation does not initiate salinity adaptation but may it self be triggered to occur as a result of the initiation of other responses to salinity stress (Hasegawa *et al.*, 1986). Many studies have indicated that adaptive role of proline is related to survival rather than maintenance of growth (Green and Munns, 1980). Recently Heuer and Nadler (1998) reported that proline accumulation may help plants adjust osmotically a positive turgor was maintained throughout the entire growth period even though leaf water and osmotic potential declined significantly as stress conditions intensified.

Stress induced protein degradation may be essential in providing amino acids for synthesis of new proteins suited for growth or survival under the modified conditions and also substrates for energy metabolism (Raymond *et al.*, 1994).

Increased accumulation of amino acids in stressed plant could be caused by : (i) Protein degradation (Becker and Fock, 1986), (ii) inhibition of protein synthesis (Dhindsa and Cleland, 1975), (iii) decrease in amino acids and amide export (Tully *et al.*, 1979) and (iv) growth inhibition of leaves (Davies and Van Volkenburgh, 1983).

Salt treatment of crop species increased activity of the proline biosynthetic enzyme proline-5-carboxylate reductase and decreased activity of proline oxidase (Ashraf, 1994). Proline can also stabilize membranes (Mansour, 1998; Gadallah, 1999). Mansour (1998) provides direct evidence that proline can protect cell membrane against salt injury. NaCl induced cellular aberrations in onion epidermis, resulting from cell membrane disruption, were mitigated by proline application. A decrease in shoot Cl^- and Na^+ accumulation and thus enhanced growth in saline environment in response to exogemous proline application was proposed to the effect of proline of membrane stabilization (Lone *et al.*, 1987).

2.1.4 Enzymatic studies

There are three main steps in nitrogen metabolism of plants, as listed below : (i) The concession of inorganic N in to organic N compounds of low molecular weight. This is irreversible step in higher plants. (ii) Synthesis of high molecular weight N compounds from low molecular weight compounds, particularly amino acids. (iii) Breakdown of N containing macromolecules by increased activity of hydrolytic enzymes.

Saline conditions influence all these step in one way or the other and thus have a considerable effect on nitrogen metabolism. Disruption of nitrogen metabolism by salinity has been attributed: (a) decreased nitrogen uptake, (b) decreased nitrate reductase activity, (c) altered amino acids synthesis, (d) impaired protein synthesis and enhanced hydrolysis of proteins, (e) slowed synthesis of nucleic acids and (f) increased activity of hydrolysing enzymes such as RNAase, DNAase , protease and several other leading to degradation of macromolecules.

Plant can take up and metabolize both NO_3^- and NH_4^+ forms of nitrogen but salinity is reported to decrease the nitrate uptake itself. A number of laboratory and green house studies have shown that salinity reduced nitrogen accumulation in plants (Garg *et al.*, 1990; Grattan and Grieve, 1994). Aslam *et al.* (1984) reported that Cl^- inhibited NO_3^- uptake more than SO_4^{2-} in barley, when these anions were present on equal osmolarity basis. Gorham *et al.* (1986) observed drastic reductions of leaf NO_3^- concentrations in response to salinity. Many other studies have indicate similar effects of Cl^- accumulation on NO_3^- uptake (Feigin, 1985; Grattan and Grieve, 1994).

Salinity causes an increase or a decrease in the activity of enzymes, depending on the nature of the enzymes, the plant part studied, and the genotypes of plant species differing in salt tolerance (Dubey, 1994). Nitrate reductase enzyme, which is involved in the reduction of nitrate to nitrite is known to be highly sensitive to all types of stresses, particularly salt and osmotic stresses. Nitrate reductase activity (NRA) is generally reported to be inhibited by salinity in number of crop plants such as wheat, pearl millet, sorghum, mustard, pea, cluster bean and tomato (Plaut, 1974; Garg *et al.*, 1990; 1993; Lahiri *et al.*, 1996) as well as in several halophytes (Heimer, 1973; Austenfeld, 1974). However, stimulation of NRA, particularly under low salinity levels has also been reported by Aslam *et al.* (1984). The reduction in NRA has been attributed to the inhibition of NO_3^- uptake by Cl^- in many investigations. At iso-osmotic concentrations of salts, suppression of NRA was greater with NaCl than with Na_2SO_4. Garg *et al.* (1983) compared the effects of different salts at equal conductivity (10 dSm^{-1}) in wheat at various growth stages. The general inhibition of NRA by all salts and particularly by Na_2CO_3 and $NaHO_3$ at the advanced stages, suggested the

possibility of performance alteration through decreased nitrogen metabolism. Likewise a progressive decline in NRA with increasing salinity points towards the disturbed at nitrogen metabolism in a number of other studies (Lahiri *et al.*, 1987; Garg *et al.*, 1993). Gouja *et al.* (1994) found that the greater sensitivity of nitrate reductase activity to NaCl in bean leaves compared to cotton leaves was due to a decreased compartmentalization of ions rather than due to a difference in salt tolerance of enzyme itself. Lahiri *et al.* (1996) found significant varietal differences in the reduction in NR activity in sensitive genotypes have been attributed to higher uptake of Na^+ and Cl^- in the shoot and lower K^+/N^+a ratios.

Ammonia either directly absorbed by plant roots or as a result of reduction of NO_3^- is further assimilated and incorporated into the amide group of glutamine by the action of the enzyme glutamine synthetase (GS) and subsequently in to glutamic acid by glutamate synthase (GOGAT). Salinity has been reported to effect GS/GOGAT pathway of ammonia assimilation and leads to either decrease or increase in their activities depending up on the plant species as well as the tissues studied (Dubey *et al.*, 1991; Garg *et al.*, 1993; Singh and Dubey, 1994). Total activity as well as specific activity of GS and GDH (glutamate dehydrogenase) were inhibited in roots of pea (Salt sensitive) while in corn (more resistant) inhibition was recorded at the stage of glutamic acid synthesis. Huber and Sankhla (1973) reported an inhibitory effect on growth and GDH activity of pearl millet seedlings by 0.5 per cent NaCl. Lahiri *et al.* (1996) found a general decline in GS activity of clustrbean genotypes at 10 dSm^{-1} soil salinity but the decrease was significantly more in sensitive as compared to tolerant genotypes. Singh and Dubey (1994) also found that high GS

activity in salt stressed seedlings in associated with salt tolerance of rice genotypes.

2.1.5 Mineral composition

Salinity stress is generally recognized as detrimental to plants by disturbing the electrolyte balance resulting in the deficiency of some essential elements and in excess of certain unwanted toxic ions in plant tissues. Changes in growth of plants under Salinity appears to be associated with reduced absorption of essential nutrient elements or high electrolyte levels contributing towards osmotic adjustment and salt tolerance and/or ion toxicity (Green and Munns, 1980; Levitt, 1980; Morgan, 1984). Salinity tended to affect the absorption, mineral composition, accumulation and balance of different major nutrients in the different organ of plant (Lal and Bhardwaj, 1987).

Salinity induced alternation in ionic composition in plant tissues in very well documented in the literature (Alfocea *et al.*, 1993; Chaudhary *et al.*, 1997; Leidi and Saiz, 1997). Reduction in growth and yield of crop plants may result either due to excess of salt ions or due to deficiency of essential nutrients. In saline environments, salt-ions competitively reduced the uptake of nutrients thus plants face situation of deprivation of essential nutrients. Salinity reduces N accumulation in plants (Feigin, 1985; Garg *et al.*, 1990, 1993). Gorham *et al.* (1986) observed that despite drastic reductions in leaf NO_3^- concentrations in response to salinity other nitrogen containing fractions either increased or only marginally reduced.

It was reported by Singh *et al.* (1979) that with increasing salinity, Na^+ content increased but that of Ca^{2+} and K^+ decreased significantly in the shoot of *Brassica juncea* L. In agreement, He and Cramer (1993b) noticed

that Na^+, mg^{+} and Cl^- concentrations increased whole that of K^+ and Ca^{2+} decreased in the shoot of Brassica species under saline conditions. Similarly, Ashraf and McNeilly (1990) observed that Na^+ and Cl^- content increased but K^+ content decreased in *Brassica campestris*, *B. juncea* and *B.napus* under NaCl stress. Under salt stress with NaCl as predominant salt, increased of Na^+ and Cl^- and reduced concentration of K^+ have been observed in wheat (Sharma *et al.*, 1994) and chickpea and lentil (Mamo *et al.*, 1996).

Sharma and Gill (1994) suggested an increase in Na^+ and decrease in K^+ concentration in the whole plant as well as leaves of salt stressed plant of *Brassica juncea*. Increase in accumulation of Na^+, Cl^- and Ca^{2+}, while decrease in K^+ and Mg^{2+} accumulation was found in leaves of Canola grown under saline conditions (Francois, 1994).

Huang and Redmann (1995) also reported an increase in Na^+ and Mg^{2+} but decrease in Ca^{2+} and K^+ concentration in the roots and shoots of Canola and wild mustard under saline condition. In conformity, Porcelli *et al.* (1995) observed that salinity caused remarkable increase in Na^+ but decrease in K^+ and Ca^{2+} concentration and consequently lowered the K^+ and Ca^{2+}/Na^+ ratio in different organs of *B. napus*.

Khan and Ashraf (1988) claimed that NaCl stress resulted a decline in K^+ and Ca^{2+} concentration but increase in Mg^{2+} concentration in sorghum. In agreement, Yaseen *et al.* (1987) noticed NaCl-caused reduction in K^+ and Ca^{2+} concentration in the leaves and roots of barley.

NaCl stress has been shown to have an inimical effect on the phosphorus absorption in sesbania acculenta (Karadge and Chavan, 1983). on the contrary, Asana and Kale (1965) observed in an increase in the uptake

of phosphorus in wheat plant even in the presence of high NaCl and $CaCl_2$. Soliman (1988) found no change in phosphorus uptake under NaCl Salinity in wheat as well as maize.

Lal and Bhardwaj (1987) claimed that under saline conditions, their was a decrease in content of total N, K^+ and Mg^{2+} while the content of P, Ca^{2+}, Na^+ and Cl^- was remarkably higher over controls. However, Rabie and Kumazawa (1988) suggested that salinity usually increased the concentration of K^+, Ca^{2+} and Mg^{2+} in the leaves but decreased in the root and stem in soybean.

Hamdia and EL-Komy (1997) observed in *Zea mays* that the total N-yield was decreased at high NaCl concentration. Zaidi and Singh (1996) found that salt stress progressively enhanced the Na^+ contents were decreased, resulting in increased Na^+/K^+ and Na^+/Ca^{2+} ratios and ultimately reduced root and leaf growth in soybean.

Garg *et al.* (1982, 1993) reported decreased phosphorus uptake by increasing salinity in wheat and mustard although P concentration was marginally affected.

Dravid and Goswami (1986) and Sharma and Manchanda (1997) reported that increasing salinity reduced the uptake of P in wheat, maize and cotton. K^+ concentration in plant tissues is reduces as the Na^+ salinity or the Na^+/Ca^{2+} ratio in the root media is increased (Subharao *et al.*, 1990; Lahiri *et al.*1998). Reduction in K^+ uptake in plants by Na^+ is a competitive process and occurs regardless of whether the solution is dominated by Na^+ salts of Cl^- or SO_4^{2-}. At low concentrations, Na^+ may actually increase K^+ uptake, though decreasing it at higher concentrations (Nimbalkar and Joshi, 1975). But in most of the studies Na^+ salt decreased dry matter production

and the leaf content of K^+ as well as its uptake (Garg and Garg, 1980). Under NaCl stress, *B.campestris* seed showed increased Na^+ and Cl^- influx and K^+ efflux (Das *et al.*, 1994). Similarly, accumulation of Na^+ with a corresponding decreasing K^+ uptake in *Phaseolus vulgaris* (Rossi *et al.*, 1997), in wheat and sugarbeet (Li jiattong and Yu Renpei, 1998) has been reported. Salinity increased shoot Na content but had little effect on K content in three chickpea cultivars. The K/Na content and N contents decreased due to salinity (Varshney *et al.*, 1998).

The higher Na^+ concentration in roots than in shoots found annual rye-grass grown with or without salinity may have been result of plant ability to avoid Na toxicity by reducing the transport of Na the shoots where it may disturbing charge balance and cause specific ion toxicity (Cramer *et al.*, 1989).

2.1.6 Yield and yield attributes

Soil salinity decreased vegetative growth more than grain yield (Francois *et al.*, 1986) several workers observed that soil salinity has adverse effect on crop yield affecting metabolic processes (Gill and Dutt, 1982; Noble, 1985; Richards *et al.*, 1986; Naryan and Rao, 1987; Peak *et al.* 1988; Taneja, 1988). Flowering and fruiting formed timely is some, wheat as delayed in others under salt stress (Datta *et al.*, 1987; Flower *et al.*, 1988; Yadav and Yadav, 1998).

The plant height, sliliquae/plant, seed yield/plant and 1000-seed weight also decreased with increase in salinity in *Brassica* species. Salinity did not decrease seeds/siliqua. However, the percentage of shrivelled seeds/ siliqua increased with an increase in the level of salinity , indicating that salinity may exert its influence by impairing the seed filling or the seed information processes (Dhawan *et al.*, 1987). High variability was observed

for secondary branches/plant, pods/plant, 1000-weight , seed yield/plant and seed yield /plot and low for seeds/ pods and primary branches/plant. Salinity increased the primary and secondary branches/plant. Seed bearing pods/plant and seed yield/plot showed high genetic coefficient of variation (Sinha, 1991). In *Pisum sativum*, plant height, number of pods/plant and seed yield/plant decreased with increasing salinity particularly when exposed to chloride salinity (Yadav and Yadav, 1998).

Kumar and Malik (1983) reported 86.9 per cent reduction in seed yield of Indian mustard following irrigation with saline water of 12 dSm^{-1} during a low rainfall year (185.5 mm) of 1979-80 against only 48.5 per cent yield reduction during the good rainfall year (969 mm) of 1978-79. Similarly, on a 5 year basis (1979-80 to 1983-84) percentage reductions in mean dry weight and seed yield of Indian mustard were noted to the extent of 51.1 and 67.2 per cent respectively, following irrigation with saline water of 16 dSm^{-1} over non- saline water (Chauhan *et al.*, 1988). Sharma *et al.*(1990) found that threshold values of soil salinity (ECe) were 4, 6 and 7 dSm^{-1} for wheat, mustard and barley crops, respectively. The relationship also indicated the yield decrease per unit increase in ECe for different crops and yield benefit to be expected form a salinity control programme work carried out at CCSRI, Karnal has shown that economic yield of Indian mustard can be obtained up to pH 9.2 and ECe 6.5 dSm^{-1} and pusa Bold, Varma and Kranti are the most suitable genotypes for saline conditions (Mishra, 1994).

Available reports indicate that rapeseed and mustard are highly susceptible to saline water during germation and early seedling growth (Kumar, 1984; Kumar and Kumar, 1990; Minhas *et al.* 1990; He and Cramer,

1993a). Saline water of even 8 dSm^{-1} proved harmful by causing significant reduction in seed yield when applied at the pre-sowing stage. Similarly Minhas *et al.* (1990) found that when saline water (12.3 dSm^{-1}), was substituted by non-saline water (0.3 dSm^{-1}), the maximum improvement in yield was due to substitution at pre-sowing (32%) followed by substitution at siliquae development (10%) compared to when saline water was applied throughout the cropping period.

Investigations conducted at CSSRI, Karnal, have shown that in mustard maximum yield loss occurred with exposure to salinity at the germination stage (Qadar, 1994). Variety Kranti showed 82 per cent reduction in seed yield at much lower EC (7.5 dSm^{-1}) when irrigated with saline water from germination to maturity compared to 85 per cent reduction at EC 10.5 dSm^{-1} at stem elongation and 86 per cent at EC 15.5 dSm^{-1} at siliquae filling stage. Noureldin *et al.* (1995) observed that different levels of salinity (EC 7.76, 10.13 or 22.4 dSm^{-1}) significantly decreased seed yield in rape seed varities. Thakral *et al.* (1996) working on *Brassica carinata* cultivars, reported that seed yield, specific leaf area, 1000- seed weight, siliquae/ plant and oil content decreased under the influence of salinity (125 meq/ litre chloride saline solution). Sharma and Gill (1994) reported that seed yield of different varities of raya did not decrease significantly at ECe 7.9 dSm^{-1} and decreased to 50 per cent at ECe 12.6 dSm^{-1}. Sharma and Manchanda (1997) worked on 'RH 30', yellow Sarson and 'sangam' toria and they found that increasing salinity levels (above 8.0 dSm^{-1}) decreased the seed yield in both raya and toria.

2.1.7 Quality test

2.1.6.1 Fatty acids composition: Oil content and quality are as important as seed yield in mustard. However, information on the impact of salinity on content and quality of oil is meagre. Kumar and Kumar (1985) observed that oil content decreased with increasing salinity in six cultivars of Indian mustard. A negative correlation existed between protein and oil content. T-59 was inferior to RL-18 in oil and protein contents but possessed higher iodine values. Kumar and Kumar (1985) found that seed yield, oil content and oil yield per plant increased up to 12 dSm^{-1} in 10 varities of Indian mustard but decreased at higher salinity level (16 dSm^{-1}). Among all the varities cv. T-59 was better than others in respect of seed and oil yield per plant. Sharma and Manchanda (1997) studied on 'RH 30' yellow sarson and 'Sangam' toria and they found that increasing salinity levels (above 8.0 dSm^{-1}) decreased oil content in both raya and toria.

Thakral *et al.* (1996) working on *Brassica carinata* cultvars and reported that oil content decreased under the influence of salinity (125 meq/litre chloride saline solution). Membranes are affected by changes in ionic environment. Increased permeability of membranes as evidenced by electrolyte leakage is reported in cowpea (Kurban *et al.*, 1998). In sugarbeet, salinity induced changes in the composition of fatty acids occur in plasma membrane (Yahya, 1998). Modification of lamellar organisation of chloroplasts by salts has been reported (Izawa and Good, 1966) Relations between salt transport, salt tolerance and lipids were observed (Kuiper, 1968 a,b).

Indian mustard which is an oilseed crop deleterious effects of salinity on lipids is understandably undesirable. Although decrease in oil content with salinity has been reported by many workers. However, quality aspects

of mustard oil in relation to salinity has received scant attention. Yellow sarson (*Brassica rapa*) showed marked decrease in oil content with salinity (Sharma and Manchanda, 1997). Salinity decreased the percentage of oil but increased iodine value of rape and linseed (Deo and Ruhal, 1971) and sunflower (Raju and Ranganayakulu, 1978). In case of sunflower, salinity decreased the oil content but the quality of the oil remained unaffected. The oil extractability became poor although fatty acid composition was not changed.

Mustard seeds contain fatty acids viz. palmitic, stearic, oleic, linoleic, linolenic, eicosenoic and erucic acids. Erucic acid is the characteristic fatty acid of mustard (Ahuja *et al.*, 1987; Gupta *et al.*, 1991; Gaur and Gupta, 1994). Recently gene coding for enzyme responsible for elongation of fatty acid chain length from C_{18} to C_{20} and C_{22} has been cloned and characterized. It expresses strongly only in the developing seed and pod wall (Venkateswari *et al.*, 1999). In mustard seed chain elongation start from palmitic acid (Downey, 1987), thereby producing a range of C_{18}, C_{20}, and C_{22} acids. With the advance of maturity, C_{22} acid viz., erucic acid increases but palmitic, oleic, linoleic and linolenic acids decrease (Gurr *et al.*, 1972; Stumpf , 1989; Gupta *et al.*, 1995).

Oil synthesis in developing seeds of *B. campestris*, *B. napus* and *B. juncea* was adversely affected by moisture stress and salinity. Stress imposed throughout the growth period or before flowering was more detrimental to lipid deposition in *B.napus* seeds than when imposed after flowering. Both moisture stress and salinity increased erucic acid content and decreased oleic acid and linoleic acid content in seeds of both species (Munshi *et al.*, 1986; Subramanian and Maheswari, 1991). However, Sukhija

et al. (1983) reported that under stress conditions, polyunsaturated fatty acids generally increase at these are related to membrane lipids which might have been altered to maintain the integrity of the cell. In yet another adaptive measure of the cell, polar lipids which are membrane lipids (Demel, 1987) general increase under the stress as the cells restore polarity by increasing synthesis of polar lipids (Gaur and Gupta, 1994).

Gupta (1982) observed that salt and moisture stress reduced oil filling by affecting the rate of photosynthesis in the leaves, transport of metabolites from the leaves to the seeds and synthesis of fatty acids at the peak period of oil filling.

2.1.6.2 Glucosinolate: High levels of glucosinolate hydrolysis products can adversely affect the feeding value of rapseed meal. Studies have shown that glucosinolates are derived from amino acids and have a common biosynthetic pathway (Fenwick *et al.*, 1983). While pod tissues appear to be the main site of synthesis of glucosinolate accumulated in the seed, some glucosinolate may be synthesized in other parts of the plant and transported to the seed (De March *et al.*, 1989).

Seed glucosinolate content can vary greatly in rape seed, depending on growing conditions and agronomic practices, with factors such as nutrition, sowing time and water regime implicated (Fenwick *et al.*, 1983). Higher seed glucosinolate contents have been associated with water stress (Mailer and Cornish, 1987), high temperature during seed growth and disease (Salisbury *et al.*, 1987).

Mailer and Cornish (1987) working on *Brassica napus* and *Brassica rapa* under water stress and found that water stress increased glucosinolate concentrations in both cultivars whether the stress was applied throughout

or only after flowering. Seeds from plants which were stressed throughout had higher concentrations of glucosinolates than those stressed after flowering.

Robbelen and Thies (1980) observed that glucosinolates accumulated in the seeds of rape progressively during their development, to reach a maximum at maturity. The water stress applied after flowering also had similar effects in the oil content as did stress applied throughout. It appears that conditions prevailing during the synthesis of seed material determines their oil and glucosinolate content.

2.2 Effect of different sources of nitrogen fertilizer under saline conditions

To alleviate the adverse effects of salinity application of different sources of nitrogen fertilizers have been employed in various crop species. Some encouraging information have been obtained so far with respect to alleviation of salinity by means of nitrogen fertilizers.

2.2.1 Growth and development

Higher plants can use either nitrate or ammonium as a source of nitrogen. However, the degree of effectiveness of these two forms of nitrogen of growth and nutrient uptake is dependent on plant type and ammonium and nitrate ratio in the nutrient medium. Contrating observations have been made with respect to preference of *Brassica* species to different source of nitrogen.

In an experiment conducted with varying soil pH, the dry matter increase of rapeseed (*B. campestris)* with nitrate nitrogen compared to ammonium nitrogen was similar on four acid soil (pH 4.6 to 5.6) and two near central soil (pH 6.4 and 6.5) reported by Malhi *et al.* (1988).

contrastingly, supply of ammonium and nitrate nitrogen at concentration of 77 ppm each brought remarkable decreasing growth of cabbage, Melon and corn. Bean seemed to be well adapted to the use of ammonia and unaffected by equal concentration of ammonium and nitrate nitrogen (Errebhi and Wilcox, 1990).

Hetch and Mohr (1990) observed a strong interaction between NO_3^- and NH_4^+ during the process of accumulation of inorganic nitrogen in *Sinapsis alpa*. Results of the experiment indicated that incorporation of inorganic nitrogen in to organic nitrogen is stimulated when both NO_3^- and NH_4^+ were supplied. Jasiewicz (1990) observed that among three forms of nitrogen fertilizer applied to rape grown in pots, ammonium sulphate yielded more dry matter as compared to sodium nitrate or ammonium nitrate. When 0.3 gm/pot was applied in 3 forms yield of above ground parts was highest with ammonium sulphate and slightly lower with sodium nitrate than with ammonium nitrate.

Uziak *et al.* (1991) reported that dry matter yield was 2-3 times higher in plants given only NO_3^- than only NH_4^+. The highest and lowest concentrations of total and protein N in both rape and maize occurred in plants given only NH_4^+ and NO_3^-, respectively. Rape plants contained lower concentrations of cations and higher concentration of anions when given NH_4^+ than when given NO_3^- whereas ionic balance was little affected by N source. Organic anion concentration in rape increased as the proportion of NO_3^- in the nutrient solution increased.

Reduction in plant growth under salinity is attributed to decrease many metabolic activities. However, supplementation of nitrogen to plant has been acceded in delaying senescence. Nitrogen being requisite for plant growth

and development is required either in form of nitrate or ammonium (Maduff and Jackson, 1991). Thus nitrogen metabolism impairment must be one of primary determinant for growth of the plants under saline conditions. Studies on nitrogen metabolism in mustard under salinity is scanty (Sharma *et al.*, 1990). Salinity induced reduction in growth, due to decrease in critical water level, ionic imbalance (Flower *et al.*, 1988) and seedling vigour as well as metabolites in root and leaf tissues, may be modulated by nitrate or ammonium supply either by replenishing the ions or through increased the metabolites (Mishra *et al.*, 1996).

Garg *et al.* (1982) reported that fertilizer induced improvement of growth of wheat under salinity stress, was associated with an increase in the concentration of nitrogen, phosphorus and potassium and a decrease in the level of chloride in the tissue.

Khan *et al.* (1994) studied on alfalfa (*Medica sativa* L.) and reported that leaf area in plants declined significantly with increasing salinity levels. The magnitude of reduction was highest at 100 mM (more than 50% over control) and lowest at 30 mM level of NaCl. By contrast, N-fertilization brought about an enhencment in control as well as in salinized plants.

Mishra *et al.* (1996) working on *Brassica juncea* cultivars in different levels of salinity (20 and 200 mM NaCl) and without nitrogen or with 10 mM nitrate or ammonium. They found that seedling length and dry weight of both cultivars were highest at 20 mM NaCl and lowest at 200 mM NaCl. Seedling growth was increased by nitrogen and dry weight was generally higher with nitrate than ammonium.

2.2.2 Water relations and gas exchange

Many natural and agricultural saline areas of the world are also N-deficient limited N-availability during plant growth causes both a reduction in the absolute levels of components of the photosynthetic apparatus and changes in the relative ratio. Salinity stress imposes additional energy requirements on plant cells (e.g., for osmotic synthesis ion extrusion). Moreover, salinity stress also resulting the diversion of metabolic carbon to storage pool (Cheesman 1988). The rationship between photosynthesis and N has been widly studied because of the importance of photosynthesis to plant productivity and the status of N as a limiting element (Chapin, 1980; Nova and Loomis, 1981). The photosynthetic capacity may be either directly through stomatal closures or as a consequence of non-stomatal inhibition of photosynthesis (Kleinkopf *et al.*, 1976; Downton, 1977). Photosynthetic rate declined when plants were subjected to increasing levels of NaCl. On the other hand, N-nutrition brought about a considerable moderation in salt induced inhibition of the photosynthetic rate (Seeman and Sharkey, 1986).

Khan *et al.*(1994) studied the interaction between salinity and nitrogen (N) forms and concentration in alfalfa, reported that salinity (0-100 mM NaCl) caused a substantial reduction in carbon assimilation rate, stomatal conductance, water use efficiency and transpiration rate also decreased. Salinity effects were considerably moderated by additional nitrogen supply, varied with form, concentration, and stage of plant growth. The decreasing trend by salinity was consistent when plants were treated with either N forms (ammonium N or as nitrate N in three concentrations i.e. 3 and 6 mM). Between the two N forms, transpiration rate in plants was

increased more by ammonium N than nitrate N. Moreover, the transpiration rate in plant was varied with concentrations as nitrate N was effective at both concentrations (3 and 6mM) while ammonium N form proved deleterious at higher concentrations. The photosynthesis was reduced more in ammonium than in nitrated fed plants. The promotive effect of N on photosynthesis and other parameters in saline as well as in non-saline conditions may be attributed to the enhanced synthesis and availability of carbon assimilatory enzymes and cofactors required for optimal photosynthesis.

According to Hsio and Lauchli (1986), the deficiency of potassium (K) as well as other nutrients can reduce transpiration by affecting stomatal closure. Moreover, plants showed a relatively higher transpiration rate under ammonium nitrogen which can be related to the depletions of other cations by the presence of an excessive ammonium (NH_4^+) ion concentration (Khan *et al.*, 1994).

Neumann *et al.* (1988) reported that salinity affects growth rate of Bean leaves by changes in turgor. One may also speculate that leaf expansion under saline conditions may be retrieved by nitrogen fertilization. A steady supply of N to peanut plants inhibited the reduction in growth caused by NaCl, since the uptake of N by the plant was renewed which allowed for the full expansion of leaves as well as the production of new ones (Silberbush and Lips, 1988).

It has been reported that under decreased N concentration, photosynthetic activity is reduced (Powell and Ryle, 1978). While under increased nitrogen levels, photosynthesis activity is enhanced (Fair *et al.*, 1971; Nichiporovich and Slobsdoskava, 1972). Nitrogen is a major

constituent of enzymes responsible for photosynthesis carbon reduction and the components of the photosynthesis including chlorophyll which generate ATP and NADPH (Edword and Walker, 1983). Thus a low nitrogen supply results in suppressed photosynthetic rate and lowering of the carbohydrate supply for growth. In fact the effect of N nutrition on photosynthesis reflects both N availability as well as the partitioning of N in the plants (Khan *et al.*, 1994).

2.2.3 Biochemical parameters

Mishra *et al.* (1996) working on *Brassica juncea* cultivars under two salinity levels (20 and 200 mM NaCl) in the presence of nitrate or ammonium. They found that higher salinity level (200 mM NaCl) reduced seed germination, seedling growth, total chlorophyll, carotenoids, chlorophyll stability index, nitrogen metabolites i.e. protein and nitrate reductase activity (NRA) in leaves and root tissues. Supplementation of nitrate or ammonium (10 mM), induced the growth further salinity caused growth inhibition to some extent. The nitrogen allocation in seedling growth under salinity was attributed to increase in the level of some nitrogen metabolites in leaf and root tissue. Garg *et al.* (1982) also reported that under both normal and saline conditions, nutritional improvement lead to higher chlorophyll concentration and increased efficiency of enzyme nitrate reductase in the leaves.

Nitrogen supplementation enhanced the pigment level under salinity stress. Alleviation of carotenoid level by either from nitrogen indicated structural repairment of chloroplast which might be deformed under salinity (Neiman and Paulson, 1971). Further more enhanced chlorophyll stability index shows the role of nitrogen in enhancing the stability of pigment may

be by reverting degradation under salinity (Mishra *et al.*, 1996).

2.2.4 Enzymatic studies

A substantial number of laboratory and green house studies have shown that salinity reduced nitrogen accumulation in plants (Feigin, 1985; Garg *et al.*, 1990, 1993). This is due to the fact that an increase in chloride uptake and accumulation is mostly accompanied by a decrease in shoot nitrate concentration (Silverbush and Ben-Asher, 1987). Gorham *et al.* (1986) observed that despite drastic reductions in leaf NO_3^- concentrations in response to salinity, other nitrogen containing fractions either increased or only marginally reduced. Lewis *et al.* (1989) found that NH_4^+ fed maize and wheat plants were more sensitive to salinity than NO_3^- fed plants, grown in solution cultures. Leidi *et al.* (1991) concluded that NO_3^- is a better source than NH_4^+ for wheat grown in salt affected areas.

It is noteworthy that reported data indicate that increased NO_3^- in the substrates decreased Cl^- uptake and accumulation (Feigin *et al.*, 1987; Garg *et al.*, 1990, 199). The decrease in Cl^- concentration has been found to be associated with decrease Na^+ concentration also. The salinity induced decrease in NO_3^- concentration is generally accompanied by a decrease in nitrate reductase (NR) activity which is responsible for NO_3^- reduction. Nitrogen supply to salt stressed wheat plant enhanced NR activity (Garg *et al.*, 1990). Similar results have been obtained with Indian mustard (Sharma *et al.*, 1989; Garg *et al.*, 1993) where improvement in soil fertility significantly enhanced the activity of NR as well as those of ammonia assimilating enzymes such as GS, GOGAT and GDH.

NaCl stress caused significant decline in protein content and nitrate reductase activity under salinity over control in leaf tissues in cultivars of

Brassica juncea. Nitrate and ammonium supplementation to the seedling enhanced the level of protein content and nitrate reductase in leaf as well as root tissues under high salinity (Mishra *et al.*, 1996). The protection of enzyme activity by nitrogen under salinity has been recorded in cultivars of *Brassica campestris* also (Mishra *et al.*, 1994).

Sharma *et al.* (1989) reported significant and positive salinity fertility interactions on the soluble protein content and activities of NR, GS, GOGAT and GDH in mustard plants grown under saline water of different concentrations (0, 50, 100 and 150 meg/l).

Garg *et al.* (1993) studied the impact of improved soil fertility on the enzymes improved soil fertiiity on the enzymes related to N-metabolism on Indian mustard firmly established the importance of a salinity fertility interaction. They reported that a progressively increasing salinity (0, 50, 100 and 150 meq/l) reduced the NR activity under both the IF and LF conditions. But the IF plants generally maintained higher NR activity compared to the LF plants up to the 50 and 100 meq/l treatment at the pre-flowering and flowering stage, respectively. Although the NR activity is highly sensitive to salt stress (Lal and Bhardwaj, 1987) also observed on studies with wheat (Garg *et al.*,1992), suggests that higher N availability in the substrate (arising from of higher N uptake) under the IF condition possibly induced a greater efficiency for this enzyme despite the salt stress up to a certain level of salinity.

Garg *et al.* (1993) studied on Indian mustard and reported that an increase in salinity also led to decreased activities of GS and GOGAT. But these activities were higher at flowering stage. Again, the fertilized plants displayed significantly higher activities for these, enzymes in compared

with unfertilized plants at all levels of salinity except at 150 meq/l treatment of saline water at the flowering stage. Such an increase in activity under salt stress in IF plants was also discernible in case of GDH at both the growth stage. However, a general decline in the activity was noticed up to the 100 meq/l salinity, and a slight increase in the IF was found at the 150 meq/l treatment. This could be a measure for accumulated ammonia under high salt stress when the GS-GOGAT system is less efficient. However, the inhibitory effect of salt stress on the level of activity for these enzymes have also reported by Lal and Bhardwaj (1987).

2.2.5 Mineral composition

As salinity may induce nutritional deficiencies or imbalances, improvement in soil nutritional status through supplemental fertilization has been shown to over come such salt induced adverse effects, particularly in susceptible crops. Garg *et al.* (1993), demonstrated that an improvement in soil fertility could favourably influence the concentration and absolute quantity of NPK and reduce the concentration of Na^+ in the mustard shoot under salinity of irrigation water.

Salt tolerance under saline condition is usually related to ability to regulate Na^+ and Cl^- uptake by plant root and their subsequent translocation to the shoots. contrast, the higher concentration of essential elements (particularly K^+ and Ca^{+2}) in leaf tissues may contribute to the salt tolerance ability of plants (Ashraf and Mc Neilly, 1990; He and Cramer, 1992). The mechanism of selectivity of ion transport appears to confer tolerance of salt stress in Indian mustard. Higher selectivity towards K and restricted uptake of Na^+ gave higher K^+ : Na^+ ratios in tolerant than in susceptible genotypes of Indian mustard (Kumar, 1984; Garg *et al.*, 1997). Similarly,

salt tolerant *B. napus* showed a greater concentration of Ca^{+2} and K^{+} than did salt sensitive *B. carinata* following irrigation with sea water of ECe 8 dSm^{-1} (He and Cramer, 1993c). The role of K^{+} in salt tolerance appears to dependent on uptake by the cell sap and protoplasmic changes leading to increased water retention in stressed plants (Singh and Tripathi, 1979).

It has renewed interest in plant nutrition and N fertilization under saline condition (Feigin, 1985). The form of N nutrition may exert a considerable influence on the mineral completion of plants (Kurvits and Kirkby, 1980; Allen *et al.*, 1985). According to cox and Reisenauer (1973), ammonium increases anion uptake, whereas nitrate increase cation concentration in wheat plants. Change in the pH of the nutrient medium and the type of N supplied, affected ion uptake.

2.2.6 Quality test

2.2.6.1 Fatty acid composition: The quality of *Brassica* seeds in terms of oil, protein content and fatty acid composition varies considerably and is influenced by nutrition, whether conditions during plant growth and at ripening stage and also degree of maturity of seeds at the harvest time. Oil accumulation in seeds crops and is the direct consequences of transformation of carbohydrates. Hence, oil status may get altered by nutritional status of the crop. There is need to increase the world supply of oils and fats. This is important particularly for those countries where the per capita availability of oils and fats is quite low. As the oil is major edible source used by a large population of the world, the fatty acid composition vitally important for its dietary effects. As more crops are grown with heavy application of fertilizer to obtain higher yields, therefore, it is of increasing importance to study possible chemical changes in the composition of oil and fats that may occur following fertilizer treatments.

It has been reported that in sesame oil content with nitrogen application, oleic acid being maximum at the highest NPK application and linoleic acid content being maximum at the intermediate fertilization (Seo *et al.*, 1986). Increasing the fertilizer dose decreased the oil content and increased linolenic and linoleic acids (Zhao *et al.*, 1990). Appelqvist (1968) could find only small effects on the fatty acid composition of rape seed with different levels of nitrogen, phosphorus and potassium dressings.

Szukalska - Golab (1985) working on five varities of winter Swedge rape and reported that higher rate of N application tended to provide higher seed yields while reducing the oil content slightly, but also reduced the erucic acid contents of oil.

Voltan and Mosco (1982) studied on oil seed rape and reported that increased level of nitrogen increased oleic acid linoleic acid and decreased palmitic acid content while linolenic acid content was unaffected and oil content decreased with increase in nitrogen rate.

Gupta and Friend (1975) working on white mustard with N, P and K and reported that all treatments increased percentage of oleic and decreased linolenic acid percentage in the oil, the percentage of palmitic, linolenic and erucic acids changed with different nutrients and their levels.

2.2.6.2 Glucosinolate: Forster (1978) found that increasing nitrogen supply increased seed glucosinolate levels of single low varities in a pot experiments. The field experiments by Bilsborrow *et al.* (1993) clearly demonstrated the effect of N in increasing seed glucosinolate content of double low varieties, where S supply was adequate.

Zhao *et al.* (1993) observed that influence of S and N application on seed yield and quality of a double low (low erucic acid and glucosinolate

content) variety of winter oilseed rape and found that a significant increase in seed glucosinalate content in response to increasing N rate.

Under conditions of sufficient S supply, increasing the N supply enhances the synthesis of amino acids particularly of S containing amino acids which are the precursors for glucosinolate synthesis (Underfaill, 1980). Therefore, given a sufficient supply of sulphate, increasing the N suply can lead to as enhanced synthesis of both protein and glucosinolates.

The specific literature on the interactive effects of salinity and different levels of nitrogen source on quality parameters is lacking.

2.2.7 Yield and yield attributes

Quantitative analysis of effect of nitrogen on growth, development and yield of oilseed rape was reported by Allen and Morgan (1972). They observed that nitrogen application affects increase in yield indirectly through an increase in supply of assimilates to flowers and young pods. Results also indicated that maintenance of large and photosynthetically efficient leaf area during flowering helps in higher yields.

Increased in total nitrogen uptake by *Brassica* species with increase in nitrogen supply has been reported by Vir and Varma (1979). While a strong correlation between nitrogen content and photosynthetic rate has been observed in *Brassica* by Tsunoda (1980). Kaur *et al.* (1992) observed increase in nitrogen content with increased application of nitrogen in *B. campestris*. This increase was attributed to cumulative effect of increase N-uptake and total dry matter as result of applied nitrogen.

The significant increases dry matter production and seed yield of fertilized plants compared to unfertilized plants at all salinity levels reported by Garg *et al.*, (1993). In a number of other reports also increased

dose of fertilizers have been advocated to alleviate the growth inhibition by salinity. Chauhan *et al.* (1988) observed 37 and 80 per cent improvement in seed yield of Indian mustard with 60 and 90 kg N ha^{-1} application, respectively in comparison with 30 kg N ha^{-1} following irrigation with saline water. Yield enhancement of Indian mustard up to 120 kg/ha observed on sodic soils at Karnal further corroborates this phenomenon (Anonymous, 1988).

Field experiments on the combined effects of saline water irrigation and nitrogen levels on the yield, WUE and NUE of Indian mustard indicated that, (a) use of saline water can boost the growth and yield of dry land mustard, and (b) with in certain limits a non-saline water supply can be substituted by applying nitrogen and saline water (Dayal *et al.*, 1995). It was suggested that fertilizer N rates should be adjusted in relation to the supply of water and its predicted salinity.

Garg *et al.* (1982) reported that fertilizer induced improvement of growth and yield of wheat (*Triticum aestivum* L.) under salinity stress, was associated with increase in the concentrations of nitrogen, phosphorus and potassium and decease in the level of chloride in the tissue.

Dayal *et al.* (1995) studied on dryland seeded Indian mustard (*Brassica juncea*) to evaluate the effects of combinations of saline irrigation (EcW 11.0 dSm^{-1}) and nitrogen levels (40, 80 and 120 kg ha^{-1}) on the yield, seasonal water use and nitrogen use efficiencies of mustard. They reported that use of saline water can increased the growth and yield of dryland mustard within certain limits at increasing level of nitrogen fertilizer.

CHAPTER - 3

MATERIALS AND METHODS

The present investigation was carried out on *Brassica juncea* (cv. RH-30) under screen house condition.

Plant material

The seeds of mustard (*Brassica juncea* L.) CV. RH-30 were procured from the Department of Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University, Hisar.

Raising of crop and treatments

The experiment was conducted during October, 1999 and 2000 in the sand culture in screen house of College of Basic Sciences and Humanities, CCS Haryana Agricultural University, Hisar. Mustard crop (cv. RH-30) was raised in cemented pots, each of which was filled with 6 kg of dune sand. Some of the physico-chemical characteristics of experimental dune sand were as follows : (i) Mechanical analysis = sand (93.3%), silt (3.0%), clay (3.7%), (ii) Texture = sand, (iii) Saturation capacity = 25%, (iv) pH = 8.2 (v) ECe = 0.8 dSm^{-1} at 25°C, (vi) $CaCO_3$ = absent, (vii) Organic carbon = 0.06%, (viii) CEC = 2.56 C mol (+) kg^{-1}, (ix) Available nutrients (ppm) = N (10.3), P (2.5), K (18.0), (x) Taxonomic class = Typic torrispamments. The pots were lined with polythene bags to avoid contamination. Before sowing of seeds the pots were supplied nitrogen through different nitrogen sources, Ca $(NO_3)_2$, $(NH_4)_2$ SO_4 and combination [$Ca(NO_3)_2 + (NH_4)_2SO_4$]

having three levels that is 40 mg kg^{-1} dune sand, 80 mg kg^{-1} dune sand and 120 mg kg^{-1} dune sand (which is equivalent to 40, 80 and 120 kg ha^{-1}). Sowing was carried out at field capacity of dune sand. Ten seeds were sown in each pot at uniform depth (1.5-2.0 cm) and distance. After 15 days of sowing thinning was done and three plants of uniform size were maintained in each pot. Each pot was supplied with equal quantity of N free nutrient solution at a regular interval of 15 days. The desired salinity levels (ECe 0, 8 and 12 dSm^{-1}) were obtained by adding Cl and SO_4 salts of Na, Ca and Mg. Non-saline control was kept. For each ECe level, the requisite amount of salts per litre was worked out and added to the pots on soil saturation basis at two stages i.e. stage I and stage II.

Stage I: The salinity solutions were applied at 35 days after sowing and sampling was done at 45 DAS (10 days after saline irrigation).

Stage II: The salinity solutions were applied at 55 DAS and sampling was done at 65 DAS (10 day after saline irrigation).

Sampling

Different observations were recorded at two stages. The plant samples were taken from each treatment at 45 and 65 days after sowing for various physiological and biochemical parameters. At maturity, the plants were sampled for mineral composition, yield attributes and quality characters.

(i) Treatments

Three levels of different sources of nitrogen were supplied in pots.

Nitrogen sources			**Nitrogen levels**
(a)	$Ca(NO_3)_2$	:	40, 80 and 120 kg ha^{-1}
(b)	$NH_4(SO_4)_2$	:	40, 80 and 120 kg ha^{-1}
(c)	Combination	:	40, 80 and 120 kg ha^{-1}

$[Ca(NO_3)_2 + (NH_4)_2SO_4]$

(ii) Salinity level

(a) Control (b) 8 dSm^{-1} (c) 12 dSm^{-1}

(iv) Sampling stages

(a) 45 days after sowing (Pre-flowering stage)

(b) 65 days after sowing (Flowering stage)

(v) Replications : Three

(vi) statistical analysis : Factorial completely randomized design (CRD)

GROWTH PARAMETERS

Plant height and number of branches per plant : Plant height and number of branches per plant were recorded at maturity.

Dry weight of different plant parts, leaf area, AGR, RGR and NAR : Dry weight of different parts (leaf, stem and root) leaf area, AGR, RGR and NAR were studied on plants at 45 and 65 DAS. These parameters were estimated based on the observations taken at a time interval of 10 days.

Leaf area per plant : The total leaf area per plant was measured by portable leaf area meter model (LI-3000 LI-COR, USA) and expressed as cm^2 $plant^{-1}$.

Absolute growth rate (AGR) : AGR is the gain in dry matter production in a unit of time.

$$AGR = \frac{\text{Dry weight}}{\text{Time in days}}$$

Relative growth rate (RGR) : The equation suggested by Fisher (1971) was used to calculated RGR.

$$RGR = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1}$$

$\log_e$ is natural logarithms W_1 and W_2 are dry weight at time t_1 and t_2, respectively. Dry weights were recorded before inducing salinity treatment at 35 and 55 DAS and ten days after salinity treatment i.e. 45 and 65 DAS.

The plants were separated in three parts viz. leaf, stem and root. The separated organs were oven dried at 60°C for 24 h or dry weight of whole plant was calculated by aggregating the dry weight of indidual parts.

Net assimilation rate (NAR) : NAR was Calculated by Williams (1948) formula :

$$NAR = \frac{W_2-W_1}{t_2-t_1} \times \frac{Log_e LA_2-Log_e LA_1}{LA_2-LA_1}$$

LA_1 and LA_2 are the leaf area (cm^2) and W_1 and W_2 are total dry weight of a plant (g) at time interval t_1 and t_2 (days), respectively.

PHYSIOLOGICAL PARAMETERS

Leaf Water Potential

Leaf water potential was determined from the third fully expanded leaf by using pressure chamber (Model -3000 series, Plant Water Status Console, Soil Moisture Equipment Corp; Santa Barbara, California, USA). The values of water potential were expressed in -MPa. This technique was introduced by Scholander *et al.* (1964) using a pressure chamber.

Leaf osmotic potential

Osmotic potential of third fully expanded leaf was measured by freezing and thawing of leaf sample. The sap was extracted by crushing the sample and filter paper disc soaked with sap were used for observing osmotic potential with a model 5100-B vapour pressure osmometer (Wescor, Inc. Logan, Utah, USA). Standard curve was prepared with the help of sodium chloride solution using 0.1, 0.2, 0.3.....1.0 molal. The values of osmotic potential of the leaf sap were recorded with the help of standard curve and expressed in -MPa.

Relative water content (RWC)

Relative water content was determined by weighing the 3rd leaf from top and floating it on water (de-ionized water) for 6 h at constant temperature in diffused light. When leaf became fully turgid, it was re-weighed, and dried and again its dry weight was determined. The RWC was calculated by the following formula (Barrs and Weatherly, 1962).

$$\text{RWC (\%)} = \frac{\text{Fresh weight-Dry weight}}{\text{Fully turgid weight- Dryweight}} \times 100$$

Gas Exchange studies

Gas exchange studies were made at 45 DAS and 65 DAS. These observaitons were made from 10.00 A.M. to 11.30 A.M. The CO_2 exchange rate (CER or A, μ mol m^{-2} s^{-1}), transpiration rate (E, mmol m^{-2} s^{-1}) and stomatal conductance (Sc, m mol $m^{-2}s^{-1}$) were determined separately from upper and lower surface of fully expanded leaf by using Portable - IRGA (PP system model CIRAS-1). Leaf cuvette was 1st clamped on upper surface (in this position PAR sensor faces sun light and was upward) of the leaf and its position was changed in such a way that :

(i) Maximum PAR was obtained .

(ii) Minimum differences in CO_2 and water vapours between reference and analytical cell were achieved.

At this stage exchange switch was pressed and when the reading were stable, the value were recorded. Whole sequences of steps were repeated by inverting the leaf cuvette and clampling the sensor on the lower surface of the leaf. Values of Sc, E and A were added where as for temperature mean was used. By using Pc-interface the print-outs were obtained. At each sampling fully expanded leaves were used for measuring these parameters.

BIOCHEMICAL PARAMETERS

Total soluble carbohydrates

Total soluble carbohydrates were estimated colorimetrically according to the phenol sulphuric acid method (Dubois *et al.*, 1956).

Extraction

The extration of soluble carbohydrates was done according to Barnett and Naylor (1966).

Two hundred mg of fresh samples were finely ground and extracted with 5 ml of 80 per cent ethanol (V/V) on a water bath at 50 ± 1°C for 15 minutes. Cooled and centrifuged at 5000 x g for 20 minutes. The supernatant was kept aside and the pellet re-extracted with 80% ethanol. The total volume was made to 7ml with 80% ethanol. This constituted the total soluble carbohydrates and free amino acids. The residue was used for starch extraction.

Reagents

(i) 2% phenol in distilled water

(ii) Conc. H_2SO_4.

Procedure

Ethanol extract (0.3 ml) was taken and evaporated to dryness. After cooling, residue was dissolved in 0.3 ml distilled water. To 0.3 ml of aqueous aliquot, 1 ml of 2% phenol and 2 ml of conc. H_2SO_4 were added and allowed to stand for 10 minutes. Test tubes were shaken and cooled and then absorbance at 475 nm against a regent blank was recorded.

Standard curve was prepared using graded conc. of glucose (20 to 100 $\mu g\ ml^{-1}$) and data was expressed as mg carbohydrate g^{-1} tissue dry weight.

Starch

Starch was hydrolyzed with $HClO_4$ and sugars were estimated by the method of Dubois *et al.* (1956) as for total carbohydrates.

Extraction

The residue left after ethanol extraction was treated with 5 ml of 0.2 N chilled perchloric acid for starch hydrolysis and kept for overnight and then centrifuged at 5000 x g for 30 minutes. The extraction was repeated once with 0.2 N chilled perchloric acid and centrifuged again. The supernatants were pooled and volume was made to 7 ml with 0.2 N perchloric acid. This constituted the starch fraction.

Procedure

To 0.3 ml of starch extract, 1 ml of 2% phenol and 2 ml of concentrated sulphuric acid were added. The rest of the procedure was same as in the total soluble carbohydrates. Standard curve was prepared using graded concentration of glucose (20 to 100 $\mu g\ ml^{-1}$). The data were expressed as mg starch g^{-1} tissue dry weight.

Free amino acids

Free amino acids were estimated colorimetrically according to the method of Yemm and Cocking (1955).

Extraction

Procedure was the same as in case of soluble carbohydrates.

Reagents

(a) 0.01M KCN : 0.1682g of KCN was dissolved in 250 ml of 60% ethanol.

(b) KCN-acetone : 5ml of reagent 'a' was diluted to 250 ml with acetone.

(c) Acetone-ninhydrin : 5% solution (w/v) of ninhydrin was prepared in acetone.

(d) KCN-acetone-ninhydrin : 50 ml of reagent 'c' was mixed with 250 of reagent 'b'.

(e) 0.2M Citrate buffer (pH 5.0) : 21.008 g of citric acid ($C_6H_8O_7.H_2O$) was dissolved in 200 ml of water, mixed with 200 ml of 1N NaOH and volume was made to 500 ml.

Procedure

One ml of extract was taken in a test tube. To it, 0.5 ml of citrate buffer (reagent 'e') and then 1.0 ml of KCN-acetone-ninhydrin (reagent 'd') were added. The mixture was heated on a boiling water bath for 20 min. After cooling, volume was made to 10 ml with distilled water. The absorbance was taken at 570 nm using reagent blank. Standard curve was prepared with graded concentration (5-50 ppm) of glycine. The data were expressed as mg amino acids g^{-1} tissue dry weight.

Free proline

Free proline was estimated spectrophotometrically according to the method of Trolet *et al.* (1996).

Extraction

Half gram of fresh sample was homogenized in 5 ml of 3% w/v sulfosalicylic acid and centrifuge at 5000 x g for 20 minutes. The supernatant was used for proline estimation.

Reagent

Ninhydrin reagent was prepared by dissolving 0.5g of ninhydrin in 30 ml acetic acid and 20 ml of distilled water with continuous stirring.

Procedure

To 0.5 ml aliquot, 1ml of ninhydrin reagent was added and the mixture was heated on a boiling water bath for 20 minutes. Then the samples were

cooled at room temperature. On attaining room temperature, the contents of test tube were shaken vigorously with 3 ml toluene. Upper pink coloured organic phase was separated from the lower aqueous phase and absorbance of organic phase was read at 520 nm using toluene as a blank.

Standard curve was prepared using graded concentration of proline and data were expressed as mg g^{-1} dry weight.

Chloroplastic pigments

Chloroplastic pigments were estimated according to the method of Hiscox and Israelstam (1979) using dimethyl sulfoxide (DMSO) as solvent.

Procedure

One hundred mg of fresh leaf portion was kept in to a test tube containing 5ml of dimethyl sulfoxide (DMSO). The test tubes were then placed in an oven at 60°C for three hours to facilitate the extraction of the pigments. Samples were cooled to room temperature and the absorbance was read at 454, 625, 645 and 665 nm on a computer aided spectrophotometer (Systronics-119) using multiple wavelength programme. DMSO was used as a blank. Calculations for different pigments were made from the following equation as given by Anderson and Boardman (1964).

$$Chl_a \text{ (mg ml}^{-1}) = 12.67\ A_{665} - 2.65\ A_{645} - 0.29\ A_{625}$$

$$Chl_b \text{ (mg ml}^{-1}) = 23.60\ A_{645} - 4.23\ A_{665} - 0.38\ A_{625}$$

Carotenoids were calculated using Goodwin (1955) equation.

$$\text{Carotenoids (mg ml}^{-1}) = A_{454}/196.$$

In all these equations A is absorbance at the specific wavelength. Amount of all these pigments were calculated in mg g^{-1} tissue dry weight and expressed as μ mol g^{-1} tissue dry weight by using the following relationship:

μ mol of Chl 'a'	=	mg Chl_a x 1.119
μ mol of Chl 'b'	=	mg Chl_b x 1.102
μ mol of total Chl	=	Chl 'a' (μmol) + Chl 'b' (μ mol)
μ mol of cartenoids	=	mg carotenoids x 1.809

PROCEDURES FOR EXTRACTION AND ASSAY FOR ENZYMES OF NITROGEN METABOLISM

Preparation of cell free extracts

For this the leaves from plants were excised from the base around 10 A.M. 3-4 hrs after their exposure to light. The leaves were washed with distilled water and dried on filter paper. One gram of the leaf material was macerated in a chilled pestle and mortar in presence of 4 ml of ice cold extraction medium composed of 0.1 M phosphate buffer (pH 7.5), 7.5 mM cysteine-HCl, PVP 1% 25 μM FAD and 10 per cent (v/v) glycerol was used for extraction of nitrate reductase and nitrite reductase, while tris-HCl buffer (0.1 M, pH 8.0) $MgCl_2$ 1 mM, cysteine HCl 1 mM, PVP 1% (w/v), EDTA 1 mM and β–ME 1 mM was used for the extraction of all other enzymes (GS, GOGAT and GDH).

The homogenate was centrifuged in refrigerated centrifuge at 10,000 x g for 20 min at 4°C. The supernatant was carefully decanted and used as the enzyme preparation.

Assay procedures for enzymes of nitrogen metabolism

Nitrate reductase (NR) (EC 1.6.6.1)

Nitrate reductase activity in the leaf extract was determined from the amount of nitrite produced using a slightly modified procedure of Hageman and Flesher (1960). The reaction mixture, in a final volume of 2 ml, contained the following :

Phosphate buffer (pH 7.5)	100 μ mol
KNO_3	10 μ mol
NADH	0.7 μ mol
Enzyme preparation	0.4 ml

The reaction was started with addition of NADH. After incubation at 30°C for 30 min, the reaction was terminated by adding 0.1 ml of 1M zinc acetate and 1.9 ml 70 % (v/v) ethanol. Blanks from which NADH was omitted were run simultaneously. The content of tubes were thoroughly mixed and centrifuged at 3000 x g for 15 min. The amount of nitrite in the supernatant was determined colorimetrically according to the procedure of Nicholas and Nason (1957). To 2 ml of the supernatant, 1ml of 1% (w/v) sulfanilamide, prepared in 1N HCl, and 1ml of 0.2% (w/v) of aqueous solution of N-1-naphthylethylenediamine-dihydrochloride were added. After 20 min the absorbance at 540 nm was recorded using Spectronic-20. The amount of nitrite in the supernatant was calculated from the standard curve prepared over a range of 10-100 n mol $NaNO_2$.

Nitrite reductase (EC 1.7.7.1)

The enzyme activity was determined from the rate of disappearance of nitrite from the reaction mixture as described by Sawhney and Naik (1973). The assay mixture in a final volume of 2 ml, contained the following:

Phosphate buffer (pH 7.5)	=	100 μ mol
Methyl-viologen	=	0.4 μ mol
$Na\ NO_2$	=	1.0 μ mol
H_2O	=	0.4 μ mol
Enzyme preparation	=	0.4 ml

The reaction was started with 0.01ml of sodium dithionite solution, prepared in 0.29 M $NaHCO_3$ solution. The assay mixture without dithionite served as blank. Care was taken to ensure that methyl viologen is maintained in reduced form throughout the assay period and when necessary an additional 0.01 ml of sodium dithionite solution was added. After incubation for 30 minutes at 30°C, the reaction was stopped by vigorously shaking the tubes to completely oxidize the methyl viologen as indicated by disappearance of blue colour. The amount of residual nitrite in a suitable aliquot (0.1 ml) of reaction mixture was then determined colorimetrically according to the procedure of Nicholas and Nason (1957) as described for NRA.

Glutamine synthetase (GS) (EC 6.3.1.2)

The transferase activities of GS was determined by measuring the amount of γ-glutamylhydroxamate produced as described by Washitani and Sato (1977). The reaction mixture for transferase activity in a final volume of 1 ml contained the following :

Tris-HCl buffer (pH 8.0)	=	100 μ mol
Glutamine	=	10 μ mol
Hydroxylamine hydrochloride (neutralized with NaOH)	=	60 μ mol
ADP	=	1 μ mol
Sodium hydrogen arsenate	=	20 μ mol
$MnCl_2$	=	1 μ mol
Enzyme extract	=	0.3 ml

The reaction was started with enzyme preparation and both the assays were carried out at 37°C for 30 minutes. The reaction was terminated by adding 2 ml of Ferric chloride reagent containing 0.67 M Ferric chloride,

0.37 M HCl and 20 per cent (w/v) TCA. After 20 minutes, absorbance at 540 nm was recorded on Spectronic-20 and the amount of γ-glutamylhydroxamate was determined from the standard curve prepared with 0-2 μ mol of γ-glutamylhydroxamate. In blank ferric chloride reagent was added prior to addition of enzyme preparation. One unit of GS activity represents 1 μ mol of γ-glutamylhydroxymate produced per 30 minutes.

Glutamate dehydrogenase (GDH) (E.C. 1.4.1.2)

Glutamate dehydrogenase activity was measured by following the oxidation of NADH (Loulakakis and Angelakis, 1990). The reaction mixture (2 ml) contained the following :

Tris-HCl buffer (pH 8.4)	=	160 μ mol
α-Ketoglutarate (pH 6.8 -7.0 neutralized with Na_2CO_3)	=	10 μ mol
NH_4Cl	=	20 μ mol
NADH	=	0.4 μ mol
Enzyme extract	=	0.15ml

The decrease in absorbance was measured at 340 nm at 30°C. A unit of enzyme was defined as the amount of enzyme required to oxidized 1 n mol of NADH per minute under assay condition.

Glutamate synthase (GOGAT) (EC 1.4.1.14) NADH dependent

Glutamate synthase activity was measured by Boland *et al.* (1978).

The reaction mixture (2.5 ml) confined following :

Tris-HCl buffer (pH 7.5)	=	160 μ mol
α-Ketoglutarate (pH 6.8-7.0 neutrilize with Na_2CO_3)	=	10 μ mol
NADH	=	0.4 μ mol

Glutamine = 10 µ mol

Enzyme extract = 0.15 µ mol

The decrease in absorbance was measured at 340 nm at 30°C. A unit of enzyme was defined as the amount of enzyme required to oxidized 1 n mol of NADH per minute under assay condition.

MINERAL COMPOSITION

Minerals like nitrogen, phosphorus, potassium, sodium, calcium, magnesium, chloride and sulphate were determined from the oven-dried material of leaves, stem and root. Except for chloride, the extraction/ digestion procedure was common for all other element (USDA Hand book, 1960). For sulphate extraction conc. HNO_3 was used instead of conc. H_2SO_4.

Digestion

Hundred milligram of oven dried and well grounded material was taken in 25 ml conical flasks to which 5 ml of 9:1 Sulphuric acid and perchloric acid mixture was added. The flasks were heated gently on a hot plate till the formation of dense white fumes. When fumes were reduced and subsided at this stage, heating was increased and digestion continued for 25-30 minutes to obtained a colourless digest. The digest thus obtained was cooled and diluted to 25 ml with distilled water. This acid digest was used for the estimation of nitrogen, phosphorus, potassium, sodium, calcium and magnesium.

Nitrogen

Nitrogen content was determined by Micro Kejldahl method (AOAC, 1975).

Phosphorus

The phosphorus content was estimated using vanado-molybophosphoric yellow colour method as described by Jackson (1973).

Reagents

(i) Vanadomolybdate reagent

(a) 25 mg of ammonium molybdate was dissolved in 300 ml distilled water.

(b) 1.25 g of ammonium metavanadate was dissolved in 300 ml of distilled water by boiling. It was then cooled and 250 ml of concentrated HNO_3 was added gradually. Reagent 'a' and 'b' were mixed and volume was made to one litre with distilled water.

(ii) Ammonia Solution

(iii) 6 N HCl

(iv) 2, 4-dinitrophenol indicator

Procedure

Using 2, 4-dinitro phenol as indicator, pH of the aliquot (2 ml) of digest was brought to 2.8-3.0 with ammonia solution till the solution become yellow, followed by the addition of 6 N HCl to make it just colourless. To it, 2 ml of vanadomolybdate reagent was added and the final volume was made to 10 ml. The absorbance was read at 440 nm against a reagent blank. The standard curve was prepared with the graded concentration of KH_2PO_4. The values were calculated and expressed as mg g^{-1} tissue dry weight.

POTASSIUM AND SODIUM ESTIMATION

Potassium and sodium contents were determined in the above acid-digest with a flame photometer (Elico) using standard KCl for potassium

and NaCl for sodium. The values were calculated and expressed as mg g^{-1} tissue dry weight.

CALCIUM AND MAGNESIUM

Calcium and magnesium were estimated by EDTA titration according to the method given in USDA, Handbook (1960).

Reagents

(i) N/100 EDTA solution: 1.86 of EDTA (di-sodium salt) was dissolved in water and volume was made one litre with distilled water

(ii) Eriochrome Black T indicator (EBT) : 4.5g of hydroxylamine hydrochloride and 0.5g EBT were dissolved in ethanol and volume was made 100 ml. This solution was prepared freshly before use.

(iii) NH_4OH-NH_4Cl buffer : 67.5 g of NH_4Cl was dissolved in small volume of distilled water and 570 ml concentrated NH_4OH was added and volume was made to one litre with distilled water.

(iv) Carbamate crystals

Procedure

One ml of aliquot was taken in China-dish and 5 ml of distilled water was added. The contents were stirred on a magnetic stirrer on slow speed. Five crystals of carbamate were added into China-dish and then to it, 2ml of NH_4OH-NH_4Cl buffer and 4 drops of EBT indicator were added. The content of the China-dish was titrated with N/100 EDTA till bluish green colour (end- point) from purple red colour appeared. The volume of EDTA solution used was noted and the following calculations were made.

$$\text{Ca+Mg (meq/ml)} = \frac{\text{EDTA used in titration (ml)} \times \text{Normality of EDTA}}{\text{Aliquot taken (ml)}}$$

DETERMINATION OF CALCIUM

Reagent

(i) N/100 EDTA (di-sodium salt) solution

(ii) Calcon indicator : 0.5 g calcon in 100 ml of methanol

(iii) 4N NaOH solution : 16 g of NaOH was dissolved in 100 ml of distilled water.

(iv) 1% PVA solution.

(v) 5% Hydroxylamine hydrochloride solution

(vi) Triethanolamine

Procedure

One ml of aliquot was taken in China-dish and to it, 5ml of distilled water was added. After stirring, 1ml of 5% hydroxylamine hydrochloride solution, 10 drops of triethanolamine, 2 ml of 4 N NaOH, 1 ml PVA solution and 4 drops of calcon indicator were added and mixed. The content was titrated against N/100 EDTA till bluish green colour (i.e. end point) appeared. Volume of EDTA consumed was recorded.

Calculation for calcium

$$\text{Ca (meq/ml)} = \frac{\text{ml of EDTA used X Normality of EDTA}}{\text{Aliquot taken (ml)}}$$

Calculation for magnesium

Mg (meq/ml) = [Ca + Mg (meq/ml)] - Ca (meq/ml)

The data for calcium and magnesium were expressed as mg g^{-1} tissue dry weight.

Chloride

Chloride was estimated by the method of Ramsay *et al.* (1955).

Digestion

Fifty milligrams of well ground material was taken in 50ml flask to which 20 ml of HNO_3 - KNO_3 solution (mixed in the ratio of 1:8 of 1 N HNO_3 and KNO_3) was added. The solution was stirred for 30 minutes and then filtered. Filtrate was used for chloride estimation.

Reagents

(a) Potassium chromate solution : Five gram of K_2CrO_4 was dissolved in 80 ml of distilled water followed by addition of saturated solution of silver nitrate ($AgNO_3$) drop wise while stirring until permanent red precipitation were produced. The solution was filtered and diluted to 100 ml.

(b) Silver nitrate solution (0.01N) : It was prepared by dissolving 1.699 g of $AgNO_3$ in H_2O and diluted to one litre. The solution was kept in brown bottle away from light.

(c) 1 N NaOH

Procedure

To 5 ml of digest, 1ml of reagent 'a' was added. The pH of the sample extract was adjusted to 8.2 with reagent 'c' the content was titrated against reagent 'b' untill the reddish brown colour (end point) was obtined. Silver nitrate ($AgNO_3$) titration was also done with reagent blank.

The amount of total chloride in the sample was calculated using following formula:

$$\text{meq chloride (per litre)} = \frac{(x-y) \times N \times 1000}{\text{ml of aliquot taken}}$$

Where,

x = ml of $AgNO_3$ solution (reagent 'b') for sample

y = ml of $AgNO_3$ solution (reagent 'b') for blank

N = Normality of $AgNO_3$ solution

The quantity of chloride was expressed as mg g^{-1} tissue dry weight.

Sulphate

The sulphate was estimated by turbidimetric method as suggested by Verma (1977).

Reagent

(I) 6 M HCl

(ii) $BaCl_2.2H_2O$ crystals

(iii) 70% aqueous solution of sorbitol (w/v)

Procedure

To a 5 ml aliquot of digest, 0.5 ml of 6 M HCl was added after which 2.5 ml of 70 % sorbitol solution and 1 gram of $BaCl_2.2H_2O$ crystals were also added and content was shaken vigorously to dissolve barium chloride and allowed to stand for at least 5 min. Turbidity of the suspension was read in turbidimeter at 470 nm against the reagents blank. Standard curve was prepared using graded concentrations of Barium sulphate. Data were expressed as mg g^{-1} tissue dry weight.

YIELD AND YIELD ATTRIBUTES

Following observations were recorded at maturity :

(i) Number of siliquae per plant

(ii) Seed yield per plant

QUALITY TEST

Fatty acid composition of total lipids by gas liquid chromatography (GLC)

Preparation of methyl esters

Methyl esters were prepared by the method of Luddy *et al.* (1968) as described below:

Reagent

(i) **0.4 N sodium methylate**: The reagent was prepared by the method of Luddy *et al.* (1960) . Metallic sodium was cut into small bright pieces under petroleum ether and added to a known volume of redistilled absolute methanol in amounts slightly in excess of that required for the desired normality (only small piece of metal was added at a time). When the addition was complete, the normality of sodium methylate was adjusted to 0.4.

(ii) **Carbon disulphide**

(iii) **Activated charcoal**

Method

Ten to fifteen mg of lipid sample was taken in a test tube and 0.4 ml of 0.4 N sodium methylate was added. It was covered with aluminium foil and then immersed in a water bath at 65°C to a depth of half inch and was shaken vigorously for 2-3 minutes. The mixture became homogeneous indicating that the esterification of the lipid sample was complete. The test tube was removed from the bath and was cooled to room temperature. One ml of carbon disulphide was added and was shaken for 1-2 minutes. Approximately 100 mg of activated charcoal was added and was shaken vigorously. It was then filtered with the help of Whatman No. 1 filter paper. The filtrate contained all the methyl esters of fatty acids.

Fractionation of methyl esters by GLC

Methyl esters of fatty acids were separated in a Nucon-5765 gas chromatograph equipped with flame ionization detector. Stainless steel column (10' x 1/8") was packed with 20 per cent diethylene glycol succinate (DEGS) on 60-80 mesh chromosorb-W. The column temperature was 190°C and the flow of the nitrogen carrier gas was maintained at 35 ml minute^{-1}. The peaks were identified by comparison of their retention times with those of standard fatty acids. The area under individual peak was calculated by the formula, half the base x height and converted directly into relative percentage.

Oil and protein content

Oil and protein content was estimated by non-destructive method using Dickey John Insta Lab 600 NIR Product Analyser.

Total of glucosinolate

Total glucosinolates were analysed as glucose by a modified method of Heaney and Fenwick (1981). The glucosinolates were extracted from defatted meal (50 mg) by adding boiling water (2 ml) and retaining for 4 min in a boiling water bath. The sample was centrifuged and the supernatant applied to a DEAE Sephadex-A-25 column. The meal was washed for a second time with boiling water (2 ml), centrifuged and the supernatant added to the column. After rinsing with pyridyl acetate (3 ml, 0.02 mol l^{-1}), was added and the column incubated for 2 h at 37°C. The hydrolysed products were washed into a 10 ml volumetric flask with 4 rinses of 1 ml distilled H_2O and made to volume. Aliquots of 1 ml were mixed with trinder's (1969) colour reagent (3 ml) and incubated at 37°C for 15 min. Colour intensity was measured at 515 nm on a Varian 634 spectrophotometer.

CHAPTER - 4

RESULTS

The present investigation was conducted on Indian mustard (*Brassica juncea* L. cv. RH-30) to evaluate whether the combined effects of salinity and N-fertilization on morpho-physiological characters, nitrogen metabolism, nutrient status, yield and quality character. The results obtained are described under appropriate heads.

GROWTH AND DEVELOPMENT

Dry weight of leaves

A close examination of data in Table 1 revealed that plant treated with combined form of nitrogen had been found to contain highest dry weight of leaves as compared to ammonical form at first sampling stage (45 DAS).

Under saline condition, the dry weight of leaves decreased significantly with every increment in salinity as compared to non saline conditions irrespective of nitrogen source used. The plant treated with ammonical form of nitrogen exhibited maximum reduction of dry weight of leaves and minimum reduction was observed with combined form as compared to non-saline plants.

The increasing levels of nitrogen (80 and 120 kg ha^{-1}) irrespective source of nitrogen showed significant increase in dry weight of leaves as

Table 1. **Effect of nitrogen source, levels and their interaction with salinity on dry weight of leaf (g) in *Brassica juncea* cv. RH-30**

Nitrogen sources	Nitrogen levels (kg ha^{-1})	Salinity levels (dSm^{-1})							
		0	8	12	**Mean**	0	8	12	**Mean**
		Pre-flowering stage (45 DAS)				Flowering stage (65 DAS)			
Nitrate form (NO_3^-)	40	0.479	0.385	0.358	**0.407**	0.552	0.466	0.437	**0.485**
	80	0.534	0.459	0.393	**0.462**	0.664	0.579	0.548	**0.597**
	120	0.572	0.495	0.432	**0.499**	0.744	0.668	0.645	**0.686**
	Mean	**0.528**	**0.446**	**0.394**	**0.456**	**0.653**	**0.571**	**0.543**	**0.589**
Ammonical form (NH_4^+)	40	0.321	0.249	0.212	**0.260**	0.455	0.375	0.339	**0.390**
	80	0.461	0.368	0.323	**0.384**	0.577	0.495	0.435	**0.502**
	120	0.266	0.207	0.171	**0.214**	0.454	0.369	0.329	**0.384**
	Mean	**0.349**	**0.274**	**0.235**	**0.286**	**0.495**	**0.413**	**0.368**	**0.425**
Combined form (NO_3^-+NH_4^+)	40	0.466	0.371	0.326	**0.387**	0.574	0.518	0.462	**0.518**
	80	0.581	0.502	0.417	**0.500**	0.681	0.593	0.568	**0.614**
	120	0.585	0.519	0.452	**0.518**	0.772	0.697	0.684	**0.717**
	Mean	**0.544**	**0.464**	**0.398**	**0.469**	**0.675**	**0.602**	**0.571**	**0.616**
Overall mean		**0.474**	**0.395**	**0.342**		**0.608**	**0.529**	**0.496**	

C.D. (P<0.05) for Ns/Nl/Sl	=	0.005	C.D. (P<0.05) for Ns/Nl/Sl = 0.007
C.D. (P<0.05) for NsxNl, Nl x Sl & Ns x Sl	=	0.009	C.D. (P<0.05) for NsxNl, Nl x Sl & Ns x Sl = 0.012
C.D. (P<0.05) for Ns x Nl x Sl	=	N.S.	C.D. (P<0.05) for Ns x Nl x Sl = N.S.

Ns = Nitrogen source; Nl = Nitrogen level; Sl =Salinity level

compared to lower level of nitrogen applications i.e. 40 kg ha^{-1}. In addition to this, the increase in dry weight of leaves was maximum with application of 120 kg ha^{-1} in combined form as compared to lower level of nitrogen application. The interactive effect between salinity and different level of nitrogen source had been to be found non-significant.

A study of data (Table 1) that plant treated with combined form of nitrogen contain highest dry weight of leaves at second sampling (65 DAS) as compared to ammonical form.

With increase in salinity level from 8 to 12 dSm^{-1} a correspondingly significant reduction in dry weight of leaves was noticed in different source of nitrogen as compared to non-saline plants. These reductions under salinity were maximum and minimum with ammonical and combined forms respectively as compared to control plants.

The increasing level of nitrogen (80 and 120 kg ha^{-1}) irrespective of source of nitrogen case increased dry weight of leaves as compared to lower level of nitrogen application i.e. 40 kg ha^{-1}. Beside this, the plant treated with combined form of nitrogen (120 kg ha^{-1}) exhibited maximum increase in dry weight of leaves as compared to a dose of 40 kg ha^{-1} application. The interaction between salinity and different levels of nitrogen had been found to be non-significant.

Dry weight of leaves showed similar trend at both the sampling stages i.e. 45 and 65 DAS. However, maximum dry weight in leaves was observed at 65 DAS.

Dry weight of stem

Critical examination of data in Table 2 revealed that plant treated with combined form of nitrogen had been found to contain highest dry

weight of stem at first sampling (45 DAS) as compared to ammonical form.

With increase in salinity level from 8 to 12 dSm^{-1} a corresponding reduction in dry weight of stem was noticed as compared to non-saline plants. These reductions were maximum and minimum with the application of ammonical and combined form of nitrogen, respectively as compared to control plants.

Increasing levels of nitrogen irrespective of source at the rate of 80 and 120 kg ha^{-1} resulted in significant increase in dry weight of stem as compared to 40 kg ha^{-1} of nitrogen application. In addition to this, the plant treated with combined form of nitrogen (120 kg ha^{-1}) under saline condition caused maximum increased as compared to lower level of nitrogen application i.e. 40 kg ha^{-1}. The interactive effect between salinity and different level of nitrogen source had been found to be non-significant.

A reference of data in Table 2 revealed that dry weight of stem was highest in plant treated with combined form of nitrogen as compared to ammonical form at second sampling stage (65 DAS).

Under saline condition, the maximum and minimum reduction was observed with ammonical and combined form, respectively as compared to non-saline plants.

Nitrogen in combined form (120 kg ha^{-1}) resulted in the relatively higher dry weight of stem under saline condition over control. However, lower dry weight was noticed with ammonical form of nitrogen. Beside this, the higher level of nitrogen (120 kg ha^{-1}) in combined form significantly increased the dry weight of stem at salinity level of 8 and 12 dSm^{-1}, respectively as compare to control plants. Such increase were notice minimum at lower level of nitrogen application (40 kg ha^{-1}).

Table 2. **Effect of nitrogen source, levels and their interaction with salinity on dry weight of stem (g) in *Brassica juncea* cv. RH-30**

Nitrogen sources	Nitrogen levels (kg ha^{-1})	Salinity levels (dSm^{-1})							
		0	8	12	**Mean**	0	8	12	**Mean**
		Pre-flowering stage (45 DAS)				Flowering stage (65 DAS)			
Nitrate form (NO_3^-)	40	1.153	0.820	0.703	**0.892**	1.867	1.437	1.027	**1.443**
	80	1.590	1.257	1.127	**1.324**	2.490	1.987	1.837	**2.104**
	120	1.757	1.510	1.317	**1.528**	3.040	2.667	2.360	**2.689**
	Mean	**1.500**	**1.196**	**1.049**	**1.248**	**2.466**	**2.030**	**1.741**	**2.079**
Ammonical form (NH_4^+)	40	0.820	0.497	0.377	**0.564**	1.337	0.897	0.720	**0.984**
	80	1.097	0.703	0.567	**0.789**	1.827	1.287	1.087	**1.400**
	120	0.887	0.557	0.417	**0.620**	1.567	1.037	0.867	**1.157**
	Mean	**0.934**	**0.586**	**0.453**	**0.658**	**1.577**	**1.073**	**0.891**	**1.180**
Combined form (NO_3^-+NH_4^+)	40	1.087	0.753	0.687	**0.842**	1.920	1.520	1.317	**1.586**
	80	1.897	1.500	1.377	**1.591**	2.647	2.217	2.057	**2.307**
	120	1.977	1.757	1.530	**1.754**	3.187	2.847	2.667	**2.900**
	Mean	**1.653**	**1.337**	**1.198**	**1.396**	**2.284**	**2.194**	**2.013**	**2.264**
Overall mean		**1.363**	**1.039**	**0.900**		**2.209**	**1.766**	**1.549**	

C.D. (P<0.05) for Ns/Nl/Sl = 0.021
C.D. (P<0.05) for NsxNl, Nl x Sl & Ns* x Sl = 0.036
C.D. (P<0.05) for Ns x Nl x Sl = N.S.

C.D. (P<0.05) for Ns/Nl/Sl = 0.029
C.D. (P<0.05) for NsxNl, Nl x Sl & Ns x Sl = 0.049
C.D. (P<0.05) for Ns x Nl x Sl = 0.086

Ns = Nitrogen source; Nl = Nitrogen level; Sl =Salinity level
*Non-significant.

Dry weight of stem showed similar trend at both the sampling stages (45 and 65 DAS). However, the magnitide was relatively higher at 65 DAS.

Total dry weight of plant

Critical examination of data in Table 3 revealed that plant treated with combined form of nitrogen had been found to contain highest total dry weight of plant at first sampling (45 DAS) as compared to ammonical form.

With increase in salinity level from 8 to 12 dSm^{-1} a significant reduction in total dry weight of plant was observed as compared to non-saline plants. However, the maximum and minimum reduction under saline conditions were notice with ammonical and combined forms of nitrogen respectively as compared to control plants.

Increasing level of different sources of nitrogen at the rate of 80 and 120 kg ha^{-1} caused significant increase in total dry weight of plant as compared to 40 kg ha^{-1} of nitrogen application. In addition to this, the plant treated with combined form (120 kg ha^{-1}) under saline condition showed maximum increase as compared to lower level of nitrogen application i.e. 40 kg ha^{-1}. However, minimum increase was noticed with ammonical form. The interactive effect between salinity and different level of nitrogen source had been found to be non-significant.

A reference of data in Table 3 revealed that total dry weight of plant was highest in plant treated with combined form of nitrogen as compared to ammonical form at second sampling stage (65 DAS).

Under saline condition, significant reduction was noticed with increasing level of salinity irrespective of nitrogen source as compared

Table 3. **Effect of nitrogen source, levels and their interaction with salinity on total dry weight of plant (g) in *Brassica juncea* cv. RH-30**

Nitrogen sources	Nitrogen levels (kg ha^{-1})	Salinity levels (dSm^{-1})							
		0	8	12	**Mean**	0	8	12	**Mean**
		Pre-flowering stage (45 DAS)				Flowering stage (65 DAS)			
Nitrate form (NO_3^-)	40	1.631	1.205	1.038	**1.291**	2.418	1.903	1.464	**1.928**
	80	2.124	1.715	1.519	**1.786**	3.154	2.565	2.384	**2.701**
	120	2.322	2.005	1.769	**2.032**	3.780	3.335	3.005	**3.373**
	Mean	**2.026**	**1.642**	**1.442**	**1.702**	**3.118**	**2.601**	**2.284**	**2.668**
Ammonical form (NH_4^+)	40	1.242	0.745	0.588	**0.859**	1.762	1.272	1.060	**1.374**
	80	1.557	1.071	0.889	**1.173**	2.404	1.781	1.522	**1.902**
	120	1.153	0.763	0.587	**0.834**	2.021	1.406	1.195	**1.541**
	Mean	**1.317**	**0.860**	**0.688**	**0.955**	**2.072**	**1.486**	**1.259**	**1.606**
Combined form (NO_3^-+NH_4^+)	40	1.553	1.111	1.012	**1.225**	2.490	2.006	1.679	**2.058**
	80	2.477	2.002	1.793	**2.091**	3.327	2.809	2.625	**2.920**
	120	2.471	2.275	1.982	**2.243**	3.958	3.543	3.350	**3.617**
	Mean	**2.167**	**1.796**	**1.596**	**1.853**	**3.259**	**2.786**	**2.551**	**2.865**
Overall mean		**1.837**	**1.432**	**1.242**		**2.816**	**2.291**	**2.031**	

C.D. (P<0.05) for Ns/Nl/Sl	=	0.030	C.D. (P<0.05) for Ns/Nl/Sl	=	0.027
C.D. (P<0.05) for NsxNl, Nl x Sl & Ns x Sl	=	0.051	C.D. (P<0.05) for NsxNl, Nl x Sl & Ns x Sl	=	0.046
C.D. (P<0.05) for Ns x Nl x Sl	=	N.S.	C.D. (P<0.05) for Ns x Nl x Sl	=	0.080

Ns = Nitrogen source; Nl = Nitrogen level; Sl =Salinity level

to non-saline plants. Such reductions were maximum and minimum with ammonical and combined forms, respectively as compared to control plants.

Application of nitrogen in combined form (120 kg ha^{-1}) resulted in significant increase the total dry weight of plant under saline condition as compared to control plants. However, minimum increase was notice with ammonical form. Beside this, the higher level of nitrogen (120 kg ha^{-1}) in combined form exhibited maximum increase in total dry weight of plant at a salinity level 8 and 12 dSm^{-1}, respectively as compared to non-saline plants. Such increase was minimum with 40 kg ha^{-1} nitrogen.

The total dry weight of plant showed similar results at both sampling at 45 and 65 DAS. However, maximum increase in total dry weight was noticed at second sampling (65 DAS).

Total leaf area per plant

A critical examination of data in Table 4 revealed that the total leaf area per plant was maximum in plants treated with combined form of nitrogen as compared to ammonical form at first sampling stage (45 DAS).

With increase in salinity level from 8 to 12 dSm^{-1} a corresponding reduction in total leaf area per plant was notice as compared to non-saline plants. These reductions were maximum (26.11 and 39.79%) and minimum (11.45 and 20.72%) with the application of ammonical and combined form of nitrogen, respectively as compared to control plants.

Nitrogen in combined form (120 kg ha^{-1}) resulted in maximum increase in total leaf over control. However, minimum increase was noticed with ammonical form of nitrogen. Beside this, the higher level of nitrogen (120 kg ha^{-1}) in combined form exhibited minimum reduction (4.61 and

Table 4. **Effect of nitrogen source, levels and their interaction with salinity on total leaf area (cm^2 $plant^{-1}$) in *Brassica juncea* cv. RH-30**

Nitrogen sources	Nitrogen levels (kg ha^{-1})	Salinity levels (dSm^{-1})							
		0	8	12	**Mean**	0	8	12	**Mean**
		Pre-flowering stage (35 -45DAS)				Flowering stage (55-65 DAS)			
Nitrate form (NO_3^-)	40	91.91	59.38	52.91	**68.07**	124.16	100.62	88.19	**104.32**
	80	130.47	112.63	101.29	**114.79**	169.95	153.34	134.95	**152.74**
	120	142.69	126.25	116.07	**128.34**	187.97	175.35	164.36	**175.89**
	Mean	**121.69**	**99.42**	**90.09**	**103.73**	**160.69**	**143.10**	**129.17**	**144.32**
Ammonical form (NH_4^+)	40	72.41	51.58	42.68	**55.55**	111.79	78.48	69.46	**86.57**
	80	94.19	75.68	68.54	**79.47**	129.82	112.54	95.03	**112.47**
	120	68.20	46.24	30.14	**48.19**	125.07	89.72	68.40	**94.39**
	Mean	**78.27**	**57.83**	**47.12**	**61.07**	**122.23**	**93.58**	**77.63**	**97.81**
Combined form (NO_3^-+NH_4^+)	40	102.28	77.74	69.40	**83.14**	134.03	110.41	102.39	**115.61**
	80	142.49	128.50	113.90	**128.30**	176.99	163.01	155.97	**165.32**
	120	153.45	146.37	132.40	**144.07**	192.24	180.05	162.10	**178.13**
	Mean	**132.74**	**117.54**	**105.23**	**118.50**	**167.76**	**151.16**	**140.15**	**153.02**
Overall mean		**110.90**	**91.59**	**80.81**		**150.22**	**129.28**	**115.65**	

C.D. (P<0.05) for Ns/Nl/Sl = 0.615
C.D. (P<0.05) for NsxNl, Nl x Sl & Ns x Sl = 1.066
C.D. (P<0.05) for Ns x Nl x Sl = 1.846
C.D. (P<0.05) for Ns/Nl/Sl = 2.178
C.D. (P<0.05) for NsxNl, Nl x Sl & Ns x Sl = 3.772
C.D. (P<0.05) for Ns x Nl x Sl = 6.534

Ns = Nitrogen source; Nl = Nitrogen level; Sl =Salinity level

13.71%) in total leaf area per plant at a salinity level of 8 and 12 dSm^{-1}, respectively as compared to non-saline plants. Such reduction was maximum (23.99 and 32.14%) at lower level of nitrogen application (40 kg ha^{-1}).

A reference of data in Table 4 revealed that plant treated with combined form of nitrogen had been found to highest total leaf area per plant at second sampling (65 DAS) as compared to ammonical form.

Under saline condition, the total leaf area per plant decreased significantly with every increment in salinity as compared to non-saline conditions irrespective of nitrogen source used. The plant treated with ammonical form of nitrogen showed maximum per cent reduction (23.43 and 36.48) in total leaf area per plant and combined form exhibited minimum per cent reduction (9.89 and 16.45) as compared to non-saline plants.

Nitrogen in combined form (120 kg ha^{-1}) resulted in relatively higher total leaf area per plant under saline conditions. However, minimum increase in total leaf area per plant was noticed with ammonical form. In addition to this, the higher level of nitrogen (120 kg ha^{-1}) in combined form exhibited minimum reduction (6.34 and 15.67%) in total leaf area per plant at a salinity level 8 and 12 dSm^{-1}, respectively as compared to control plants. Such reductions were maximum (17.62 and 23.60%) at lower level of fertilizer application i.e. 40 kg ha^{-1}.

The total leaf area per plant showed that similar trends exist at both sampling stages i.e. 45 and 65 DAS. However, the highest total leaf area per plant occurred at 65 DAS.

Absolute growth rate (AGR)

A study of data (Table 5) that absolute growth rate was maximum in plant treated with combined form of nitrogen as compared to ammonical forms at first sampling i.e. 45 DAS.

With increase in salinity level from 8 to 12 dSm^{-1} a significant reduction in absolute growth rate was noticed as compared to non-saline plants. These reduction under salinity were maximum (54.46 and 76.53%) and minimum (30.32 and 47.24%) with the application of ammonical and combined form of nitrogen, respectively as compared to control plants.

Nitrogen in combined form (120 kg ha^{-1}) resulted minimum reduction in absolute growth rate under saline condition. However, maximum reduction occured with ammonical form. Beside this, the higher level of nitrogen (120 kg ha^{-1}) in combined form showed minimum per cent reduction (19.19 and 38.88) in absolute growth rate at a salinity level of 8 and 12 dSm^{-1}, respectively as compare to non saline plants. Such reduction was maximum (43.46 and 59.35%) at lower level of nitrogen application i.e. 40 kg ha^{-1}.

A reference of data in Table 5 revealed that plant treated with combined form of nitrogen had been found to exhibit maximum absolute growth rate at second sampling (65 DAS) as compared to ammonical form.

Under saline condition, the absolute growth rate decreased significantly with every increment in salinity as compare to non-saline plants. The plant treated with ammonical form of nitrogen resulted in maximum per cent reduction (50.05 and 73.95) and in combined form minimum per cent reduction (29.60 and 44.25) as compared to control plants.

Table 5. Effect of nitrogen source, levels and their interaction with salinity on absolute growth rate (mg day^{-1}) in *Brassica juncea* cv. RH-30

Nitrogen sources	Nitrogen levels (kg ha^{-1})	Salinity levels (dSm^{-1})							
		0	8	12	**Mean**	0	8	12	**Mean**
		Pre-flowering stage (35 -45DAS)				Flowering stage (55-65 DAS)			
Nitrate form (NO_3^-)	40	92.83	50.10	33.46	**58.80**	114.53	62.90	49.00	**75.47**
	80	121.80	80.93	61.33	**88.02**	156.83	94.63	76.53	**109.33**
	120	134.93	103.33	79.73	**106.00**	186.06	141.50	108.53	**145.36**
	Mean	**116.52**	**78.12**	**58.17**	**84.27**	**152.47**	**99.67**	**78.02**	**110.05**
Ammonical form (NH_4^+)	40	79.46	39.93	24.23	**47.87**	78.83	46.83	22.23	**49.30**
	80	87.43	38.80	20.63	**48.95**	121.03	68.80	42.83	**77.55**
	120	66.43	27.50	9.90	**34.61**	91.33	29.83	10.80	**43.98**
	Mean	**77.77**	**35.41**	**18.25**	**43.81**	**97.06**	**48.48**	**25.28**	**56.94**
Combined form (NO_3^-+NH_4^+)	40	91.03	51.46	37.00	**59.83**	119.26	70.70	38.10	**76.02**
	80	141.70	94.13	73.30	**103.04**	163.30	111.50	93.03	**122.61**
	120	149.00	120.40	91.06	**120.15**	197.06	155.43	136.23	**162.91**
	Mean	**127.24**	**88.66**	**67.12**	**94.34**	**159.87**	**112.54**	**89.12**	**120.51**
Overall mean		**107.18**	**67.40**	**47.85**		**136.47**	**86.90**	**64.14**	

C.D. (P<0.05) for Ns/Nl/Sl = 1.646
C.D. (P<0.05) for NsxNl, Nl x Sl & Ns x Sl = 2.850
C.D. (P<0.05) for Ns x Nl x Sl = 4.937

C.D. (P<0.05) for Ns/Nl/Sl = 2.276
C.D. (P<0.05) for NsxNl, Nl x Sl & Ns x Sl = 3.943
C.D. (P<0.05) for Ns x Nl x Sl = 6.829

Ns = Nitrogen source; Nl = Nitrogen level; Sl =Salinity level

Nitrogen in combined form (120 kg ha^{-1}) showed the minimum per cent reduction in absolute growth rate under saline condition over control plants. However, maximum per cent reductions were observed with ammonical form of nitrogen. In addition to this, the higher level of nitrogen (120 kg ha^{-1}) in combined form caused minimum reduction (21.12 and 30.86%) at a salinity level at 8 and 12 dSm^{-1}, respectively, as compare to control plants. Such reduction was maximum (40.71 and 68.05%) at a lower level of nitrogen application.

The absolute growth rate exhibited similar trend at both the sampling stages i.e. 45 and 65 DAS. However, the highest AGR occurred at 65 DAS.

Net assimilation rate (NAR)

A critical examination of data in Table 6 revealed that the net assimilation rate of plant was maximum in plant treated with combined form of nitrogen as compared to ammonical form at first sampling (45 DAS).

With increase in salinity level from 8 to 12 dSm^{-1} a corresponding reduction in net assimilation rate was notice as compared to control plant, irrespective of nitrogen source used. These reduction under salinity were maximum (38.37 and 59.87%) and minimum (21.03 and 39.90%) with the application of ammonical form and combined form of nitrogen, respectively as compared to non-saline plants.

Nitrogen in combined form (120 kg ha^{-1}) resulted maximum increase in net assimilation rate of plant under saline condition over control. However, minimum increase was notice with ammonical form of nitrogen. Beside this, the higher level of nitrogen (120 kg ha^{-1}) in combined form

Table 6. **Effect of nitrogen source, levels and their interaction with salinity on net assimilation rate (g m^{-2} day^{-1}) in *Brassica juncea* cv. RH-30**

Nitrogen sources	Nitrogen levels (kg ha^{-1})	Salinity levels (dSm^{-1})							
		0	8	12	**Mean**	0	8	12	**Mean**
		Pre-flowering stage (35 -45DAS)				Flowering stage (55-65 DAS)			
Nitrate form (NO_3^-)	40	11.41	8.19	6.14	**8.58**	11.27	6.92	2.23	**6.80**
	80	12.04	8.93	7.00	**9.32**	11.42	7.42	6.18	**8.34**
	120	12.37	10.11	8.16	**10.21**	11.98	8.83	7.88	**9.56**
	Mean	**11.94**	**9.08**	**7.10**	**9.37**	**11.56**	**7.72**	**5.43**	**8.24**
Ammonical form (NH_4^+)	40	10.52	7.18	5.45	**8.71**	8.60	3.49	0.77	**4.28**
	80	11.79	7.88	5.63	**8.43**	10.50	4.97	2.53	**6.00**
	120	12.01	6.09	2.70	**6.93**	8.92	4.12	1.27	**4.77**
	Mean	**11.44**	**7.05**	**4.59**	**7.69**	**9.34**	**4.19**	**1.52**	**5.02**
Combined form (NO_3^-+NH_4^+)	40	11.38	8.79	5.68	**8.61**	10.96	7.20	4.03	**7.39**
	80	12.00	9.13	7.58	**9.57**	11.67	8.32	7.11	**9.03**
	120	12.72	10.58	8.43	**10.58**	12.05	10.19	7.95	**10.06**
	Mean	**12.03**	**9.50**	**7.23**	**9.59**	11.56	8.57	6.36	**8.83**
Overall mean		**12.14**	**8.54**	**6.31**		**10.82**	**6.83**	**4.44**	

C.D. ($P<0.05$) for Ns/Nl/Sl = 0.195
C.D. ($P<0.05$) for NsxNl, Nl x Sl & Ns x Sl = 0.338
C.D. ($P<0.05$) for Ns x Nl x Sl = 0.586

C.D. ($P<0.05$) for Ns/Nl/Sl = 0.224
C.D. ($P<0.05$) for NsxNl, Nl x Sl & Ns x Sl = 0.388
C.D. ($P<0.05$) for Ns x Nl x Sl = 0.672

Ns = Nitrogen source; Nl = Nitrogen level; Sl =Salinity level

exhibited minimum per cent reduction (16.82 and 33.72) in net assimilation rate at a salinity level of 8 and 12 dSm^{-1}, respectively as compare to non-saline plants. Such per cent reduction was maximum (22.75 and 50.08) at lower level of nitrogen fertilizer dose i.e. 40 kg ha^{-1}.

A reference of data in Table 6 revealed that plant treated with combined form of nitrogen had been found to exhibit highest net assimilation rate of plant at 65 DAS as compared ammonical form.

Under saline condition, the net assimilation rate of plant decreased significantly with every increment in salinity as compared to non saline conditions irrespective of nitrogen used. The plant treated with ammonical and combined forms of nitrogen resulted in maximum per cent reduction (55.13 and 83.72) and minimum per cent reduction (25.86 and 44.98), respectively as compared to control plants.

Nitrogen in combined form (120 kg ha^{-1}) relatively lesser reduction in net assimilation rate of plant under saline condition (8 and 12 dSm^{-1}) over control plants. However, maximum reduction occurred with ammonical form. In addition to this, the higher level of nitrogen (120 kg ha^{-1}) in combined form showed minimum per cent reduction (15.43 and 34.02) in net assimilate rate of plant at a salinity level 8 and 12 dSm^{-1}, respectively, as compared to non-saline condition. Such per cent reduction (34.30 and 63.22) was maximum at 40 kg ha^{-1} nitrogen application.

The net assimilation rate of plant exhibited similar trend at both sampling trend at both sampling i.e. 45 and 65 DAS. However, highest net assimilation rate of plant was observed at 65 DAS.

Relative growth rate (RGR)

A critical examination of data in Table 7 revealed that the relative growth rate of plant was highest in plant treated with combined form of nitrogen as compared to ammonical form at first sampling (45 DAS).

With increase in salinity level from 8 to 12 dSm^{-1} a corresponding reduction in relative growth rate of plant was observed as compared to non-saline plants. These reductions under salinity were maximum (41.18 and 64.62%) and minimum (13.64 and 29.43%) with the application of ammonical and combined form of nitrogen, respectively as compared to control plants.

Nitrogen in combined form (120 kg ha^{-1}) showed minimum reduction in relative growth rate of plant under saline condition (8 and 12 dSm^{-1}) over control. However, maximum reduction was noticed with ammonical form of nitrogen. In addition to this, the higher level of nitrogen (120 kg ha^{-1}) in combined form exhibited lesser reduction (13.64 and 29.43%) at a salinity level 8 and 12 dSm^{-1}, respectively as compared to non-saline to plants. Such reduction occured maximum (32.64 and 46.00%) at a lower level of fertilizer application.

A reference of data in Table 7 revealed that plant treated with combined form of nitrogen had been found to exhibit maximum relative growth rate of plant at second sampling (65 DAS) as compare to ammonical form.

Under saline conditions (8 and 12 dSm^{-1}) the relative growth rate of plant decreased significantly with every increment in salinity as compared to non-saline condition irrespective of nitrogen source used. The plant treated with ammonical form of nitrogen resulted maximum reduction

Table 7. **Effect of nitrogen source, levels and their interaction with salinity on relative growth rate (mg g^{-1} day^{-1}) in *Brassica juncea* cv. RH-30**

Nitrogen sources	Nitrogen levels (kg ha^{-1})	Salinity levels (dSm^{-1})							
		0	8	12	**Mean**	0	8	12	**Mean**
		Pre-flowering stage (35 -45DAS)				Flowering stage (55-65 DAS)			
Nitrate form (NO_3^-)	40	84.10	53.76	38.93	**58.93**	64.10	40.13	13.90	**39.37**
	80	85.20	63.86	51.70	**66.92**	66.66	46.03	38.70	**50.46**
	120	87.13	72.43	59.93	**73.16**	67,.76	55.20	44.80	**55.92**
	Mean	**85.47**	**63.35**	**50.18**	**66.34**	**66.17**	**47.12**	**32.46**	**48.58**
Ammonical form (NH_4^+)	40	80.30	46.53	33.10	**53.31**	58.00	23.73	12.06	**31.26**
	80	82.40	55.00	36.40	**57.93**	61.96	31.96	16.26	**36.73**
	120	85.86	44.66	18.43	**49.65**	60.13	23.86	7.66	**30.55**
	Mean	**82.85**	**48.73**	**29.31**	**53.63**	**60.03**	**26.52**	**12.00**	**32.85**
Combined form (NO_3^-+NH_4^+)	40	84.26	56.96	45.50	**62.24**	65.20	43.50	25.73	**44.81**
	80	85.86	64.53	53.53	**67.97**	67.50	50.05	43.76	**53.92**
	120	87.20	75.30	61.53	**74.67**	68.90	57.80	52.20	59.63
	Mean	**85.77**	**65.60**	**53.52**	**68.30**	**67.10**	**50.60**	**40.56**	**52.78**
Overall mean		**84.70**	**59.23**	**44.34**		**64.47**	**41.41**	**28.34**	

C.D. (P<0.05) for Ns/Nl/Sl = 1.161
C.D. (P<0.05) for NsxNl, Nl x Sl & Ns x Sl = 2.010
C.D. (P<0.05) for Ns x Nl x Sl = 3.482

C.D. (P<0.05) for Ns/Nl/Sl = 1.071
C.D. (P<0.05) for NsxNl, Nl x Sl & Ns x Sl = 1.855
C.D. (P<0.05) for Ns x Nl x Sl = 3.212

Ns = Nitrogen source; Nl = Nitrogen level; Sl =Salinity level

(55.82 and 60.00%) and in combined form minimum reduction (24.70 and 39.64%) as compared to control plants.

Nitrogen in combined form (120 kg ha^{-1}) resulted in the relatively lesser per cent reduction in relative growth rate of plant under saline condition (8 and 12 dSm^{-1}) over control plants. However, maximum per cent reduction was noticed with ammonical form. Beside this, the higher level of nitrogen (120 kg ha^{-1}) in combined form showed minimum reduction (16.11 and 24.23 %) in relative growth rate at a salinity level of 8 and 12 dSm^{-1}, respectively, as compare to non-saline plants. Such reduction was maximum (33.28% and 60.53%) at a lower level of nitrogen application (40 kg ha^{-1}).

The relative growth rate of plant showed similar trend at both sampling i.e. 45 and 65 DAS. However, the magnitude was relatively higher at 65 DAS.

Plant height

Table 8 showed that plant treated with combined form of nitrogen and exposed to salinity at 35 days after sowing (Stage I) contained maximum plant height at harvest as compared to ammonical form.

Under saline condition, the plant height decreased significantly with every increment in salinity as compared to non-saline conditions irrespective of nitrogen source used. The plant treated with ammonical form of nitrogen exhibited maximum per cent reduction (15.50 and 24.11) and minimum per cent reduction (14.19 and 19.60) with combined form as compared to non-saline plants.

Nitrogen in combined form (120 kg ha^{-1}) resulted in relatively higher plant height under saline condition over control. However, short plant

Table 8. **Effect of nitrogen source, levels and their interaction with salinity on plant height (cm) in *Brassica juncea* cv. RH-30 at harvest**

Nitrogen sources	Nitrogen levels (kg ha^{-1})	Salinity levels (dSm^{-1})							
		0	8	12	**Mean**	0	8	12	**Mean**
		Stage-I				Stage-II			
Nitrate form (NO_3^-)	40	99.1	84.5	75.6	**86.4**	94.6	78.1	69.1	**80.6**
	80	113.0	99.4	91.5	**101.3**	111.8	94.3	85.6	**97.2**
	120	119.0	107.7	99.3	**108.6**	115.4	101.2	93.4	**103.3**
	Mean	**110.3**	**97.2**	**88.8**	**98.7**	**107.2**	**91.2**	**82.7**	**93.7**
Ammonical form (NH_4^+)	40	94.7	84.8	70.9	**83.4**	89.0	70.1	61.4	**73.5**
	80	113.9	95.0	87.8	**98.9**	108.1	91.7	83.1	**94.3**
	120	105.1	85.2	79.3	**89.8**	99.2	79.5	73.5	**84.0**
	Mean	**104.5**	**88.3**	**79.3**	**90.7**	**98.7**	**80.4**	**72.6**	**83.9**
Combined form (NO_3^-+NH_4^+)	40	110.3	96.3	86.1	**97.5**	106.4	87.1	79.3	**90.9**
	80	127.1	103.3	101.7	**110.7**	122.6	104.8	95.7	**107.7**
	120	128.4	114.5	106.2	**116.3**	126.3	110.5	101.5	**112.7**
	Mean	**121.9**	**104.6**	**98.0**	**108.1**	**118.4**	**100.8**	**92.1**	
Overall mean		**112.0**	**96.0**	**88.0**		**108.1**	**90.8**	**82.4**	

C.D. (P<0.05) for Ns/Nl/Sl = 2.637
C.D. (P<0.05) for NsxNl, Nl x Sl & Ns x Sl = 4.566
C.D. (P<0.05) for Ns x Nl x Sl = 7.908

C.D. (P<0.05) for Ns/Nl/Sl = 2.661
C.D. (P<0.05) for NsxNl, Nl x Sl & Ns x Sl = 4.611
C.D. (P<0.05) for Ns x Nl x Sl = N.S.

Ns = Nitrogen source; Nl = Nitrogen level; Sl =Salinity level

height was notice with ammonical form of nitrogen. Beside this, the higher level of nitrogen (120 kg/ha) in combined form showed minimum reduction (10.82 and 17.28%) in the plant height at salinity level of 8 and 12 dSm^{-1} respectively as compare to control plants. Such reduction was observe maximum (12.78 and 21.94%) at lower level of fertilizer application i.e. 40 kg ha^{-1}.

A refercnce of data in Table 8 revealed that the highest plant height at harvest was observed when plant treated with combined form of nitrogen and exposed to salinity at 55 days after sowing (Stage II), as compared ammonical form.

With increase in salinity level from 8 to 12 dSm^{-1} a corresponding reduction in plant height was noticed as compared to non-saline plants. These reductions were maximum (18.54 and 26.44%) and minimum (14.86 and 22.21%) with the application of ammonical and combined form of nitrogen respectively as compared to control plants.

Increasing level of nitrogen source at the rate of 80 and 120 kg ha^{-1} significant increase in plant height as compared to 40 kg ha^{-1} of nitrogen application. In addition to this, the plant treated with combined form @ 120 kg ha^{-1} under saline condition caused maximum increase as compared to lower level of nitrogen application i.e. 40 kg ha^{-1}. The interactive effect between salinity and different levels of nitrogen source had been found non-significant.

The plant treated with basal dose of nitrogen fertilizer and saline water application at 35 and 55 days after sowing (Stage I and II), showed similar trend in plant height at harvest. However, the maximum plant height was observed in plant treated at 35 days after sowing (Stage I).

Table 9. **Effect of nitrogen source, levels and their interaction with salinity on number of branches per plant in *Brassica juncea* cv. RH-30 at harvest**

Nitrogen sources	Nitrogen levels (kg ha^{-1})	Salinity levels (dSm^{-1})							
		0	8	12	**Mean**	0	8	12	**Mean**
		Stage-I				Stage-II			
Nitrate form (NO_3^-)	40	5.00	4.66	4.00	**4.55**	4.66	4.00	3.33	**4.00**
	80	5.33	5.00	4.33	**4.88**	5.00	4.33	4.00	**4.44**
	120	6.00	5.66	5.00	**5.55**	5.33	5.00	4.66	**5.00**
	Mean	**5.44**	**5.11**	**4.44**	**5.00**	**5.00**	**4.44**	**4.00**	**4.48**
Ammonical form (NH_4^+)	40	4.66	4.00	3.66	**4.11**	4.33	3.66	3.00	**3.66**
	80	5.00	4.33	4.00	**4.44**	4.66	4.00	3.33	**4.00**
	120	5.00	4.00	3.33	**4.11**	4.66	3.33	2.66	**3.55**
	Mean	**4.88**	**4.11**	**3.66**	**4.22**	**4.55**	**3.66**	**3.00**	**3.74**
Combined form (NO_3^-+NH_4^+)	40	5.00	4.66	4.33	**4.66**	4.66	4.00	3.66	**4.11**
	80	5.66	5.33	5.00	**5.33**	5.33	4.66	4.33	**4.77**
	120	6.33	6.00	5.66	**6.00**	6.00	5.33	5.00	**5.44**
	Mean	**5.66**	**5.33**	**5.00**	**5.33**	**5.33**	**4.66**	**4.33**	**4.77**
Overall mean		**5.33**	**4.85**	**4.37**		**4.96**	**4.25**	**3.77**	

C.D. (P<0.05) for Ns/Nl/Sl = 0.710
C.D. (P<0.05) for NsxNl, Nl x Sl & Ns x Sl = N.S.
C.D. (P<0.05) for Ns x Nl x Sl = N.S.

C.D. (P<0.05) for Ns/Nl/Sl = 0.639
C.D. (P<0.05) for NsxNl, Nl x Sl & Ns x Sl = N.S.
C.D. (P<0.05) for Ns x Nl x Sl = N.S.

Ns = Nitrogen source; Nl = Nitrogen level; Sl =Salinity level

Number of branches per plant

A close examination of data in Table 9 revealed that plant treated with combined form of nitrogen and exposed salinity at 35 and 55 DAS had been found to retain maximum number of branches per plant at harvest as compared to ammonical form. The interactive effect of salinity x level of nitrogen and salinity x level of nitrogen x source found to be non-significant.

WATER RELATIONS OR PHYSIOLOGICAL OBSERVATIONS

Physiological observations were recorded after salinization (Stage I and II) and sampling at 45 and 65 DAS. The observations were recorded ten days after salinization of plants. The physiological parameters such as, relative water content, water potential, osmotic potential, photosynthetic rate, transpiration rate and stomatal conductance were studied. The results obtained are given below:

Relative water content

A critical examination of data in Table 10 revealed that plant treated with combined form of nitrogen had been found to high relative water cotnent in leaves at 45 DAS as compared to ammonical form.

With increase in salinity level from 8 to 12 dSm^{-1} a corresponding reduction in relative water content in leaves was noticed as compared to non-saline plants. These reductions under salinity were maximum (17.58 and 27.95%) and minimum (13.49 and 22.16%), with the application of ammonical and combined form of nitrogen, respectively as compared to control plants.

Different source of nitrogen at the rate of 80 and 120 kg ha^{-1} resulted in significant increase relative water content in leaves as compared to 40

Table 10. **Effect of nitrogen source, levels and their interaction with salinity on relative water content (%) in leaf of *Brassica juncea* cv. RH-30**

Nitrogen sources	Nitrogen levels (kg ha^{-1})	Salinity levels (dSm^{-1})							
		0	8	12	**Mean**	0	8	12	**Mean**
		Pre-flowering stage (45 DAS)				Flowering stge (65 DAS)			
Nitrate form (NO_3^-)	40	87.37	73.33	62.63	**74.74**	87.06	70.46	60.82	**72.78**
	80	89.54	75.87	68.57	**77.99**	88.62	74.50	65.26	**76.12**
	120	92.58	80.67	73.52	**82.26**	90.23	77.95	69.94	**79.37**
	Mean	**89.83**	**76.62**	**68.24**	**78.23**	**88.64**	**74.30**	**65.34**	**76.09**
Ammonical form (NH_4^+)	40	86.51	70.04	59.81	**72.12**	85.45	64.34	57.24	**69.01**
	80	88.73	74.79	65.46	**76.32**	87.64	72.57	62.62	**74.28**
	120	89.59	73.45	65.53	**76.19**	87.19	70.83	62.22	**73.41**
	Mean	**88.28**	**72.76**	**63.60**	**74.88**	**86.76**	**69.25**	**60.69**	**72.23**
Combined form (NO_3^-+NH_4^+)	40	88.64	74.50	63.35	**75.49**	87.69	72.46	63.26	**74.47**
	80	90.53	78.43	72.17	**80.38**	87.93	75.70	69.49	**77.70**
	120	93.23	82.72	76.50	**84.15**	91.54	82.38	74.61	**82.84**
	Mean	**90.80**	**78.55**	**70.67**	**80.01**	**89.05**	**76.84**	**69.12**	**78.34**
Overall mean		**89.64**	**75.98**	**67.50**		**88.15**	**73.46**	**65.05**	

C.D. (P<0.05) for Ns/Nl/Sl = 0.934
C.D. (P<0.05) for NsxNl, Nl x Sl & Ns x Sl = 1.617
C.D. (P<0.05) for Ns x Nl x Sl = N.S.

C.D. (P<0.05) for Ns/Nl/Sl = 1.112
C.D. (P<0.05) for NsxNl, Nl x Sl & Ns x Sl = 1.926
C.D. (P<0.05) for Ns x Nl x Sl = N.S.

Ns = Nitrogen source; Nl = Nitrogen level; Sl =Salinity level

kg/ha nitrogen application. In addition to this, the plant treated with combined form (120 kg ha^{-1}) under saline condition (12 dSm^{-1}) showed maximum per cent increase (11.47) as compared to lower level of nitrogen application (40 kg ha^{-1}). However, minimum increase was noticed with ammonical form of nitrogen application.

A reference of data in table 10 revealed that relative water content in leaves was highest in plant treated with combined form of nitrogen as compared to ammonical form at 65 DAS.

Under saline condition the maximum (20.18 and 30.04%) and minimum (13.71 and 22.38%) reduction was observed with ammonical and combined form, respectively as compared to non-saline plants.

With increase in the level of nitrogen fertilizer, significant increase in relative water content irrespective of different level of nitrogen source. The applicaiton of 120 kg/ha nitrogen in combined form resulted maximum increase (11.23%) as compared to lower level of nitrogen application (40 kg ha^{-1}). However, minimum increase was noticed with ammonical form of nitrogen applicaiton. The interaction was found non-significant.

Relative water content in leaves showed similar trend at both sampling stages. However, magnitude was relatively higher at 45 DAS.

Leaf water potential

Critial examination of data in Table 11 revealed that plant treated with combined form of nitrogen had been found to contain higher water potential at first sampling (45 DAS) as compared to ammonical form.

With increase in salinity level from 8 to 12 dSm^{-1} a significant declined in water potential as comapred to non-saline plants. However, the maximum (25.08 and 32.33%) and minimum (12.21 and 19.36%)

Table 11. ***Effect of nitrogen source, levels and their interaction with salinity on water potential (-Mpa) in leaf of Brassica juncea* cv. RH-30**

Nitrogen sources	Nitrogen levels (kg ha^{-1})	Salinity levels (dSm^{-1})							
		0	8	12	**Mean**	0	8	12	**Mean**
		Pre-flowering stage (45 DAS)				Flowering stge (65 DAS)			
Nitrate form (NO_3^-)	40	0.575	0.700	0.751	**0.675**	0.625	0.774	0.825	**0.741**
	80	0.475	0.550	0.574	**0.533**	0.524	0.625	0.651	**0.600**
	120	0.451	0.500	0.524	**0.491**	0.474	0.551	0.574	**0.533**
	Mean	**0.500**	**0.583**	**0.616**	**0.566**	**0.541**	**0.650**	**0.683**	**0.625**
Ammonical form (NH_4^+)	40	0.600	0.751	0.800	**0.717**	0.651	0.825	0.874	**0.783**
	80	0.524	0.625	0.674	**0.607**	0.574	0.701	0.725	**0.667**
	120	0.575	0.750	0.775	**0.700**	0.625	0.825	0.851	**0.767**
	Mean	**0.566**	**0.708**	**0.749**	**0.674**	**0.617**	**0.784**	**0.817**	**0.739**
Combined form (NO_3^-+NH_4^+)	40	0.550	0.650	0.700	**0.633**	0.620	0.725	0.774	**0.706**
	80	0.475	0.524	0.551	**0.516**	0.525	0.534	0.625	**0.561**
	120	0.400	0.425	0.450	**0.425**	0.451	0.501	0.525	**0.492**
	Mean	**0.475**	**0.533**	**0.567**	**0.524**	**0.532**	**0.587**	**0.641**	**0.587**
	Overall mean	**0.513**	**0.608**	**0.644**		**0.563**	**0.673**	**0.714**	

C.D. (P<0.05) for Ns/Nl/Sl = 0.026
C.D. (P<0.05) for NsxNl, Nl x Sl & Ns x Sl = 0.063
C.D. (P<0.05) for Ns x Nl x Sl = 0.095

C.D. (P<0.05) for Ns/Nl/Sl = 0.035
C.D. (P<0.05) for NsxNl, Nl x Sl & Ns x Sl = 0.078
C.D. (P<0.05) for Ns x Nl x Sl = 0.105

Ns = Nitrogen source; Nl = Nitrogen level; Sl =Salinity level

reduction under saline condition was noticed with ammonical and combined forms of nitrogen, respectively as comapred to control plants.

The treatment with combined form of nitrogen (120 kg ha^{-1}) exhibtied minimum reduction of water potential in leaves as compared to non-saline plants. But maximum reduction was noticed with ammonical form of nitrogen. Beside this, application of 120 kg/ha nitrogen in combined form showed less decline (6.25 and 12.50%) in water potential at salinity level of 8 and 12 dSm^{-1} as compared to non-saline plants. However, higher reduction was observed at 40 kg ha^{-1} nitrogen application.

Plant treated with combined form of nitrogen resulted more upsurge in water potential of leaf at 65 DAS as compared to ammonical form of nitrogen. With increase in salinity, a corresponding reduction in water potential of leaf as compared to non-saline plants. These reductions under salinity was maximum (27.06 and 32.41%) and minimum (10.33 and 20.48%) with ammonical and combined forms, respectively as compared to control plants. Application of nitrogen (120 kg ha^{-1}) in combined form had been found to cause minimum reduction in water potential as comapred to non-saline plants. However, maximum reduction was osberved with ammonical form. In addition to this, nitrogen at the rate of 120 kg ha^{-1} in the combined form showed minimum reduction (11.08 and 16.40%) of water potential as compared to control plants at salinity level of 8 and 12 dSm^{-1}, respectively. However, maximum reduction (16.93 and 24.83%) was noticed at lower level of nitrogen application i.e. 40 kg ha^{-1}.

Water potential in leaf exhibited similar trend when plant treated with basal dose of nitrogen fertilizer and exposed to salinity at two stages i.e. Stage I and Stage II. However, the magnitude was relatively higher at 45 DAS.

Osmotic Potential

Critial examination of data in Table 12 revealed that plant treated with combined form of nitrogen had been found to contain higher water potential at first sampling (45 DAS) as compared to ammonical form.

With increase in salinity levle from 8 to 12 dSm^{-1} a significant declined in water potential as comapred to non-saline plants. However, the maximum (25.31 and 41.89%) and minimum (13.62 and 27.60%) reduction under saline condition was noticed with ammonical and combined forms of nitrogen, respectively as compared to control plants.

The treatment with combined form of nitrogen (120 kg ha^{-1}) exhibtied minimum reduction of water potential in leaves as compared to non-saline plants. But maximum reduction was noticed with ammonical form of nitrogen. Beside this, application of 120 kg ha^{-1} nitrogen in combined form showed less decline in water potential at salinity level of 8 and 12 dSm^{-1} as compared to non-saline plants. However, higher reduction was observed at 40 kg ha^{-1} nitrogen application.

Plant treated with combined form of nitrogen resulted more upsurge in water potential of leaf at 65 DAS as compared to ammonical form of nitrogen. With increase in salinity, a corresponding reduction in water potential of leaf as compared to non-saline plants. These reductions under salinity was maximum (25.21 and 44.85%) and minimum (18.06 and 25.73%) with ammonical and combined forms, respectively as compared to control plants. Application of nitrogen (120 kg ha^{-1}) in combined form had been found to cause minimum reduction in water potential as compared to non-saline plants. However, maximum reduction was observed with ammonical form. In addition to this, nitrogen at the rate of 120 kg ha^{-1} in

Table 12. **Effect of nitrogen source, levels and their interaction with salinity on osmotic potential (-Mpa) in leaf of *Brassica juncea* cv. RH-30**

Nitrogen sources	Nitrogen levels (kg ha^{-1})	Salinity levels (dSm^{-1})							
		0	8	12	**Mean**	0	8	12	**Mean**
		Pre-flowering stage (45 DAS)				Flowering stge (65 DAS)			
Nitrate form (NO_3^-)	40	1.109	1.300	1.488	**1.299**	1.224	1.578	1.696	**1.499**
	80	1.173	1.339	1.492	**1.335**	1.296	1.634	1.765	**1.565**
	120	1.233	1.404	1.540	**1.392**	1.410	1.687	1.724	**1.607**
	Mean	**1.172**	**1.348**	**1.507**	**1.342**	**1.310**	**1.633**	**1.729**	**1.557**
Ammonical form (NH_4^+)	40	1.213	1.588	1.692	**1.497**	1.247	1.627	1.753	**1.542**
	80	1.329	1.619	1.716	**1.555**	1.368	1.694	1.765	**1.609**
	120	1.511	1.871	2.346	**1.908**	1.585	1.939	2.566	**2.030**
	Mean	**1.351**	**1.693**	**1.917**	**1.653**	**1.400**	**1.753**	**2.028**	**1.727**
Combined form (NO_3^-+NH_4^+)	40	0.987	1.149	1.364	**1.167**	1.207	1.495	1.594	**1.432**
	80	1.167	1.324	1.454	**1.315**	1.277	1.516	1.637	**1.477**
	120	1.215	1.354	1.479	**1.349**	1.385	1.557	1.635	**1.526**
	Mean	**1.123**	**1.276**	**1.433**	**1.277**	**1.220**	**1.523**	**1.622**	**1.478**
Overall mean		**1.215**	**1.439**	**1.619**		**1.333**	**1.636**	**1.793**	

C.D. (P<0.05) for Ns/Nl/Sl = 0.045
C.D. (P<0.05) for NsxNl, Nl x Sl & Ns x Sl = 0.075
C.D. (P<0.05) for Ns x Nl x Sl = 0.120

C.D. (P<0.05) for Ns/Nl/Sl = 0.020
C.D. (P<0.05) for NsxNl, Nl x Sl & Ns x Sl = 0.055
C.D. (P<0.05) for Ns x Nl x Sl = 0.087

Ns = Nitrogen source; Nl = Nitrogen level; Sl =Salinity level

the combined form showed minimum reduction of water potential as compared to control plants at salinity level of 8 and 12 dSm^{-1}, respectively. However, maximum reduction was noticed at lower level of nitrogen application i.e. 40 kg ha^{-1}.

Water potential in leaf exhibited similar trend when plant treated with basal dose of nitrogen fertilizer and exposed to salinity at two stages i.e. Stage I and Stage II. However, the magnitude was relatively higher at 45 DAS.

Photosynthetic rate

Fig. 1A represents that the photosynthetic rate in leaves was maximum in plants treated with nitrate form of nitrogen as compared to ammonical form at first sampling stage (45 DAS).

With increase in salinity level from 8 and 12 dSm^{-1} a corresponding reduction in rate of photosynthesis in leaves was noticed as compared to non-saline plants. These reduction under salinity were maximum (27.81 and 39.20%t) and minimum (16.76 and 32.70%) with the application of ammonical and nitrate forms of nitrogen, respectively as compared to control plants.

Application of nitrogen in nitrate form (120 kg ha^{-1}) resulted maximum increase in photosynthetic rate in leaves under saline condition over control. However, minimum increase was noticed with ammonical form of nitrogen. Beside this, the higher level of nitrogen (120 kg ha^{-1}) in nitrate form exhibited minimum reduction (13.92 and 27.10%) in photosynthetic rate at a salinity level of 8 and 12 dSm^{-1}, respectively as compared to non-saline plants. Such reduction was maximum (18.99 and 39.94%) at lower level of nitrogen fertilizer application.

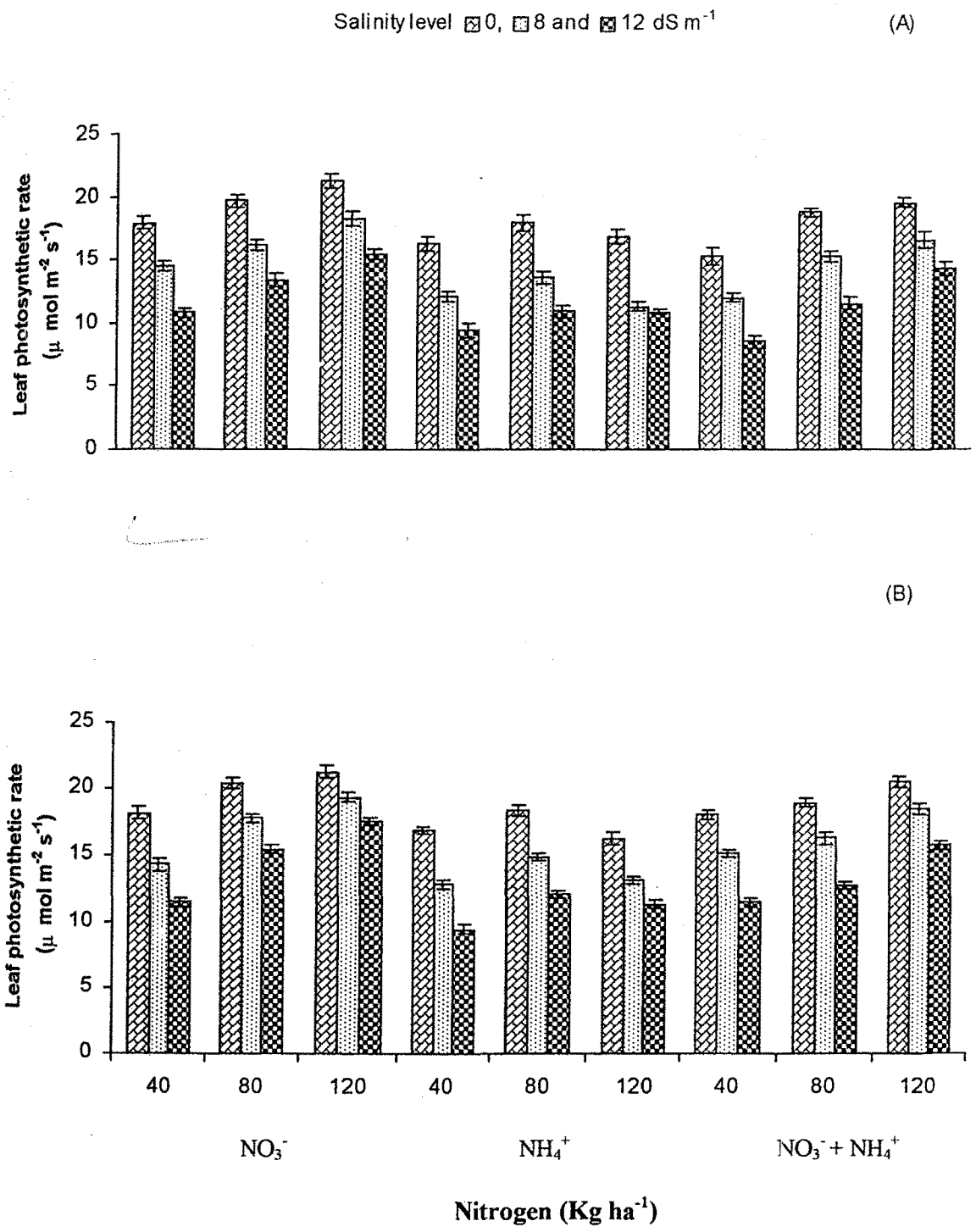

Fig. 1 Effect of nitrogen source, levels and their interaction with salinity on leaf photosynthetic rate at (A) 45 DAS and (B) 65 DAS in *Brassica juncea* cv. RH-30.

The plant treated with nitrate form of nitrogen had been found to be highest photosynthetic rate in leaves at 65 DAS as compared to ammonical form (Fig. 1B).

Under saline condition, the photosynthetic rate in leaves decreased significantly with every increment in salinity as compared to non-saline conditions irrespective of nitrogen source used. The plant treated with ammonical form of nitrogen showed maximum reduction (20.67 and 36.23 %) in photosynthetic rate and with nitrate form resulted minimum reduction (13.92 and 25.55%) as compared to non-saline plants.

Increasing level of nitrogen (80 and 120 kg ha^{-1}) irrespective of source of nitrogen showed significantly increase in photosynthetic rate of leaves except application of ammonical form at 120 kg ha^{-1}, as compared to lower level of nitrogen dose i.e. 40 kg ha^{-1}. In addition to this, the photosynthetic rate was maximum (22.08 and 32.37%) with the application of 80 and 120 kg ha^{-1} in nitrate form, respectively as compared to lower level of nitrogen i.e 40 kg ha^{-1}. The interactive effect between salinity and different levels of nitrogen sources had been found to be non-significant.

The photosynthetic rate in leaves showed that similar trend when plant treated with basal dose of nitrogen fertilizer and exposed to salinity. However, the magnitude was relativley higher at 65 DAS.

Transpiration rate

Fig. 2 A shows that transpiration rate in leaves was minimum in plant treated with nitrate form of nitrogen as compared to ammonical form at first sampling stage i.e. 45 DAS.

With increase in salinity level from 8 and 12 dSm^{-1} a significant reduction in transpiration rate was observed as compared to non-saline

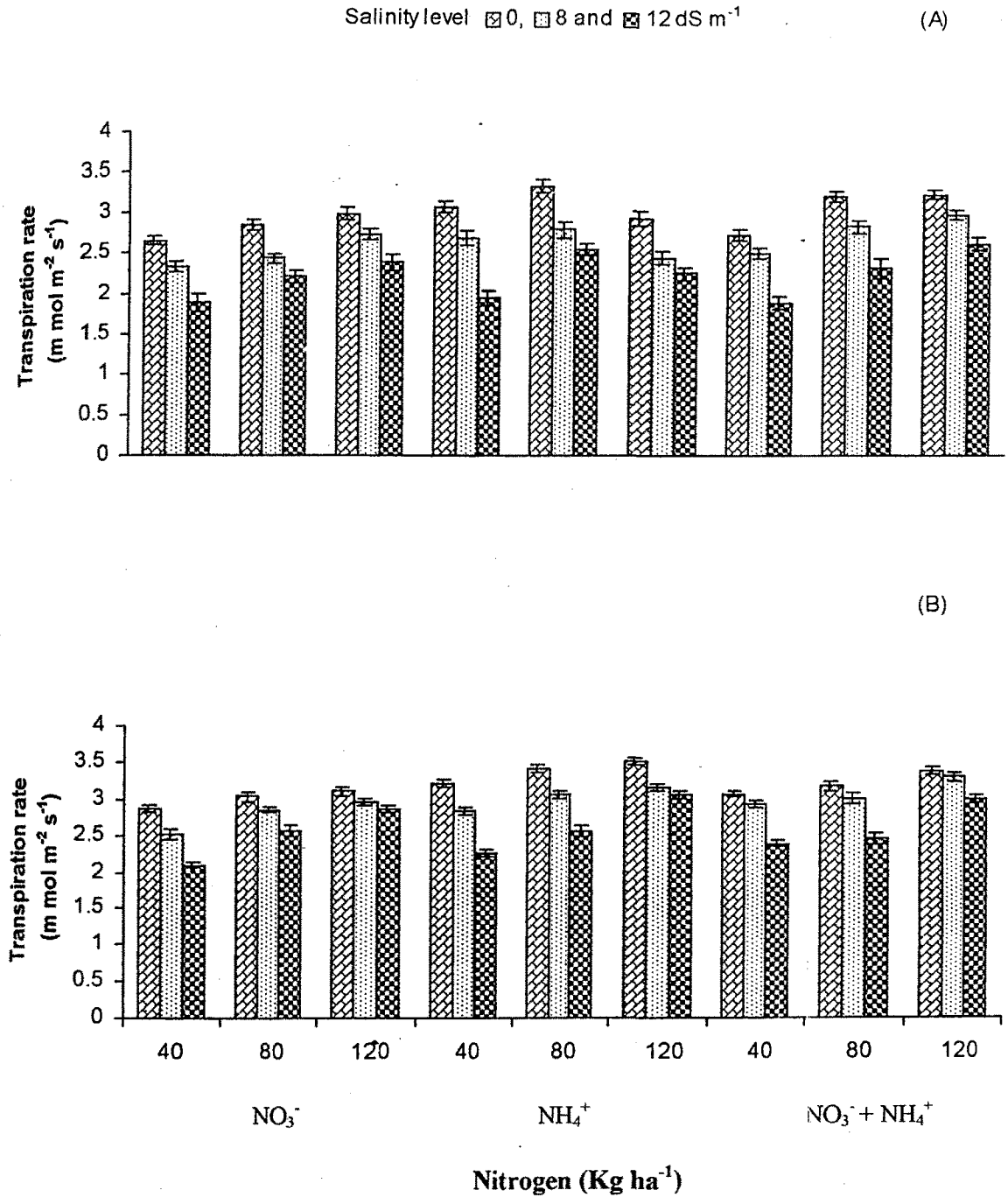

Fig. 2 Effect of nitrogen source, levels and their interaction with salinity on transpiration rate at (A) 45 DAS and (B) 65 DAS in *Brassica juncea* cv. RH-30.

plants. These reduction under salinity were maximum (14.79 and 27.65%) and minimum (11.66 and 23.67%) with application of ammonical and nitrate form of nitrogen, respectively as compared to control plants.

Application of nitrogen in nitrate form (120 kg ha^{-1}) resulted in the relatively lesser reduction in transpiration rate under saline condition (8 and 12 dSm^{-1}) over control plants. However, maximum reduction was observed with ammonical form. Beside this, the higher level of nitrogen (120 kg ha^{-1}) in nitrate form showed minimum (8.36 and 20.06%) reduction in transpiration rate at a salinity level of 8 and 12 dSm^{-1}, respectively, as compared to non-saline plants. Such reductions were maximum (12.07 and 29.05%) at lower level or nitrogen i.e. 40 kg ha^{-1}.

The plant treated with nitrate form of nitrogen had been found to cause lowest transpiration rate in leaves at 65 DAS as compared to ammonical form (Fig. 2B).

Under saline condition, the transpiration rate in leaves decreased significantly with every increment in salinity as compared to non-saline plants. The plant treated with ammonical form of nitrogen resulted in maximum reduction (10.35 and 22.18%) in transpiration rate and nitrate form of nitrogen showed minimum reduction (7.64 and 16.61%) as compared to non-saline plants.

Nitrogen in nitrate form (120 kg ha^{-1}) showed the minimum reduction in transpiration rate of leaves under saline condition over control plants. However, maximum reduction was observed with ammonical form of nitrogen. In addition to this, the higher level of nitrogen (120 kg ha^{-1}) in nitrate forms resulted in minimum reduction (4.18 and 8.03%) in transpiration rate at salinity levels of 8 and 12 dSm^{-1}, respectively , as

compare to control plants. Such reduction was maximum (12.19 and 26.82%) at lower level of nitrogen application i.e. 40 kg ha^{-1}.

The transpiration rate in leaves showed similar trend at both the sampling stages (45 and 65 DAS). However, the magnitude was relatively higher at 65 DAS.

Stomatal conductance

Fig. 3A represents that the stomatal conductance in leaves was highest in plant treated with nitrate form of nitrogen as compared to ammonical form at first sampling (45 DAS).

With increase in salinity level from 8 and 12 dSm^{-1} a corresponding reduction in stomatal conductance of leaves was observed as compared to non-saline plants. These reduction under salinity were maximum (18.45 and 37.85%) and minimum (15.86 and 26.56%) with the application of ammonical and nitrate form of nitrogen, respectively as compared to control plants.

Nitrogen in nitrate form (120 kg ha^{-1}) showed minimum reduction in stomatal conductance in leaves under saline condition (8 and 12 dSm^{-1}) over control. However, maximum reduction was observed with ammonical form of nitrogen. In addition to this, the higher level of nitrogen (120 kg ha^{-1}) in nitrate form exhibited lower reduction (10.54 and 21.57%) at salinity levels 8 and 12 dSm^{-1} respectively as compared to non-saline plants. Such reduction was observed maximum (19.05 and 31.60%) at lower level of fertilizer application.

The plant treated with combined form of nitrogen had been found to maximum stomatal conductance in leaves at 65 DAS as compared to ammonical form (Fig. 3B).

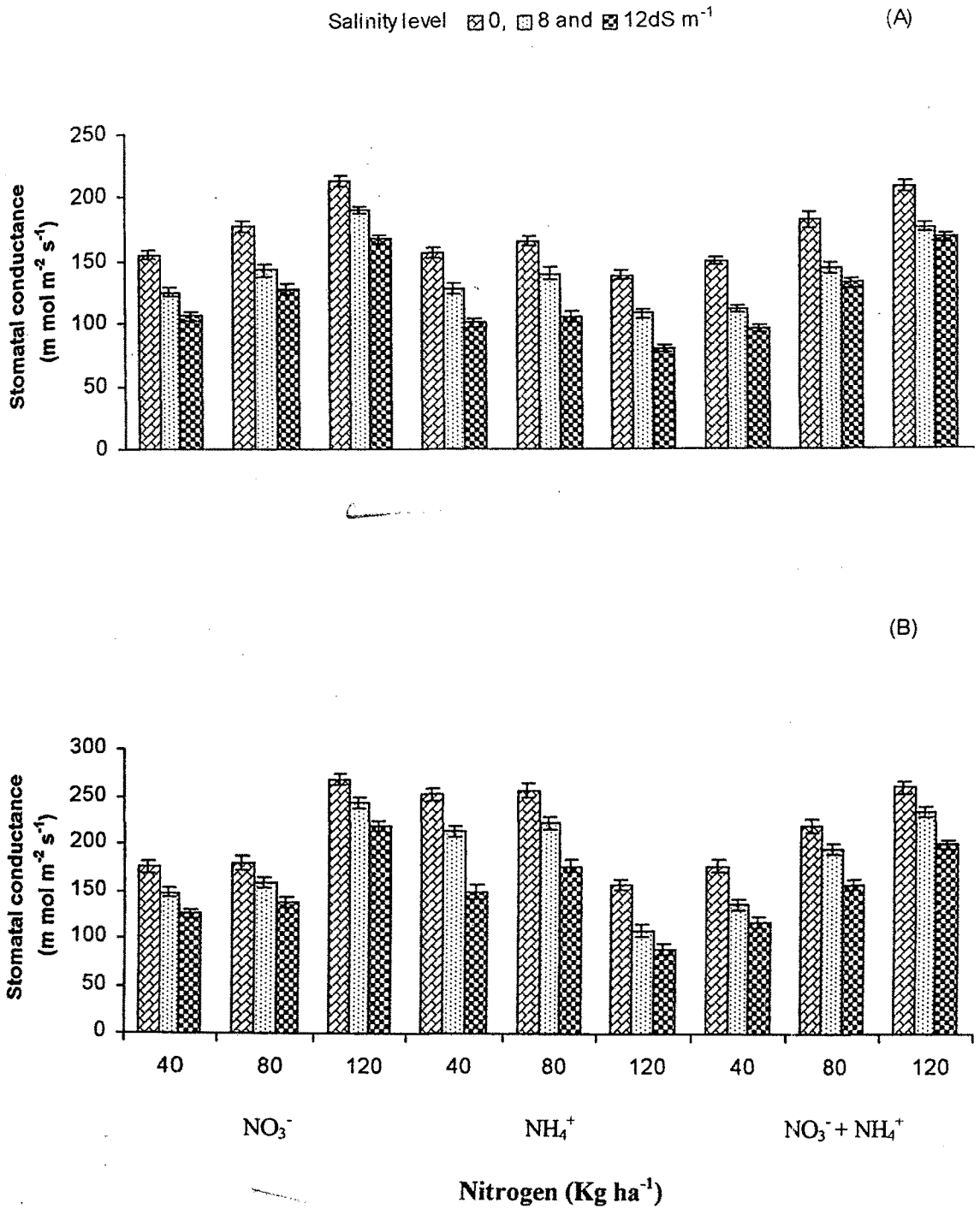

Fig. 3 Effect of nitrogen source, levels and their interaction with salinity on stomatal conductance at (A) 45 DAS and (B) 65 DAS in *Brassica juncea* cv. RH-30.

Under saline condition (8 and 12 dSm^{-1}), the stomatal conductance in leaves decreased significantly with every increment in salinity as compared to non-saline condition irrespective of nitrogen source used. The plant treated with ammonical form of nitrogen resulted in maximum reduction (18.33 and 37.77%) and minimum reduction (11.68 and 22.25%) with nitrate form as compared to control plants.

Application of nitrogen in nitrate form (120 kg ha^{-1}) resulted in the relatively lower reduction in stomatal conductance under saline condition (8 and 12 dSm^{-1}) over control plants. However, maximum reduction was noticed with ammonical form. Beside this, the higher level of nitrogen (120 kg ha^{-1}) in nitrate form showed minimum reduction (9.45 and 18.16%) in stomatal conductance at salinity levels 8 and 12 dSm^{-1}, respectively, as compare to non-saline plants. Such reduction was maximum (15.26 and 28.05%) at lower level of nitrogen i.e. 40 kg ha^{-1}.

The stomatal conductance in leaves showed similar trend at both the sampling stages (45 and 65 DAS). However, the magnitude was relatively higher at 65 DAS.

BIOCHEMICAL PARAMETERS

The biochemical estimations were made after 10 days of salinity treatment. The experimental findings were as given below:

Total chlorophyll content

A critical examination of data in Table 13 revealed that plant treated with combined form of nitrogen had been found to contain highest chlorophyll content in the leaves at 45 DAS as compared to ammonical form.

Table 13. **Effect of nitrogen source, levels and their interaction with salinity on total chlorophyll content (mg g^{-1} dry weight) in leaf of *Brassica juncea* cv. RH-30**

Nitrogen sources	Nitrogen levels (kg ha^{-1})	Salinity levels (dSm^{-1})							
		0	8	12	**Mean**	0	8	12	**Mean**
		45 DAS				65 DAS			
Nitrate form (NO_3^-)	40	18.89	13.64	10.72	**14.42**	14.29	11.80	8.91	**11.67**
	80	19.23	14.71	11.92	**15.29**	16.56	14.02	12.17	**14.25**
	120	19.43	15.76	14.25	**16.48**	18.35	16.39	14.70	**16.48**
	Mean	**19.18**	**14.70**	**12.30**	**15.39**	**16.40**	**14.07**	**11.93**	**14.13**
Ammonical form (NH_4^+)	40	14.33	10.21	7.75	**10.76**	13.51	11.02	7.95	**10.83**
	80	15.73	11.92	9.33	**12.32**	14.25	11.94	9.63	**11.94**
	120	16.25	12.47	11.29	**13.34**	14.61	11.49	9.82	**11.97**
	Mean	**15.44**	**11.53**	**9.46**	**12.14**	**14.12**	**11.48**	**9.13**	**11.58**
Combined form (NO_3^-+NH_4^+)	40	19.13	14.31	11.72	**15.05**	15.80	13.22	10.79	**13.27**
	80	20.79	16.32	13.56	**16.89**	16.19	13.93	12.10	**14.07**
	120	22.43	18.02	16.04	**18.83**	18.23	15.82	14.71	**16.25**
	Mean	**20.78**	**16.21**	**13.77**	**16.92**	**16.74**	**14.32**	**12.53**	**14.53**
Overall mean		**18.49**	**14.15**	**11.84**		**15.75**	**13.29**	**11.20**	

C.D. (P<0.05) for Ns/Nl/Sl	= 0.208	C.D. (P<0.05) for Ns/Nl/Sl	= 0.187
C.D. (P<0,05) for NsxNl, Nl x Sl & Ns x Sl	= 0.360	C.D. (P<0.05) for NsxNl, Nl x Sl & Ns x Sl	= 0.323
C.D. (P<0.05) for Ns x Nl x Sl	= N.S.	C.D. (P<0.05) for Ns x Nl x Sl	= N.S.

Ns = Nitrogen source; Nl = Nitrogen level; Sl =Salinity level

With increase in salinity level from 8 to 12 dSm^{-1} a corresponding reduction in chlorophyll content in leaves was noticed as compared to non-saline plants. These reductions under salinity were maximum (25.32 and 38.73%) and minimum (21.99 and 33.73%) with the application of ammonical and combined form of nitrogen, respectively as compared to control plants.

Different source of nitrogen at the rate of 80 and 120 kg ha^{-1} resulted in significant increase in chlorophyll content in leaves as compared to 40 kg/ha of nitrogen application. In addition to this, the plant treated with combined form (120 kg ha^{-1}) under saline condition (12 dSm^{-1}) showed maximum per cent increase (25.11) as compared to lower level of nitrogen application i.e. 40 kg ha^{-1}. The interactive effect between salinity and different level of nitrogen source had been found to be non significant.

A reference of data in Table 13 revealed that chlorophyll content in leaves was hightest in plants treated with combined form of nitrogen as compared to ammonical form at second sampling stage (65 DAS).

Under saline condition the maximum (18.69 and 35.33%) and minimum (14.45 and 25.14%) reduction was observed with ammonical and combined form, respectively as compared to non-saline plants.

With increase in the level of nitrogen, significant increase in chlorophyll content irrespective of different level of nitrogen source. The applciation of 120 kg/ha in combined form resulted maximum increase as compared to lower level of nitrogen application (40 kg ha^{-1}). However, minimum increase was noticed with ammonical form of nitrogen.The interaction was found non-significant.

Chlorophyll content in leaves showed the similar trend at both the sampling stages (45 and 65 days). However, the magnitude was relatively higher at 45 DAS.

Carotenoid content

A close examination of data in Table 14 revealed that plant treated with combined form of nitrogen had been found to contain highest carotenoid content in the leaves as compared to ammonical form at first sampling stage (45 DAS).

Under saline condition (8 and 12 dSm^{-1}), the treatment with combined form of nitrogen resulted in minimum reduction (11.93 and 15.34 %) over non-saline plants in carotenoid content of leveas . However, the maximum reduction (18.23 and 16.35%) was noticed with ammonical form.

The higher level of nitrogen (80 and 120 kg ha^{-1}) irrespective of nitrogen source increase the carotenoid content of leaves significantly as compared to lower level of nitrogen application i.e. 40 kg ha^{-1}. In addition to this, the increased in carotenoid content was maximum with application of nitrogen @ 120 kg ha^{-1} in combined form as compared to lower level of nitrogen. However, minimum increase was observed with ammonical form of nitrogen. The interactive effect between salinity and different level of nitrogen sources had been found to be non-significant.

A study of data in Table 14 revealed that carotenoid content in leaves at 65 DAS was observed maximum with combined form of nitrogen as compared to ammonical form.

With increase in salinity level from 8 to 12 dSm^{-1} a corresponding reduction in carotenoid content in leaves was noticed as compared to non-

Table 14. Effect of nitrogen source, levels and their interaction with salinity on carotenoid content (mg g^{-1} dry weight) in leaf of *Brassica juncea* cv. RH-30

Nitrogen sources	Nitrogen levels (kg ha^{-1})	Salinity levels (dSm^{-1})							
		0	8	12	**Mean**	0	8	12	**Mean**
		45 DAS				65 DAS			
Nitrate form (NO_3^-)	40	1.62	1.38	1.29	**1.43**	1.75	1.42	1.32	**1.50**
	80	1.74	1.51	1.49	**1.58**	1.86	1.64	1.53	**1.68**
	120	1.82	1.60	1.61	**1.67**	1.97	1.83	1.72	**1.84**
	Mean	**1.72**	**1.50**	**1.46**	**1.56**	**1.86**	**1.63**	**1.52**	**1.67**
Ammonical form (NH_4^+)	40	1.65	1.28	1.57	**1.50**	1.73	1.37	1.29	**1.46**
	80	1.67	1.34	1.28	**1.43**	1.79	1.49	1.43	**1.57**
	120	1.46	1.30	1.13	**1.29**	1.55	1.33	1.26	**1.38**
	Mean	**1.59**	**1.30**	**1.33**	**1.41**	**1.69**	**1.40**	**1.33**	**1.47**
Combined form (NO_3^-+NH_4^+)	40	1.63	1.39	1.26	**1.43**	1.76	1.44	1.33	**1.51**
	80	1.75	1.55	1.51	**1.61**	1.88	1.68	1.54	**1.70**
	120	1.89	1.71	1.68	**1.76**	1.98	1.84	1.74	**1.85**
	Mean	**1.76**	**1.55**	**1.49**	**1.60**	**1.87**	**1.65**	**1.54**	**1.69**
Overall mean		**1.69**	**1.45**	**1.42**		**1.81**	**1.56**	**1.46**	

C.D. (P<0.05) for Ns/Nl/Sl	=	0.071	C.D. (P<0.05) for Ns/Nl/Sl = 0.025
C.D. (P<0.05) for NsxNl, Nl x Sl & Ns x Sl	=	0.124	C.D. (P<0.05) for NsxNl, Nl x Sl & Ns x Sl = 0.043
C.D. (P<0.05) for Ns x Nl x Sl	=	N.S.	C.D. (P<0.05) for Ns x Nl x Sl = N.S.

Ns = Nitrogen source; Nl = Nitrogen level; Sl =Salinity level

saline plants. These reduction under salinity were maximum (17.15 and 21.30%) and minimum (11.76 and 17.64%) with combined and ammonical forms, respectively as compared to control plants.

The increasing level of nitrogen irrespective of source exhibited significant increase in carotenoid content as compared to lower level of nitrogen. Plant treated with 120 kg ha^{-1} nitrogen in combined form resulted in maximum increase (22.51%) as compared to 40 kg ha^{-1} nitrogen. However, such increase was observed minimum in ammonical form. The interaction between salinity and different level of nitrogen sources had been found to be non- significant.

The plant treated with basal dose of nitrogen fertilizer and saline water application at 45 and 65 DAS showed similar trend in carotenoid content of leaves. However, the magnitude was higher at 65 DAS.

Total soluble carbohydrate

A study of data in Table 15 revealed that total soluble carbohydrate content in leaves at 45 DAS was highest in plant treated with nitrate form of nitrogen as compared to ammonical form.

Under saline condition, the treatment with ammonical form of nitrogen resulted in maximum per cent reduction (42.16 and 87.85) in total soluble carbohydrate content of leaves over control plants. However, per cent reduction was minimum (29.84 and 47.71) with nitrate form of nitrogen application.

The treatment with nitrate form of nitrogen (120 kg ha^{-1}) exhibited minimum reduction of total soluble carbohydrate in leaves as compared to non-saline plants. But maximum reduction was noticed with ammonical form of nitrogen. Beside this, treatment with 120 kg ha^{-1} of nitrate form

Table 15. **Effect of nitrogen source, levels and their interaction with salinity on total soluble carbohydrates (mg g^{-1} dry weight) in leaf of *Brassica juncea* cv. RH-30**

Nitrogen sources	Nitrogen levels (kg ha^{-1})	Salinity levels (dSm^{-1})							
		0	8	12	**Mean**	0	8	12	**Mean**
		45 DAS				65 DAS			
Nitrate form (NO_3^-)	40	30.92	44.12	48.49	**41.18**	36.53	51.02	59.36	**48.97**
	80	46.64	59.60	68.77	**58.34**	47.67	60.61	69.84	**59.37**
	120	58.12	72.48	83.18	**71.26**	67.13	82.48	94.19	**81.27**
	Mean	**45.23**	**58.73**	**66.81**	**56.92**	**50.44**	**64.70**	**74.46**	**63.20**
Ammonical form (NH_4^+)	40	29.83	45.08	55.83	**43.58**	31.30	52.44	61.12	**48.29**
	80	36.45	49.79	58.82	**48.35**	39.48	61.42	67.39	**56.10**
	120	39.23	55.15	65.58	**53.32**	47.18	65.34	75.56	**62.69**
	Mean	**35.17**	**50.00**	**66.07**	**48.41**	**39.32**	**59.73**	**68.02**	**55.69**
Combined form (NO_3^-+NH_4^+)	40	32.34	42.85	50.31	**41.43**	35.96	53.10	60.42	**49.83**
	80	40.78	54.70	64.34	**53.27**	43.67	58.54	66.36	**56.19**
	120	56.12	71.90	84.82	**70.84**	63.23	78.90	91.53	**77.89**
	Mean	**43.08**	**56.48**	**66.39**	**55.32**	**47.62**	**63.51**	**72.77**	**61.30**
Overall mean		**41.16**	**55.07**	**62.65**		**45.79**	**62.65**	**71.75**	

C.D. (P<0.05) for Ns/Nl/Sl = 0.344
C.D. (P<0.05) for NsxNl, Nl x Sl & Ns x Sl = 0.551
C.D. (P<0.05) for Ns x Nl x Sl = 0.779

C.D. (P<0.05) for Ns/Nl/Sl = 0.240
C.D. (P<0.05) for NsxNl, Nl x Sl & Ns x Sl = 0.416
C.D. (P<0.05) for Ns x Nl x Sl = 0.720

Ns = Nitrogen source; Nl = Nitrogen level; Sl =Salinity level

showed less reduction (24.70 and 43.11%) in total soluble carbohydrate at salinity level 8 and 12 dSm^{-1} as compared to non-saline plants. However, highest reduction was observed at 40 kg ha^{-1} nitrogen application.

A reference of data in Table 15 revealed that plant treated with nitrate form of nitrogen resulted more upsurge in total soluble carbohydrate content in leaves at 65 DAS as compared to ammonical form of nitrogen.

With increase in salinity, a corresponding reduction in total soluble carbohydrate content in leaves was observed as compared to non-saline plants. These reduction under salinity were maximum (51.90 and 72.99 %) and minimum (28.27 and 47.62%) with ammonical and nitrate form respectively as compared to control plants.

Application of nitrogen (120 kg ha^{-1}) in nitrate form had been found to cause maximum accumulation of total soluble carbohydrate content in leaves as compared to non-saline plants. However, minimum accumulation was observed with ammonical form of nitrogen. In addition to this, nitrogen at the rate of 120 kg ha^{-1} in the nitrate form showed minimum reduction (22.86 and 40.30%) of total soluble carbohydrate content as compared to control plants. However, maximum reduction (39.66 and 62.49%) was noticed at lower level of nitrogen application i.e. 40 kg ha^{-1}.

Total soluble carbohydrate content in leaves exhibited similar trend when plant treated with basal dose of nitrogen fertilizer and exposed to salinity at two stages. However, the magnitude was relatively higher at 65 DAS.

Starch content

A critical examination of data in Table 16 revealed that plant treated with combined form of nitrogen had been found to contain maximum starch content in the leaves at 45 DAS as compared to ammonical form.

With increase in salinity level from 8 and 12 dSm^{-1} a corresponding reduction in starch content in leaves was noticed as compared to non-saline plants. These reduction under salinity were maximum (34.05 and 51.52 %) and minimum (27.93 and 43.24%) with application of ammonical and combined form of nitrogen respectively as compared to control plants.

Nitrogen in combined form (120 kg ha^{-1}) resulted in the relatively higher content level of starch under saline condition over control plants. However, minimum content was observed with ammonical form of nitrogen. Beside this, the highest level of nitrogen (120 kg ha^{-1}) in combined form showed minimum (17.05 and 33.81%) reduction in starch content at a salinity level of 8 and 12 dSm^{-1}, respectively as compare to control plants. Such reduction was maximum (43.4 and 61.60%) at lower level of nitrogen i.e. 40 kg ha^{-1}.

A reference of data in Table 16 revealed that starch content in leaves at 65 DAS was noticed highest in combined form of nitrogen treated plants as compared to ammonical form.

Under saline condition, the maximum (35.06 and 52.63%) and minimum (24.24 and 39.29%) reduction was observed with ammonical and combined form, respectively as compared to non-saline plants.

The application of higher level of nitrogen in combined form (120 kg ha^{-1}) resulted in the relatively higher content of starch in leaves as compared to lower level of nitrogen application. However, minimum

Table 16. **Effect of nitrogen source, levels and their interaction with salinity on starch content (mg g^{-1} dry weight) in leaf of *Brassica juncea* cv. RH-30**

Nitrogen sources	Nitrogen levels (kg ha^{-1})	Salinity levels (dSm^{-1})							
		0	8	12	**Mean**	0	8	12	**Mean**
		45 DAS				65 DAS			
Nitrate form (NO_3^-)	40	32.69	18.34	10.95	**20.66**	37.32	20.51	22.18	**26.67**
	80	49.64	33.47	25.74	**36.28**	56.64	35.43	27.48	**39.85**
	120	63.12	46.09	32.19	**47.13**	69.40	49.62	35.17	**51.40**
	Mean	**48.48**	**32.63**	**22.96**	**34.69**	**54.45**	**35.19**	**28.27**	**39.30**
Ammonical form (NH_4^+)	40	30.44	17.79	11.15	**19.79**	32.13	21.20	12.48	**21.93**
	80	46.29	29.77	22.96	**33.01**	53.55	31.12	24.85	**36.50**
	120	55.32	39.54	29.91	**41.59**	61.64	43.35	32.44	**45.81**
	Mean	**44.02**	**29.03**	**21.34**	**31.46**	**49.11**	**31.89**	**23.26**	**34.75**
Combined form (NO_3^-+NH_4^+)	40	34.85	19.72	13.38	**22.65**	40.97	33.54	24.01	**32.84**
	80	57.13	39.23	32.47	**42.94**	70.16	44.23	35.80	**50.06**
	120	67.49	55.98	44.67	**56.05**	83.49	69.66	58.33	**70.49**
	Mean	**53.16**	**38.31**	**30.17**	**40.55**	**64.87**	**49.14**	**39.38**	**51.13**
Overall mean		**48.55**	**33.33**	**24.82**		**56.14**	**38.74**	**30.30**	

C.D. (P<0.05) for Ns/Nl/Sl = 0.200
C.D. (P<0.05) for NsxNl, Nl x Sl & Ns x Sl = 0.347
C.D. (P<0.05) for Ns x Nl x Sl = 0.601

C.D. (P<0.05) for Ns/Nl/Sl = 0.365
C.D. (P<0.05) for NsxNl, Nl x Sl & Ns x Sl = 0.632
C.D. (P<0.05) for Ns x Nl x Sl = 1.094

Ns = Nitrogen source; Nl = Nitrogen level; Sl =Salinity level

content was noticed with ammonical form of nitrogen. In addition to this, the higher level of nitrogen (120 kg ha^{-1}) in combined form exhibited minimum reduction (16.56 and 30.13%) in starch content of leaves as compared to non-saline plants. Such reduction was observed maximum (18.13 and 41.39 %) at lower level of nitrogen application.

The plant treated with basal dose of nitrogen fertilizer and saline water application at two stages showed similar trend in starch content. However, the magnitude was relatively higher at second sampling stage.

Free proline content

Table 17 showed that plant treated with combined form of nitrogen highest accumulation of proline content in leaves was observed at 45 DAS as compared to ammonical form.

Under the salinity level of 8 and 12 dSm^{-1} the plant treated with combined form of nitrogen further enhanced the proline content around 1.46 and 2.16 times, respectively as compared to non-saline plants. However such increase was observed maximum (2.90 and 3.91 times) in ammonical form.

Nitrogen in combined form (120 kg ha^{-1}) resulted in the lesser increase in proline content in leaves under saline condition over control. However, maximum increase was noticed with ammonical form. Beside this, the higher level of nitrogen (120 kg ha^{-1}) in combined form exhibited minimum increase (1.19 and 1.54 times) in proline content at a salinity level of 8 and 12 dSm^{-1}, respectively as compared to control plants. Such increase was maximum (2.65 and 5.20 times) at lower level of nitrogen fertilizer application (40 kg ha^{-1}).

Table 17. **Effect of nitrogen source, levels and their interaction with salinity on proline content (mg g^{-1} dry weight) in leaf of *Brassica juncea* cv. RH-30**

Nitrogen sources	Nitrogen levels (kg ha^{-1})	Salinity levels (dSm^{-1})							
		0	8	12	**Mean**	0	8	12	**Mean**
		45 DAS				65 DAS			
Nitrate form (NO_3^-)	40	0.99	3.45	6.15	**3.53**	1.11	5.05	7.55	4.57
	80	3.50	5.63	8.74	**5.96**	3.95	8.48	10.10	**7.51**
	120	5.93	7.20	9.05	**7.39**	7.80	9.26	11.94	**9.67**
	Mean	**3.47**	**5.43**	**7.98**	**5.63**	**4.29**	**7.60**	**9.86**	**7.25**
Ammonical form (NH_4^+)	40	1.04	3.86	6.28	**3.73**	1.27	5.46	7.64	**4.79**
	80	3.33	8.44	10.95	**7.57**	3.73	10.14	12.75	**8.87**
	120	1.22	3.92	4.63	**3.25**	0.97	3.43	10.33	**4.91**
	Mean	**1.86**	**5.41**	**7.28**	**4.85**	**1.99**	**6.34**	**10.24**	**6.19**
Combined form (NO_3^-+NH_4^+)	40	1.60	4.25	8.33	**4.73**	1.28	5.84	9.69	**5.60**
	80	4.48	6.45	8.95	**6.63**	5.63	9.21	11.58	**8.81**
	120	6.45	7.70	9.94	**8.03**	8.18	9.90	12.24	**10.11**
	Mean	**4.18**	**6.13**	**9.07**	**6.46**	**5.03**	**8.32**	**11.17**	**8.17**
Overall mean		**3.17**	**5.66**	**8.11**		**3.77**	**7.42**	**10.43**	

C.D. (P<0.05) for Ns/Nl/Sl = 0.031
C.D. (P<0.05) for NsxNl, Nl x Sl & Ns x Sl = 0.053
C.D. (P<0.05) for Ns x Nl x Sl = 0.093

C.D. (P<0.05) for Ns/Nl/Sl = 0.029
C.D. (P<0.05) for NsxNl, Nl x Sl & Ns x Sl = 0.049
C.D. (P<0.05) for Ns x Nl x Sl = 0.086

Ns = Nitrogen source; Nl = Nitrogen level; Sl =Salinity level

A reference of data in Table 17 revealed that the highest proline accumulation in leaves at 65 DAS was observed when plant treated with combined form of nitrogen as compared to nitrate and ammonical forms.

With increase in salinity level from 8 and 12 dSm^{-1} a progressive increase in proline content in leaves was noticed as compared to non-saline plants. These increase under salinity were maximum (3.18 and 5.14 times) and minimum (1.65 and 2.22 times) with ammonical and combined form, respectively as compared to control plants.

The increase in proline content of leaves was relatively low with the application of nitrogen in combined form (120 kg ha^{-1}) as compared to control plants. However, it was maximum increase with ammonical form of nitrogen. In addition to this, higher level of nitrogen (120 kg ha^{-1}) in combined form resulted in minimum increase (1.21 and 1.49 times) in proline content at salinity level of 8 and 12 dSm^{-1} , respectively as compared to non-saline plants. Such increase was noticed maximum (4.56 and 7.57 times) at lower level of nitrogen fertilizer application i.e. 40 kg ha^{-1}.

The plant treated with basal dose of nitrogen fertilizer and saline water water application showed similar trend in proline content of leaves. However, the magnitude was relatively higher at 65 DAS.

Total free amino acids

A close examination of data in Table 18 revealed that the maximum accumulation of free ammino acids in levels at 45 DAS was noticed, when plant treated with ammonical form of nitrogen as compared to nitrate form.

Under saline condition, when plant treated with ammonical form of nitrogen showed highest accumulation (46.43 and 83.31%) of free amino

Table 18. ***Effect of nitrogen source, levels and their interaction with salinity on free amino acids (mg g^{-1} dry weight) in leaf of Brassica juncea* cv. RH-30**

Nitrogen sources	Nitrogen levels (kg ha^{-1})	Salinity levels (dSm^{-1})							
		0	8	12	**Mean**	0	8	12	**Mean**
		45 DAS				65 DAS			
Nitrate form (NO_3^-)	40	35.79	52.60	57.62	**48.67**	44.45	63.25	66.24	**57.98**
	80	58.38	68.90	100.85	**76.04**	63.80	74.56	108.64	**82.33**
	120	78.79	99.67	147.76	**108.74**	89.52	114.58	170.80	**124.97**
	Mean	**57.65**	**73.72**	**102.07**	**77.82**	**65.92**	**84.13**	**115.23**	**88.43**
Ammonical form (NH_4^+)	40	39.69	57.16	61.82	**52.89**	40.98	60.47	65.62	**55.69**
	80	59.27	73.83	105.06	**79.39**	65.39	79.93	112.11	**85.81**
	120	80.70	132.10	162.50	**125.10**	88.26	143.63	188.16	**140.01**
	Mean	**59.89**	**87.70**	**109.79**	**85.79**	**64.88**	**94.67**	**121.96**	**93.84**
Combined form (NO_3^-+NH_4^+)	40	36.70	53.58	59.96	**50.08**	44.87	62.25	67.05	**58.05**
	80	61.22	71.59	104.09	**78.97**	72.73	78.95	113.09	**88.26**
	120	84.66	124.86	155.22	**121.58**	101.28	142.70	190.05	**144.68**
	Mean	**60.86**	**83.34**	**106.42**	**83.54**	**72.96**	**94.63**	**123.40**	**97.00**
Overall mean		**59.47**	**81.59**	**106.10**		**67.92**	**91.14**	**120.20**	

C.D. (P<0.05) for Ns/Nl/Sl = 0.780
C.D. (P<0.05) for NsxNl, Nl x Sl & Ns x Sl = 1.350
C.D. (P<0.05) for Ns x Nl x Sl = 2.339

C.D. (P<0.05) for Ns/Nl/Sl = 0.788
C.D. (P<0.05) for NsxNl, Nl x Sl & Ns x Sl = 1.365
C.D. (P<0.05) for Ns x Nl x Sl = 2.364

Ns = Nitrogen source; Nl = Nitrogen level; Sl =Salinity level

acids in leaves and the extent of accumulation was less with combined form (36.93 and 74.86%) as compared to non-saline plants. Nitrogen in ammonical form (120 kg ha^{-1}) resulted in the relatively higher per cent accumulation of free amino acids in leaves under saline condition over control plants. However, minimum per cent accumulation was noticed with combined form of nitrogen. Beside this, the highest level of nitrogen (120 kg ha^{-1}) in ammonical form exhibited maximum accumulation in free amino acids at a salinity level of 8 and 12 dSm^{-1}, respectively as compared to control plants. Such increase was observed minimum at lower level of fertilizer application.

A reference of data in Table 18 revealed that maximum accumulation in free amino acids content was noticed at 65 DAS in plant treated with combined form of nitrogen as compared to ammonical form.

With increase in salinity level from 8 and 12 dSm^{-1} a corresponding increase in free amino acid content in leaves was observed as compared to non-saline plants. These increases were maximum (45.91 and 69.13%) with ammonical and minimum (29.70 and 69.13%) with combined form, respectively as compared to control plants.

Nitrogen in ammonical form (120 kg ha^{-1}) resulted in relatively higher increase of free ammino acids in leaves under saline condition over control plants. However, minimum increase was observed with nitrate form of nitrogen. In addition to this, the highest level of nitrogen (120 kg ha^{-1}) in ammonical form resulted in maximum accumulation of free amino acids under saline condition as compared to control plants. Such increase was observed minimum at a lower level of fertilizer application.

The plant treated with based dose of nitrogen fertilizer and saline water application two sampling stages (45 and 65 DAS) showed similar trend in free amino acids accumulation in leaves. However, the magnitude of accumulation was relatively higher in plant at 65 DAS.

ENZYMATIC STUDIES

Nitrate reductase activity

Fig. 4A shows that the nitrate reductase (NR) activity in leaves enhanced more when plants treated with nitrate form of nitrogen as compared to ammonical form at first sampling (45 DAS).

Salinity exhibited the significant inhibition in nitrate reductase activity (NR) as compared to non-saline plants and inhibition was more with increasing level of salinity i.e.. 8 and 12 dSm^{-1}. The per cent reduction (31.57 and 39.91) in NR activity was maximum with ammonical form and minimum reduction (17.43 and 26.52%) noticed with nitrate form of nitrogen application at higher level of salinity (8 and 12 dSm^{-1}) as compared to control plants.

Increasing level of nitrogen (80 and 120 kg ha^{-1}) irrespective source of nitrogen showed significant increase in NR activity of leaves as compared to lower level of nitrogen dose i.e. 40 kg ha^{-1}. In addition to this, the NR activity was maximum (70.38 and 128.64%) with application of 80 and 120 kg ha^{-1} nitrogen in nitrate form, respectively as compared to lower level of nitrogen i.e. 40 kg ha^{-1}. The interactive effect between salinity and different levels of nitrogen sources had been found to be non-significant.

Fig. 4B represents that NR activity in leaves was highest in plant grown with nitrate form of nitrogen as compared to ammonical form at second sampling (65 DAS).

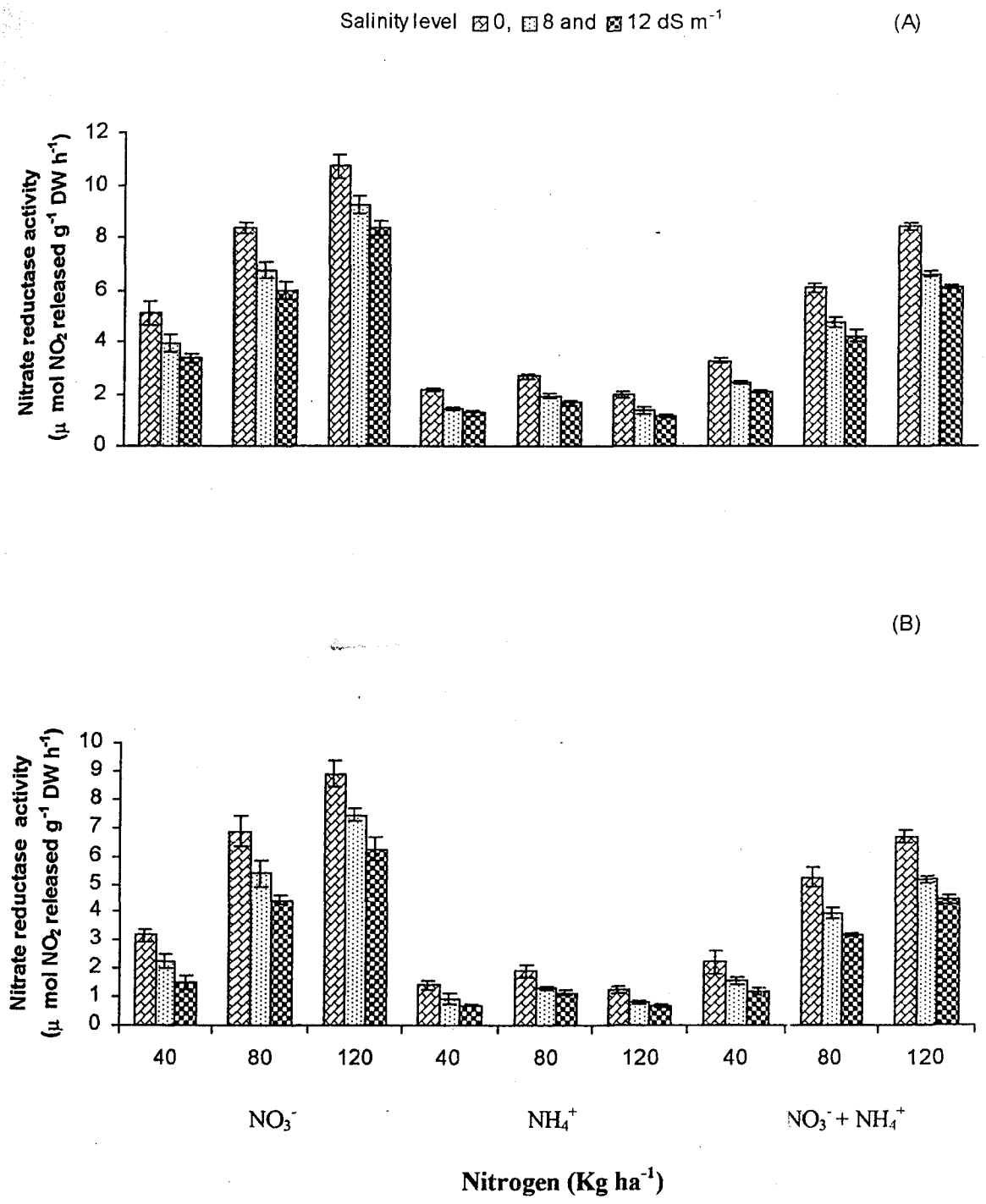

Fig. 4 Effect of nitrogen source, levels and their interaction with salinity on nitrate reductase activity at (A) 45 DAS and (B) 65 DAS in *Brassica juncea* cv. RH-30.

Under saline condition, NR activity in leaves declined drastically with each increment of salinity (8 and 12 dSm^{-1}) irrespective of nitrogen source used as compared to non-saline plants. Under salinity the per cent reduction (33.76 and 44.80) was maximum with ammonical form and the minimum (20.25 and 35.91%) with nitrate form as compared to control plants.

Nitrate form of nitrogen (120 kg ha^{-1}) resulted in maximum alleviation of deleterious effect of salinity on NR activity in leaves. However, minimum alleviation was observed with ammonical form of nitrogen used. With 120 kg ha^{-1} of nitrogen in nitrate form showed the minimum reduction (15.97 and 29.80%) at 8 and 12 dSm^{-1} of salinity level over control plants. Such reduction was maximum (28.61 and 53.14%) at a lower level of fertilizer application i.e. 40 kg ha^{-1}.

The NR activity in leaves exhibited similar trend at both sampling stages i.e. 45 and 65 DAS. However, the magnitude was relatively higher at 45 DAS.

Nitrite reductase

Nitrite reductase (NiR) activity in leaves was highest in plant treated with nitrate form as compared to ammonical form at first sampling stage i.e. 45 DAS (Fig. 5A).

Under different levels of salinity (8 and 12 dSm^{-1}) the NiR activity in leaves decreased significantly as compared to non-saline plants. With the increase in salinity levels, the per cent reduction over control was maximum (40.68 and 49.25%) with ammonical form and minimum (16.20 and 26.80%) with nitrate form of nitrogen.

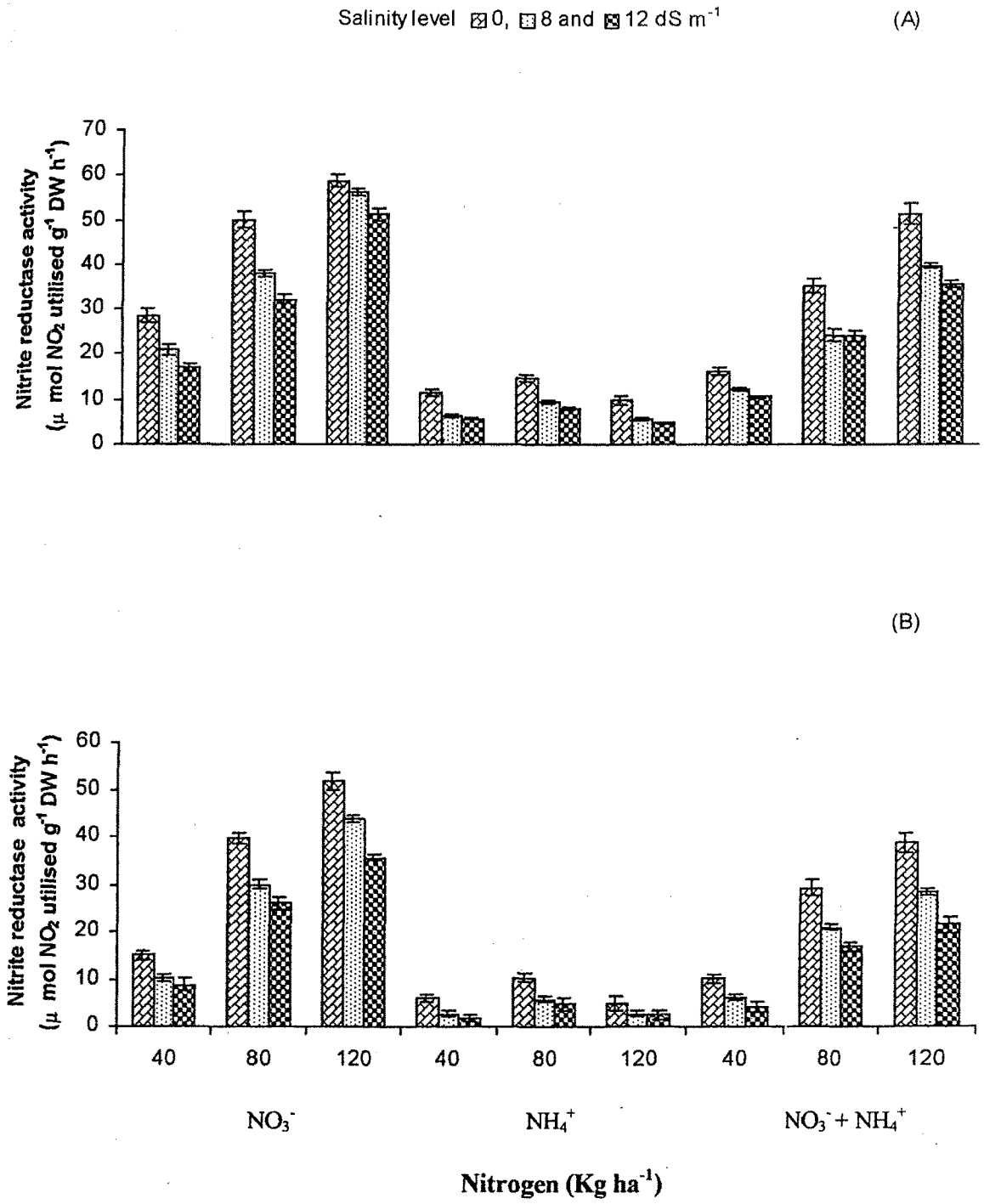

Fig. 5 Effect of nitrogen source, levels and their interaction with salinity on nitrite reductase activity at (A) 45 DAS and (B) 65 DAS in *Brassica juncea* cv. RH-30.

Application of 120 kg ha^{-1} nitrogen in nitrate form mitigated the adverse effect of salinity on NiR activity in leaves. While minimum alleviation was noticed with ammonical form. NiR activity in leaves showed the minimum reduction (4.41 and 12.63%) under saline condition (8 and 12 dSm^{-1}) as compared to control plants with the application of 120 kg ha^{-1} nitrate form of nitrogen. However, with a dose 40 kg/ha resulted maximum reductions (26.49 and 40.23%).

Fig. 5B illustrates that nitrite reductase (NiR) activity in leaves were highest in plant treated with nitrate form of nitrogen as compared to ammonical form at second sampling i.e. 65 DAS.

NiR activity in leaves reduced significantly with each increment in salinity levels (8 and 12 dSm^{-1}) as compared to non-saline plants. Under saline condition, treatment with ammonical form exhibited maximum per cent reduction (46.58 and 56.55) in NiR activity in leaves. However, the minimum per cent reduction (21.01 and 33.84) was observed with nitrate form of nitrogen irrespective of levels of salinity over control.

The nitrate form of nitrogen with a dose of 120 kg ha^{-1} resulted in maximum alleviation in deleterious effect of salinity on NiR activity in leaves, while minimum alleviation of deleterious effect of salinity was observed with ammonical form of nitrogen. Nitrate form of nitrogen with 120 kg ha^{-1} showed the minimum reduction of 15.17 and 31.10 per cent at the salinity level of 8 and 12 dSm^{-1}, respectively as compared to control plants. Such reduction noticed maximum (32.20 and 40.93%) with 40 kg ha^{-1} of nitrogen application.

Similar trend in NiR activity observed at both the sampling stages (45 and 65 DAS). However, the magnitude was relatively higher at 45 DAS.

Glutamine synthetase activity

Fig. 6A shows that the glutamine synthetase (GS) activity in leaves was maximum in plant treated with ammonical form of nitrogen as compared to nitrate form at first sampling (45 DAS).

With increase in salinity level from 8 to 12 dSm^{-1} a corresponding inhibition in GS activity in leaves was noticed as compared to non-saline plants. These reductions under salinity were maximum (24.74 and 33.16%) and minimum (17.90 and 28.31%) with the application of nitrate and ammonical form of nitrogen, respectively as compared to control plants.

Nitrogen in ammonical form (120 kg ha^{-1}) caused maximum enhancement in GS activity in leaves under saline condition over control. However, minimum increase was notice with nitrate form of nitrogen. Beside this, the higher level of nitrogen (120 kg ha^{-1}) in ammonical form exhibited minimum reduction (18.45 and 28.81%) in GS activity at a salinity level of 8 and 12 dSm^{-1}, respectively as compared to non-saline plants. Such reduction was maximum (22.55 and 30.48%) at lower level of nitrogen application.

Fig. 6B represents that the plant treated with ammonical form of nitrogen had been found to be highest glutamine synthetase (GS) activity in leaves at second sampling (65 DAS) as compared to nitrate form.

Under saline condition, the GS activity in leaves decreased significantly with every increment in salinity as compared to non-saline condition irrespective of nitrogen source used. The plant treated with nitrate form of nitrogen showed maximum reduction (27.95 and 39.65%) in GS activity and minimum reduction (21.62 and 32.47%) in ammonical form as compared to control plants.

Nitrogen in ammonical form (120 kg ha^{-1}) resulted in the relatively lesser reduction in GS activity under saline condition (8 and 12 dSm^{-1}) over control plants. However, maximum reduction was notice with nitrate form. Beside this, the higher level of nitrogen (120 kg ha^{-1}) in ammonical form showed minimum inhibition (19.56 and 31.00%) in GS activity at a salinity level 8 and 12 dSm^{-1}, respectively, as compare to non-saline plants. Such reduction was maximum (26.42 and 37.78%) at lower level of nitrogen i.e. 40 kg ha^{-1}.

The glutamine synthetase (GS) activity in leaves showed similar trend at both sampling stages (45 and 65 DAS). However the maximum activity glutamine synthetase in leaves was notice at 65 DAS.

Glutamate synthase activity

Fig. 7A represents that GOGAT activity in leaves was highest in plant treated with ammonical form as compared to nitrate form at first sampling i.e. 45 DAS.

Under different levels of salinity (8 and 12 dSm^{-1}) the GOGAT activity in leaves decreased significantly as compared to non-saline plants. With the increase in salinity levels the reduction over control was maximum (23.3 and 45.34%) with nitrate form and minimum (21.44 and 37.14%) with ammonical form of nitrogen.

Application of 120 kg ha^{-1} nitrogen in ammonical form resulted in minimum inhibition of GOGAT activity of leaves under saline condition as compare to non-saline plants. While maximum reduction was notice with nitrate form of nitrogen. Beside this, GOGAT activity in leaves showed the minimum per cent inhibition (18.99 and 34.91%) under saline condition (8 and 12 dSm^{-1}) as compared to control plants with the

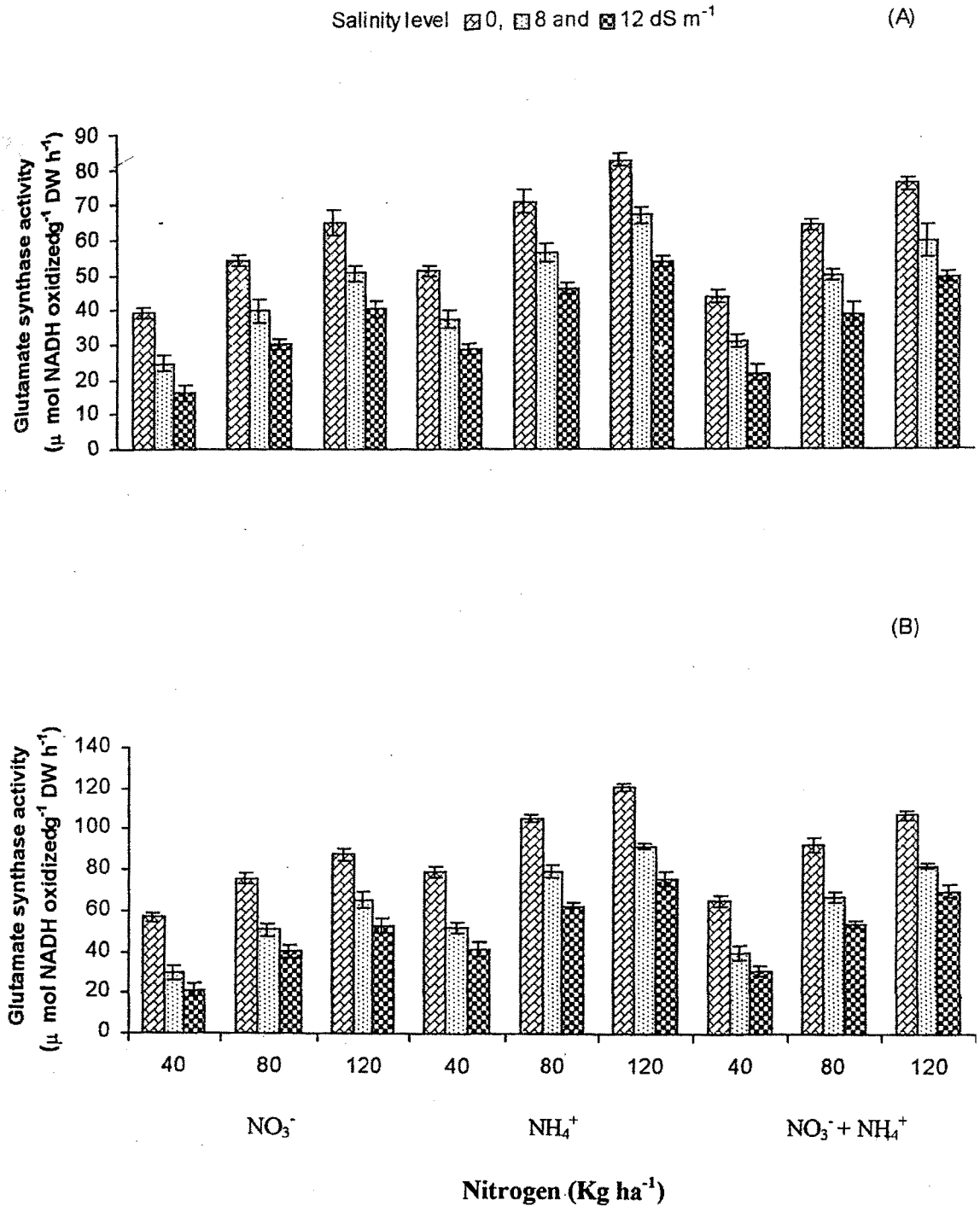

Fig. 7 Effect of nitrogen source, levels and their interaction with salinity on glutamate synthase activity at (A) 45 DAS and (B) 65 DAS in *Brassica juncea* cv. RH-30.

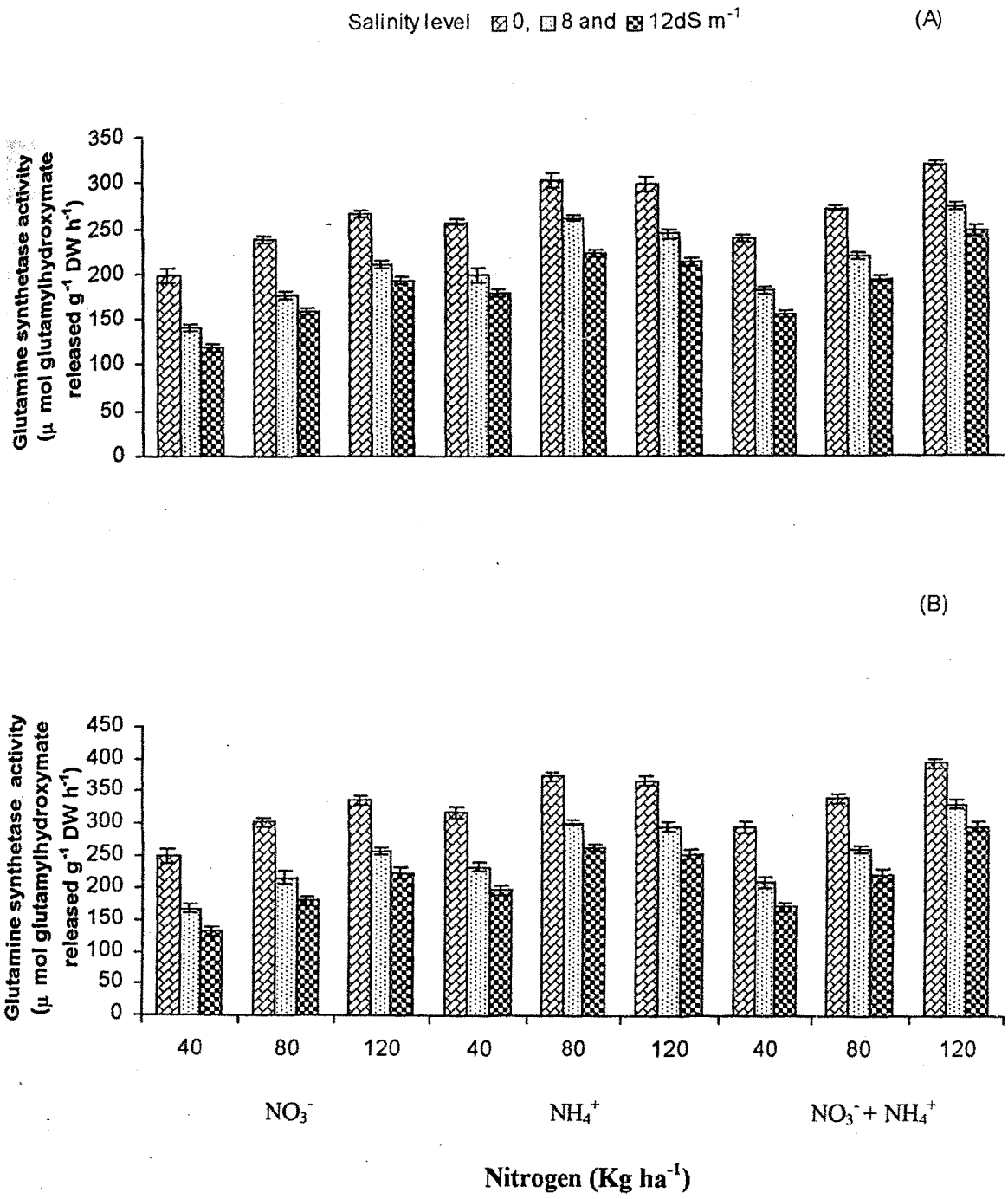

Fig. 6 Effect of nitrogen source, levels and their interaction with salinity on glutamine synthetase activity at (A) 45 DAS and (B) 65 DAS in *Brassica juncea* cv. RH-30.

application of 120 kg ha^{-1} ammonical form of nitrogen. However, with 40 kg ha^{-1} dose maximum per cent reduction (27.16 and 43.82).

Similar trend was observed at both sampling stages (45 and 65 DAS). However, the highest GOGAT activity was noticed at second sampling i.e. 65 DAS.

Fig. 7B shows that glutamate synthase (GOGAT) activity in leaves was maximum in plant grown with ammonical form of nitrogen as compared to nitrate form at second sampling (65 DAS).

Under saline condition, GOGAT activity in leaves declined drastically with each increment of salinity (8 and 12 dSm^{-1}) irrespective of nitrogen source used as compared to non-saline plants. At higher level of salinity the reduction (33.61 and 48.12%) was maximum with nitrate form and the minimum (26.76 and 41.03%) with ammonical form as compared to control plants.

Ammonical form of nitrogen (120 kg ha^{-1}) resulted in minimum inhibition in GOGAT activity in leaves under saline condition, as compared to control plants. However, maximum reduction was notice with nitrate form of nitrogen used. In addition to this, with 120 kg ha^{-1} of nitrogen in ammonical form showed the minimum reduction (23.90 and 37.29%), respectively at 8 and 12 dSm^{-1} levels of salinity over control plants. Such reduction was maximum (33.84% and 47.42%) at lower level of fertilizer application i.e. 40 kg ha^{-1}.

Glutamate dehydrogenase

Fig. 8A shows that glutamate dehydrogenase (GDH) activity in leaves was maximum in plant treated with ammonical form of nitrogen as compared to nitrate form at first sampling (45 DAS).

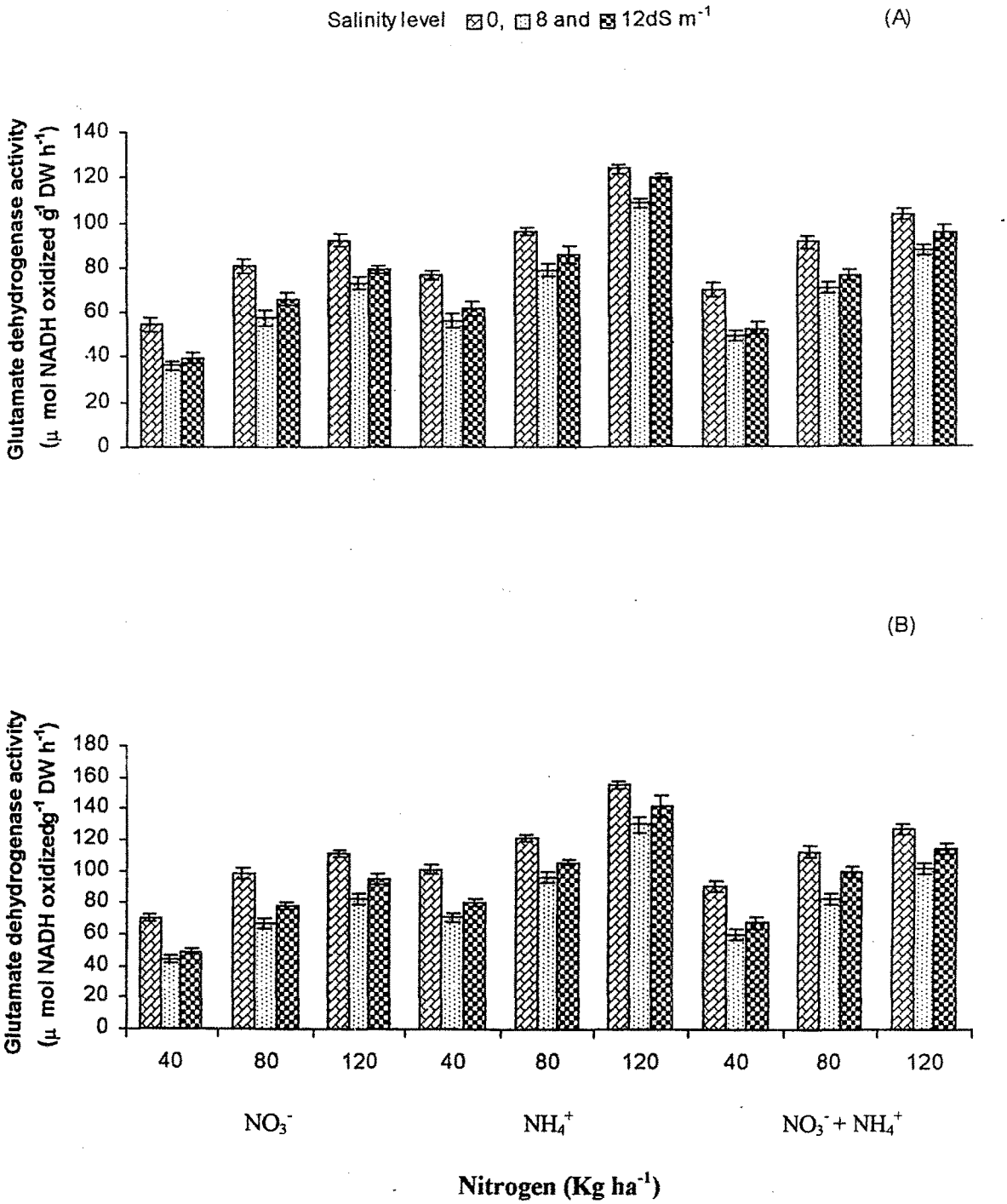

Fig. 8 Effect of nitrogen source, levels and their interaction with salinity on glutamate dehydrogenase activity at (A) 45 DAS and (B) 65 DAS in *Brassica juncea* cv. RH-30.

With increase in salinity level from 8 to 12 dSm^{-1} a significant inhibition in glutamate dehydrogenase (GDH) activity in leaves observed as compared to non-saline plants. However, a marginal enhancement in GDH activity was noticed at 12 dSm^{-1} as compared to 8 dSm^{-1}. The reduction under salinity were maximum (26.81 and 18.62%) and minimum (17.74 and 9.54%) with application of nitrate and ammonical form of nitrogen respectively as compere to control plants.

Nitrogen in ammonical form (120 kg ha^{-1}) resulted in relatively less inhibition in GDH activity under saline condition (8 and 12 dsm^{-1}) over control plants. However, maximum inhibition was observed with nitrate form. Beside this, the higher level of nitrogen (120 kg ha^{-1}) in ammonical form resulted in minimum reduction of 12.13 and 2.62 per cent in GDH activity at salinity level of 8 and 12 dSm^{-1}, respectively, as compared to non-saline plants. Such reduction was observed maximum (26.6 and 19.50%) at lower level of nitrogen i.e. 40 kg ha^{-1}.

Plant treated with ammonical form of nitrogen had been found to enhanced maximum glutamate dehydrogenase (GDH) activity in leaves at second sampling (65 DAS) as compared to nitrate form (Fig. 8B).

Under saline condition, the glutamate dehydrogenase activity in leaves decreased significantly with every increment in salinity as compared to non-saline plants. However, GDH activity increased slightly at 12 dSm^{-1} as compared to 8 dSm^{-1}. The plant treated with nitrate form of nitrogen resulted in maximum inhibition (30.62 and 20.58%) in GDH activity and minimum reduction (21.38 and 13.17%) with ammonical form as compared to control plants.

Nitrogen in ammonical form (120 kg ha^{-1}) showed the minimum inhibition in glutamate dehydrogenase activity under saline condition over control plants. However, maximum inhibition was observed with nitrate form of nitrogen. In addition to this, the higher level of nitrogen (120 kg ha^{-1}) in ammonical form resulted in minimum reduction of 16.23 and 8.63 per cent in GDH activity at salinity level of 8 and 12 dSm^{-1}, respectively as compared to control plants. Such reduction was maximum (29.75 and 20.72%) at lower level of nitrogen application i.e. 40 kg ha^{-1}.

The glutamate dehydrogenase activity in leaves showed similar trend at both the sampling stages (45 and 65 DAS). However, the highest GDH activity in leaves was observed at second sampling i.e. 65 DAS.

MINERAL COMPOSITION

N, P, K, Mg, Ca, Na, Cl and SO_4 were studied in different parts of plant i.e. leaves, stem and root at harvest.

Nitrogen content of leaves

Fig. 9A represents that nitrogen content was highest in leaves followed by stem and root under different level of salinity and fertilizer treatment. The nitrogen content in leaves was highest (19.76 and 24.12%) in plants grown with combined form of nitrogen over nitrate and ammonical form, respectively.

Salinity exhibited significant reduction in nitrogen content over non-saline plants and reduction was more with increasing level of salinity i.e. 8 and 12 dSm^{-1}. The per cent reduction (43.30 and 50.66) in nitrogen content was maximum with ammonical form and minimum reduction (21.63 and 33.78%) was observed with combined form of nitrogen application at higher level of salinity (8 and 12 dSm^{-1}) as compared to control plants.

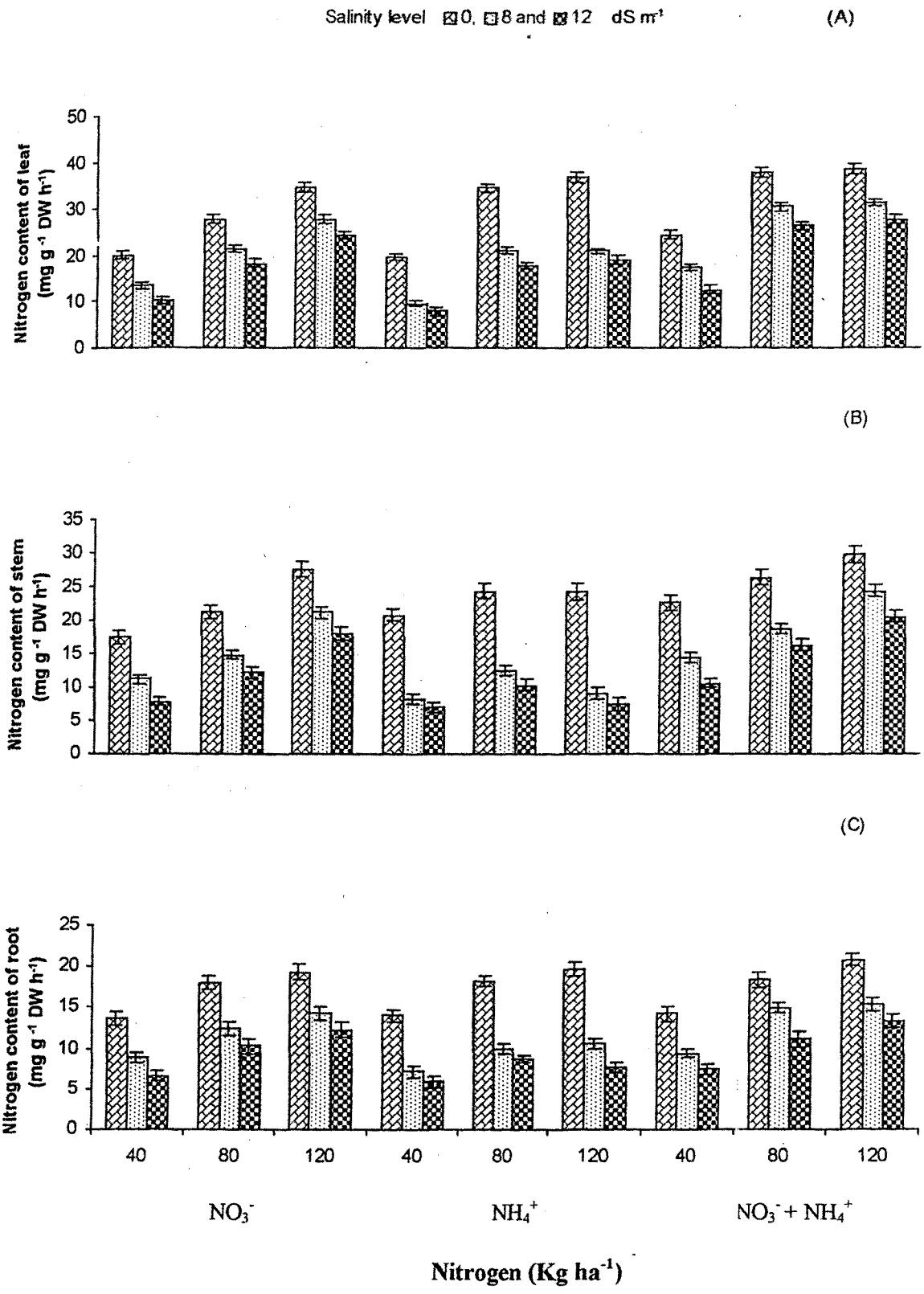

Fig. 9 Effect of nitrogen source, levels and their interaction with salinity on nitrogen content of (A) leaf, (B) stem and (C) root in *Brassica juncea* cv. RH-30 at harvest.

Under saline condition, combined nitrogen form (120 kg ha^{-1}) exhibited to alleviate the deleterious effect of salinity on nitrogen content in leaves as compare to non-saline condition. But minimum alleviation was observed with ammonical form of nitrogen applied. With 120 kg ha^{-1} of nitrogen in the combined form showed the minimum per cent reduction i.e. 18.94 and 27.87, respectively at 8 and 12 dSm^{-1} of salinity level over control. Such reduction was maximum (29.16 and 48.54%) at lower level of fertilizer application i.e. 40 kg ha^{-1}.

Nitrogen content of stem

Fig. 9B shows that nitrogen content in stem was highest (17.50 and 32.45%) in plant grown with combined form of nitrogen source as compared to nitrate and ammonical form of nitrogen treatment.

Under saline condition, nitrogen content in stem declined drastically with each increment of salinity (8 and 12 dSm^{-1}) irrespective of nitrogen source used as compared to non-saline condition. At higher level of salinity (8 and 12 dSm^{-1}) the per cent reduction (57.06 and 64.20) was maximum with ammonical form and the minimum reduction (27.01 and 39.74%) was observed with combined form as compared to control plants.

Nitrogen in combined form (120 kg ha^{-1}) mitigated maximum the detrimental effect of salinity on nitrogen content in stem under saline condition over control. However, minimum alleviation the adverse effect of salinity was observed with ammonical form. Application of nitrogen (120 kg ha^{-1}) in combined form resulted in minimum per cent reduction (18.11 and 30.92) under saline condition as compared to control plants. Such reduction was maximum (36.33 and 53.42%) at lower level of fertilizer application i.e. 40 kg ha^{-1}.

Nitrogen content of root

The nitrogen content in root was highest (18.42%) in plants grown with combined form of nitrogen as compared to other two forms of nitrogen (Fig. 9C).

Nitrogen content in root decreased significantly with increasing level of salinity as compared to non-saline condition. At higher level of salinity i.e. 8 and 12 dSm^{-1} the per cent reduction (46.66 and 56.98) in nitrogen content was more in plants treated with ammonical form and minimum reduction (25.69 and 40.39%) was observed with combined form of plants as compared to non-saline control plants.

Under saline condition, application of combined form of nitrogen (120 kg ha^{-1}) resulted in maximum alleviation adverse effect of salinity on nitrogen content in root as compared to non saline plants. While minimum alleviation on detrimental effect of salinity was noticed with ammonical form of nitrogen treatment. With the application of 120 kg ha^{-1} of nitrogen exhibited the minimum per cent reduction (25.80 and 36.05) at 8 and 12 dSm^{-1} of levels of salinity, respectively as compared to control plants. However, the application of 40 kg ha^{-1} nitrogen the reduction (34.36 and 47.99%) was observed maximum.

Phosphorus content of leaves

Fig. 10A represents that the combined form of nitrogen resulted in maximum increase (13.82 and 21.22%) in the phosphorus content in leaves as compared to nitrate and ammonical form of nitrogen treatment.

Phosphorus content in leaves decreased under different levels of salinity (8 and 12 dSm^{-1}) as compare to control plants. With the increase in salinity levels of the per cent reduction over control was maximum

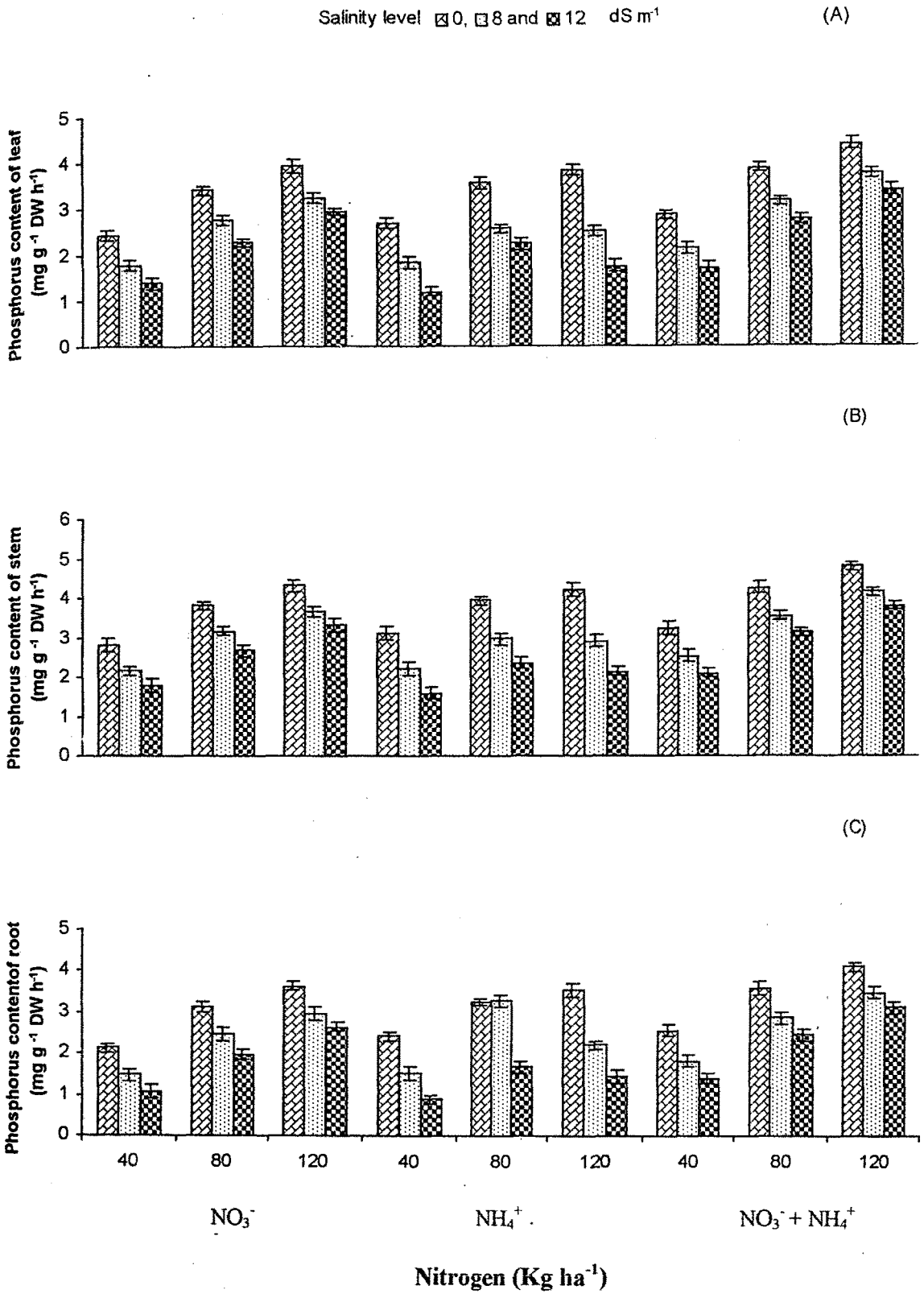

Fig. 10 Effect of nitrogen source, levels and their interaction with salinity on phosphorus content of (A) leaf, (B) stem and (C) root in *Brassica juncea* cv. RH-30 at harvest.

(31.64 and 48.65%) with ammonical form and minimum (18.64 and 22.55%) was observed with combined form of nitrogen.

Under saline condition, application of 120 kg ha^{-1} nitrogen in combined form maximum mitigated the adverse effect of salinity on phosphorus content in leaves as compare to non-saline plants. While minimum alleviation was noticed with ammonical form. Phosphorus content in leaves showed minimum per cent reduction (14.57 and 22.55%) under saline condition (8 and 12 dSm^{-1}) as compared to non-saline plants with the application of 120 kg ha^{-1} combined form of nitrogen. However, with 40 kg ha^{-1} dose, maximum reduction (25.61 and 40.35%) was observed.

Phosphorus content of stem

Phosphorus content was highest in stem followed by leaves and root under different level of salinity and fertilizer treatment. The combined form of nitrogen increased (12.25 and 19.37%) phosphorus content in stem as compare to plant grown with nitrate and ammonical form (Fig. 10B).

Under saline condition, treatment with ammonical form exhibited maximum per cent reduction (28.45 and 46.01) in phosphorus content of stem. However the minimum reduction (16.08 and 26.58%) was observed with combined form of nitrogen irrespective of salinity level over control.

The combined form of nitrogen with dose of 120 kg ha^{-1} resulted in maximum alleviation on phosphorus content in stem under saline condition as compared to non-saline condition. While minimum alleviation of deleterious effect of salinity was observed with ammonical form of nitrogen. Combined form of nitrogen with 120 kg ha^{-1} showed the minimum reduction (13.36 and 20.66%) at the salinity level of 8 and 12 dSm^{-1}

respectively as compared to non-saline plants. Such reduction was observed maximum (22.46 and 35.38%) with 40 kg ha^{-1} nitrogen application.

Phosphorus content of root

Fig. 10C shows that significant increase (15.30 and 20.28%) in phosphorus content of root in plant grown with combined form of nitrogen treatment was observed as compared to nitrate and ammonical form of nitrogen treatment.

Phosphorus content in root was significantly reduced under saline condition as compare to non-saline plants. The maximum per cent reduction (23.8 and 56.53) in phosphorus content of root was observed in plants treated with ammonical form and minimum reduction (20.29 and 32.05%) was noticed in combined form over control.

The maximum alleviation in adverse effect of salinity on phosphorus content of root was exhibited with combined form of nitrogen at different level of salinity (8 and 12 dSm^{-1}) used as compared to control plants. However, minimum alleviation was noticed with ammonical form of nitrogen. The minimum per cent reduction (15.64 and 24.20%) in phosphorus content of root was observed with 120 kg ha^{-1} of combined form of nitrogen at 8 and 12 dSm^{-1} level of salinity, respectively as compared to non-saline plants. Such reduction was maximum (28.62 and 45.09%) at lower level of fertilizer dose i.e. 40 kg ha^{-1}.

Potassium content of leaves

Plant treated with combined form had been found to contain maximum (24.88%) potassium content in leaves as compared to ammonical form (Fig. 11A).

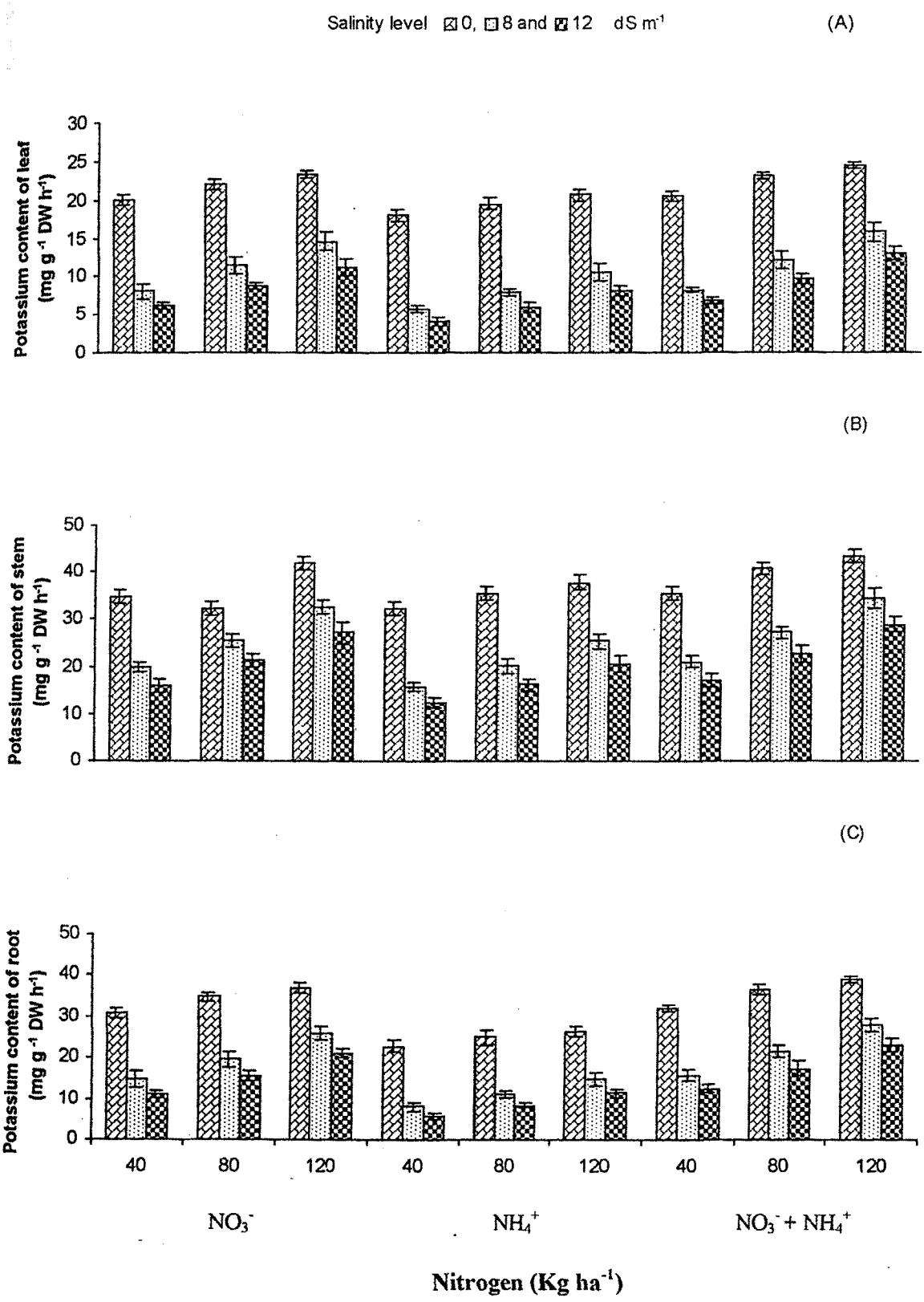

Fig. 11 Effect of nitrogen source, levels and their interaction with salinity on potassium content of (A) leaf, (B) stem and (C) root in *Brassica juncea* cv. RH-30 at harvest.

Under saline condition (8 and 12 dSm^{-1}), potassium content decreased with enhancement of salinity level, over control plants irrespective of nitrogen source applied. The per cent reduction in potassium content was highest (58.41 and 68.71) in ammonical form and lowest (46.77 and 56.55%) in combined form over non-saline plants. The higher level of nitrogen application (120 kg ha^{-1}) exhibited significantly increased potassium content as compared to lower level of nitrogen i.e. 40 kg ha^{-1}. However, maximum increase was observed in combined form of nitrogen and minimum with ammonical form of nitrogen. Interactive effect of salinity and different level of nitrogen source on potassium content had been found non-significant.

Potassium content of stem

Fig. 11B shows that potassium concentration was highest in stem followed by root and leaves under different level of salinity and fertilizer treatment. The potassium content was highest (20.27%) in plants grown with combined form of nitrogen as compared to ammonical form of nitrogen.

With increase in salinity level, a corresponding reduction in potassium content was observed as compare to non-saline plants. These reduction under salinity were maximum (41.95 and 53.46%) and minimum (30.88 and 42.55%) with ammonical and combined form of nitrogen, respectively as compared to control plants.

The higher level of nitrogen application (120 kg ha^{-1}) significantly increase potassium content as compared to lower level of i.e. 40 kg ha^{-1} under different levels of nitrogen source used. However, the interactive effect of salinity and different level of nitrogen source on potassium content had been found to be non-significant.

Potassium content of root

Fig. 11C represents that plant treated with combined form had been found to contain maximum (41.09%) potassium content as compared to ammonical form of nitrogen.

Under saline condition (8 and 12 dSm^{-1}), potassium content reduced with enhancement of salinity level over control plants irrespective of nitrogen source applied. These reduction under salinity were maximum (54.06 and 65.65%) and minimum (39.45 and 50.92%) with ammonical and combined form, respectively as compare to control plants.

The reduction in potassium content under saline condition (8 and 12 dSm^{-1}) resulted in maximum alleviate with application of 120 kg ha^{-1} nitrogen in combined form as compare to non-saline plants. But it was minimum with ammonical form. Beside this, plant treated with combined form (120 kg/ha) showed the minimum reduction (28.28 and 41.15%) at the salinity level of 8 and 12 dSm^{-1}, respectively as compare to control plants. However, reduction was notice maximum (50.90 and 61.11%) with 40 kg ha^{-1} nitrogen application.

Magnesium content of leaves

Fig. 12A represents that significant increase (29.53%) in magnesium content of leaves was observed in plant grown with combined farm of nitrogen as compared to ammonical form of nitrogen.

Significant reduction in magnesium content was observed with each increment of salivity level i.e. 8 and 12 dSm^{-1} as compare to non-saline plants. The highest per cent reduction (35.81 and 51.66) in magnesium content was observe in plant treated with ammonical form and minimum reduction (18.69 and 30.75%) with combined form of nitrogen over control.

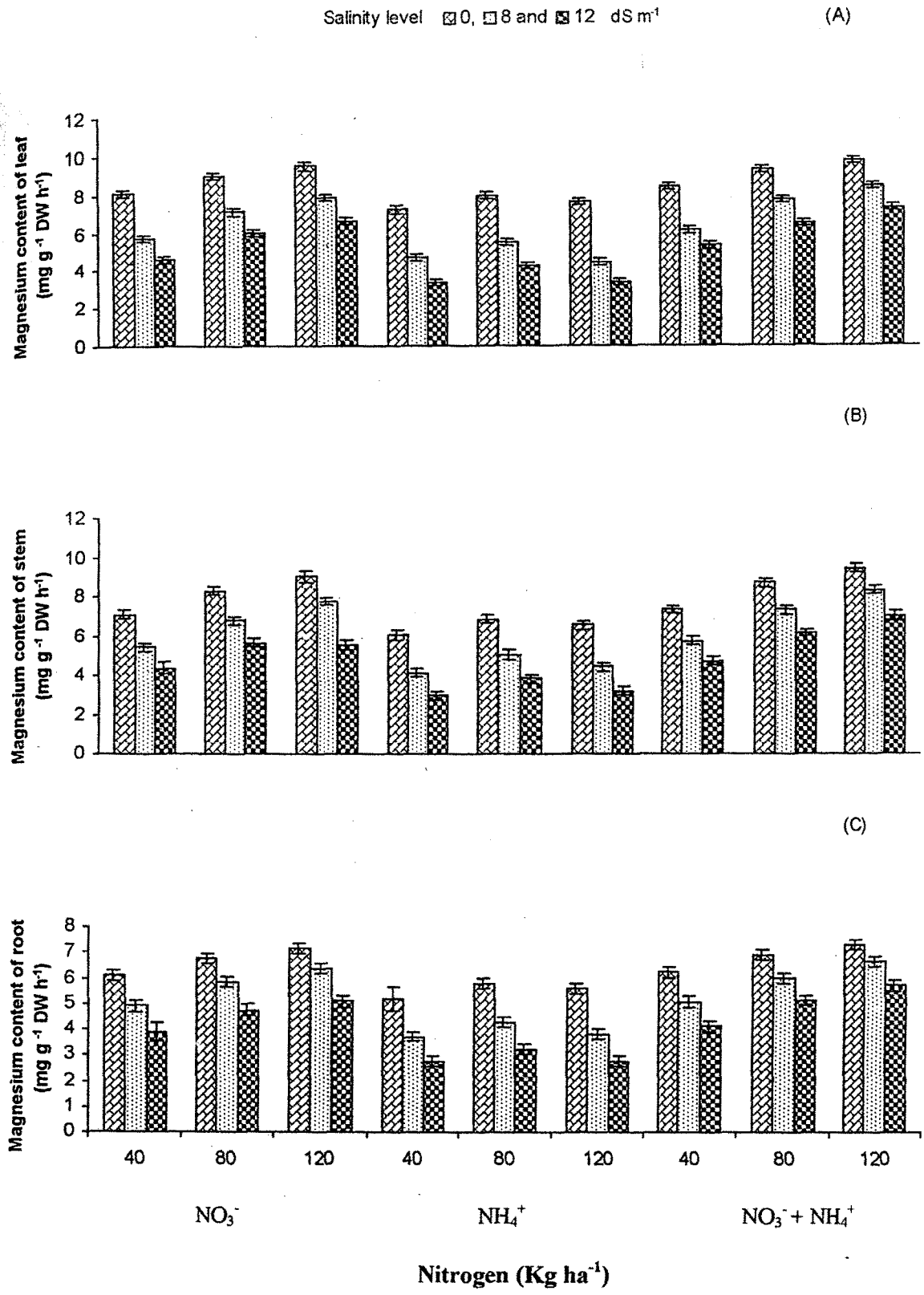

Fig. 12 Effect of nitrogen source, levels and their interaction with salinity on magnesium content of (A) leaf, (B) stem and (C) root in *Brassica juncea* cv. RH-30 at harvest.

The maximum alleivation in reduction of magnesium content of leaves with combined form of nitrogen at different level of salinity (8 and 12 dSm^{-1} as compared to control plants. However, minimum alleviation was noticed with ammonical form. In addition to this, the minimum per cent reduction (13.11 and 25.39) in magnesium content was observed with use of 120 kg ha^{-1} combined form of nitrogen at 8 and 12 dSm^{-1} level of salinity, respectively as compared to non-saline plants. Such reduction was maximum (26.86 and 37.10%) at lower level of fertilizer application i.e. 40 kg ha^{-1}.

Magnesium content of stem

Fig. 12B shows that when plan treated with combined form of nitrogen increased (7.25 and 33.61%) magnesium content as compare to plant grown with nitrate and ammonical forms.

Under saline condition, treatment with ammonical form exhibited maximum per cent reduction (30.03 and 48.76) in magnesium content of leaves. However, the minimum reduction (11.91 and 25.53%) observed in combined form of nitrogen treatment irrespective level of salinity over control.

Application of 120 kg ha^{-1} nitrogen in combined form under saline condition resulted relatively less reduction in magnesium content as compare to control plants. But reduction was greater with ammonical form of nitrogen. Plant treated with combined form (120 kg ha^{-1}) showed the minimum reduction of 11.91 and 25.53 per cent at the salinity level of 8 and 12 dSm^{-1}, respectively as compared to control plants. However, reduction was notice maximum (21.72 and 35.79%) with 40 kg ha^{-1} nitrogen application.

Magnesium content of root

Plant treated with combined form of nitrogen exhibited maximum magnesium content in root in comparison to nitrate and ammonical form of nitrogen (Fig. 12C).

Under saline condition (8 and 12 dSm^{-1}) the magnesium content decreased significantly irrespective of nitrogen source applied as compare to control plants. The per cent reduction (29.16 and 46.92) in magnesium content was highest in ammonical form and the lowest (13.52 and 26.76%) in combined form as compared to non-saline plants.

The combined form of nitrogen with a dose of 120 kg ha^{-1} resulted in maximum alleviation in reduction of magnesium content under saline condition (8 and 12 dSm^{-1}) as compared to control plants. While the minimum alleviation was observed with ammonical form of nitrogen. Beside this, highest level of nitrogen (120 kg ha^{-1}) in combined form resulted in the minimum per cent reduction (9.05 and 21.94) in magnesium content at salinity level of 8 and 12 dSm^{-1}, respectively as compared to control plants. Such reductions were noticed maximum (18.67 and 33.97%) at lower level of fertilizer application.

Calcium content of leaves

Fig. 13A shows that calcium content was highest in leaves followed by stem and root under different level of salinity and fertilizer treatment. Plant treated with combined form of nitrogen had been found to contain maximum (31.84%) calcium content in leaves as compared to ammonical form.

With the increase in salinity level from 8 and 12 dSm^{-1} a corresponding increase in calcium content in leaves was observed as

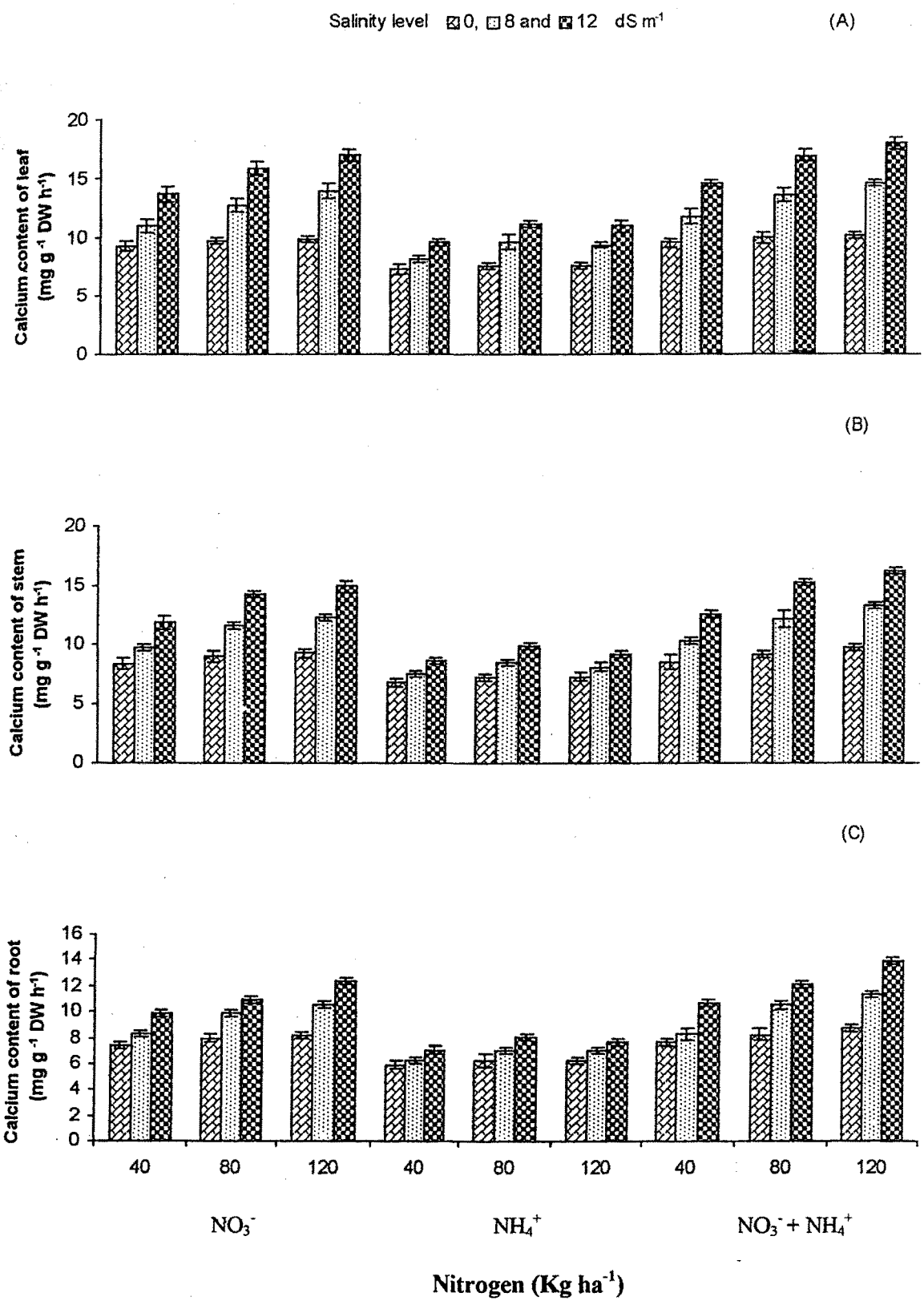

Fig. 13 Effect of nitrogen source, levels and their interaction with salinity on calcium content of (A) leaf, (B) stem and (C) root in *Brassica juncea* cv. RH-30 at harvest.

compared to non-saline plants. These increases under salinity were maximum (34.71 and 66.80%) and minimum (21.01 and 40.96%) with combined and ammonical form, respectively as compared to control plants.

The treatment with combined form of nitrogen (120 kg ha^{-1}) resulted in the maximum accumulation of calcium content under saline condition as compared to control plants. However, minimum accumulation was observed with ammonical form of nitrogen. In addition to this, highest level of nitrogen used (120 kg ha^{-1}) in the combined form exhibited the maximum upsurge (44.41 and 77.64%) in calcium content under salinity (8 and 12 dSm^{-1}) as compared to control plants. Such increase was noticed minimum (23.37 and 53.03%) at lower level of fertilizer application i.e. 40 kg ha^{-1}.

Calcium content of stem

Fig. 13B represents that when plant treated with combined form of nitrogen resulted maximum increase (31.95%) in calcium content of stem as compared to ammonical form of nitrogen.

Under saline condition, the treatment with combined form of nitrogen resulted in maximum per cent increase (30.15 and 60.85) over control in calcium content of stem. However , per cent increase was minimum (13.33 and 30.78) with ammonical form of nitrogen.

The treatment with combined form of nitrogen (120 kg ha^{-1}) exhibited the highest accumulation of calcium content irrespective of salinity level as compared to non-saline plants. However, lowest accumulation was observed with ammonical form of nitrogen. Combined form of nitrogen (120 kg ha^{-1}) showed the maximum increase (36.32 and 67.59%) at 8 and 12 dSm^{-1} salinity level, respectively as compared to

control plants. Such increase was lowest (21.03 and 47.70%) at lower level of fertilizer application i.e. 40 kg ha^{-1}.

Calcium content of root

When plant treated with combined form of nitrogen had been found to contain maximum (33.10%) calcium content in root as compared to ammonical form (Fig. 13C).

Under saline condition (8 and 12 dSm^{-1}), calcium content increased corresponding to enhancement in salinity level over control plants irrespective of nitrogen source applied. The per cent upsurge (25.21 and 48.84%) in calcium accumulation was highest in combined form and lowest (10.24 and 23.90%) in ammonical form as compare to non-saline plants.

The combined form of nitrogen with a application of 120 kg ha^{-1} resulted in relatively higher accumulation of calcium content in root under saline condition as compared to control plants. While the lowest accumulation was observed with ammonical form of nitrogen. Besides this, highest level of nitrogen (120 kg ha^{-1}) in combined form exhibited the maximum per cent increase (30.50 and 59.51) in calcium concentration at a salinity level 8 and 12 dSm^{-1} , respectively as compared to non-saline plants. Such increase were noticed minimum (17.25 and 39.73%) at lower level of fertilizer application i.e. 40 kg ha^{-1}.

Sodium content of leaves

Fig. 14A shows that plant treated with ammonical form of nitrogen had been found highest sodium concentration in leaves at harvest as comapred to combined form of nitrogen.

Under saline condition, the sodium cotnent in leaves increase with every increment in salinity as compared to non-saline conditions

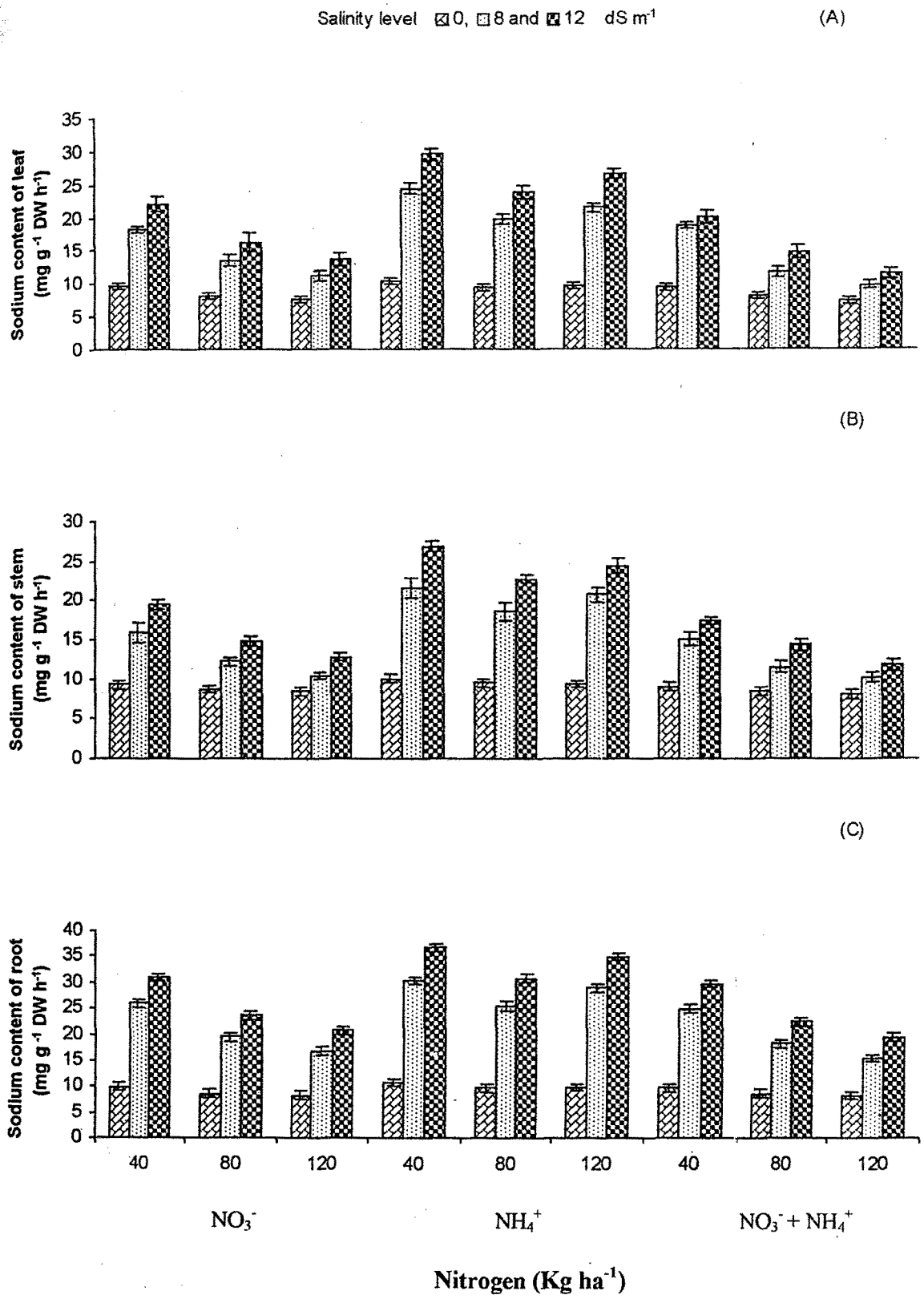

Fig. 14 Effect of nitrogen source, levels and their interaction with salinity on sodium content of (A) leaf, (B) stem and (C) root in *Brassica juncea* cv. RH-30 at harvest.

irrespective of nitrogen source used. The plant treated with ammonical form of nitrogen exhibited highest accumulation (124.17 and 173.96%) of sodium concentration and combined form caused lesser content of sodium accumulation (62.46 and 86.79%) as compared to non-saline plants.

Nitrogen in combined form (120 kg ha^{-1}) resulted in the relatively less accumulation of sodium content in leaves under saline condition over control. However, maximum accumulation was noticed with ammonical form of nitrogen. Beside this, the higher level of nitrogen (120 kg ha^{-1}) in combined form exhibited minimum per cent increase (32.19 and 55.38%) in sodium cotnent at a salinity levle of 8 and 12 dSm^{-1}, respectively as compared to control plants. Such increase was observed maximum (102.38 and 116.61%) at lower level of fertilizer application.

Sodium content of stem

Plant treated with ammonical form resulted in highest (55.26%) sodium content at harvest over combined form of nitrogen (Fig. 14B).

Under saline condition (8 and 12 dSm^{-1}), sodium concentration increased with enhancement salinity levels over control plants irrespective of nitrogen source applied. The per cent upsurge (111.05 and 156.72%) in sodium accumulation was highest in ammonical form and the lowest (44.43 and 71.09%) in combined form as compared to non-saline plants.

The combined form of nitrogen with 120 kg ha^{-1} exhibited relatively less accumulation of sodium concentration in stem under saline condition as compared to control plants. While the highest accumulation was observed with ammonical form of nitrogen. Besides this, use of 120 kg ha^{-1} nitrogen in combined form showed the minimum per cent increase (26.27 and 46.32%) in sodium concentrations at a salinity level of 8 and

12 dSm^{-1}, respectively as compared to non-saline plants. However, at a lower level of fertilizer application noticed maximum increase (67.97 and 93.72%).

Sodium content of root

Fig. 14C represents that sodium concentration was maximum in root followed by leaves and stem under different level of salinity and fertilizer treatment. Plant treated with ammonical form had been found to accumulate highest Na as compared to combined form.

With increase in salinity level from 8 to 12 dSm^{-1} a corresponding increase in sodium concentration in root was noticed as comapred to non-saline plants. These increase under salinity were maximum (181.13 and 239.52%) and minimum (120.61 and 170.61%) with ammonical and combined form , respectively as compared to non-saline plants.

The extent of accumulation Na in root under saline condition was relatively less with application of nitrogen in combined form (120 kg ha^{-1}) as compared to control plants. However, it was highest with ammonical form of nitrogen. In addition to this, highest level of nitrogen (120 kg ha^{-1}) in combined form resulted in less per cent upsurge (87.23 and 137.05%) in sodium content at salinity level of 8 and 12 dSm^{-1}, respectively as compared to control plants. Such increase was noticed maximum (157.96 and 207.80%) at lower level of fertilizer application i.e. 40 kg ha^{-1}.

Chloride content of leaves

Fig. 15A represents that ammonical form of nitrogen had been found to cause more accumulation of chloride content in leaves as compared to nitrate and combined form of nitrogen.

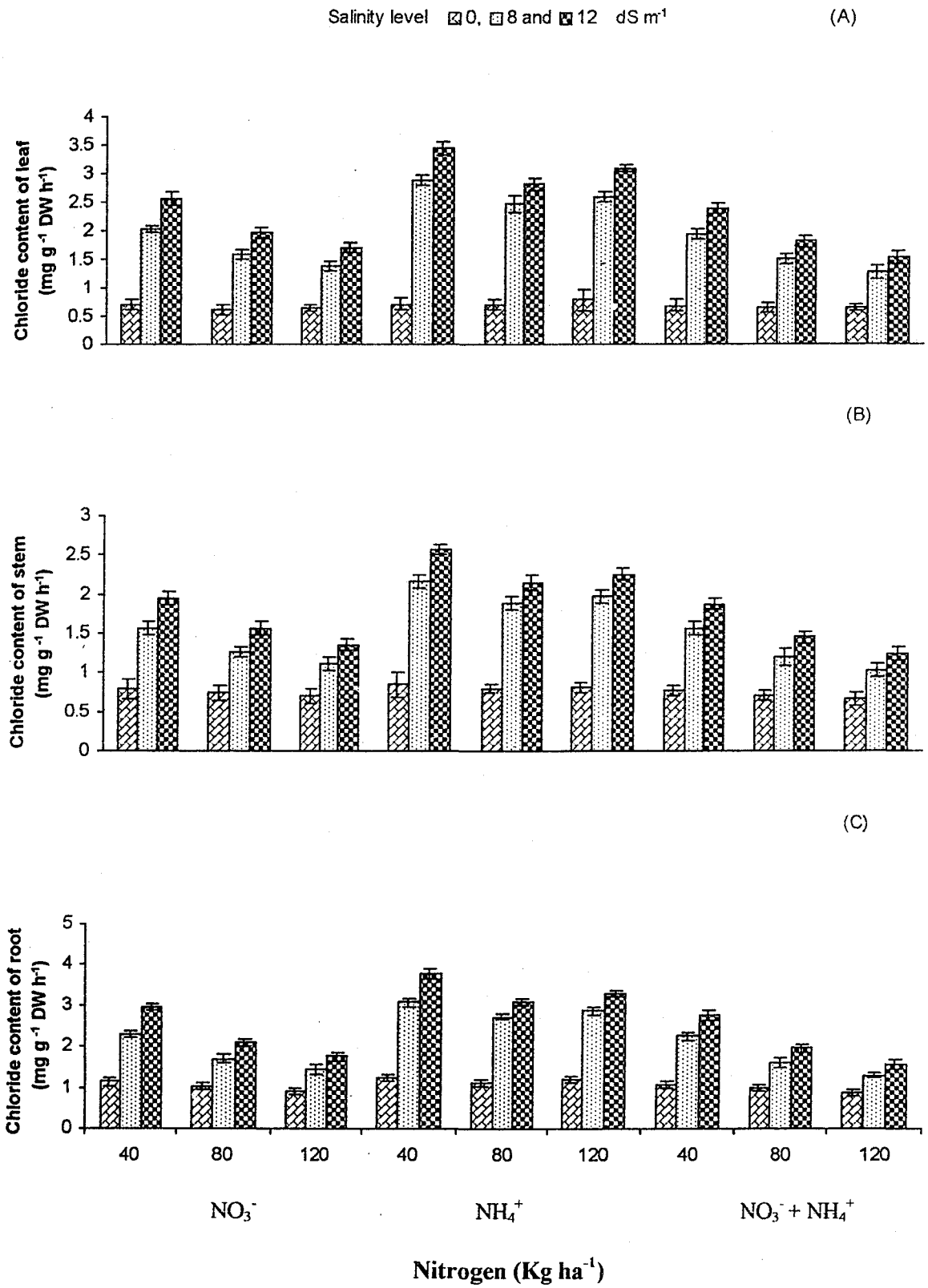

Fig. 15 Effect of nitrogen source, levels and their interaction with salinity on chloride content of (A) leaf, (B) stem and (C) root in *Brassica juncea* cv. RH-30 at harvest.

Chloride concentrations in leaves under salinity were significantly higher as compared to non-saline control plants. The ammonical form had tremendously increased (261.64 and 326.02%) the chloride concentration under different levels of salinity (8 and 12 dSm^{-1}) as compared to control. But the increase (136.36 and 187.87%) in chloride content was to a lesser extent with combined form of nitrogen irrespective of salinity levels.

Application of 120 kg/ha nitrogen in combined form caused relatively less accumulation in chloride content of leaves as compare to non-saline plants, whereas it was relatively higher with ammonical form under saline condition. The minimum per cent increase (93.84 and 136.92) in chlorides content of leaves was observed with each increment of salinity level (8 and 12 dSm^{-1}) on account of nitrogen treatment (120 kg ha^{-1}) in combined form over control. However, with a dose of 40 kg ha^{-1}, the increase (182.60 and 243.47%) in chloride content was maximum.

Chloride content of stem

Fig. 15B shows that chloride content in stem was relatively higher in ammonical form than the remaining two other forms of nitrogen.

Under saline condition, the chloride content in stem increased consistently with each increment of salinity (8 and 12 dSm^{-1}) irrespective of nitrogen source used as compare to non-saline condition. The per cent increase (143.90 and 182.92) was observed maximum in ammonical form and the minimum (77.46 and 114.08%) in combined form as compared to control plants.

Nitrogen in combined form (120 kg ha^{-1}) exhibited the maximum alleviation in the deleterious effect of salinity in chloride content of stem under saline condition over control. However, minimum alleviation was

observed with ammonical for nitrogen. Application of nitrogen (120 kg ha^{-1}) in combined form under saline condition resulted in minimum per cent increased (53.73 and 85.07). Such increases were noticed maximum (103.89 and 142.85%) at lower level of fertilizer application i.e. 40 kg ha^{-1} as compared to control plants.

Chloride content of root

Fig. 15C represents that chloride content was highest in root followed by leaves and stem under different level of salinity and fertilizer treatment. Plants treated with ammonical form of nitrogen resulted in maximum chloride content in root in comparison to nitrate and combined form of nitrogen.

Under saline condition (8 and 12 dSm^{-1}), the chloride content in root increased significantly irrespective of nitrogen source applied as compare to control plants. The per cent increase (145.29 and 188.03) in chloride accumulation was highest in ammonical form and the lowest (78.12 and 117.70%) observed in combined form as compare to non-saline plants.

The combined form of nitrogen with a dose of 120 kg ha^{-1} resulted in relatively less accumulation of chloride content in root under saline condition as compare to control plants. While the highest accumulation was observed with ammonical form of nitrogen. Moreover, higher level of nitrogen (120 kg ha^{-1}) in the combined form resulted in minimum per cent increase (51.76 and 83.52%) in chloride content at a salinity level of 8 and 12 dSm^{-1}, respectively as compared to control plants in roots. Such increase was noticed maximum (109.34 and 157.94%) at lower of fertilizer application i.e. 40 kg ha^{-1}.

Sulphate content of leaves

Fig. 16A represents that sulphate concentration was highest in leaves followed by stem and root under different level of salinity and fertilizer treatment. Although sulphate accumulation was noticed under different source of nitrogen used. However, ammonical and combined form caused considerably higher accumulation (35.57 and 13.55%) of sulphate content in leaves over nitrate form of nitrogen.

With increase in salinity level, a corrosponding increase in sulphate content in leaves was observed as compared to non-saline plants. These increase under salinity were maximum (59.71 and 78.37%) and minimum (41.70 and 62%) with ammonical and nitrate form, respectively as compared to control plants.

Application of nitrogen in nitrate form (120 kg ha^{-1}) mitigated the detrimental effect of salinity to a maximum extent in term of sulphate accumulation in leaves as compared to control plants. However, it was minimum with ammonical form of nitrogen. In addition to this highest level of nitrogen (120 kg ha^{-1}) in the nitrate form resulted in lowest upsurge (34.67 and 54.16%) in sulphate content at a senility level 8 and 12 dSm^{-1}, respectively as compared to control plants. Such increase was noticed maximum (51.07 and 73%) at lower level of fertilizer application i.e. 40 kg ha^{-1}.

Sulphate content of stem

Fig. 16B shows that when plant treated with different nitrogen source, the increase in sulphate content was maximum with ammonical form followed by combined form of nitrogen source.

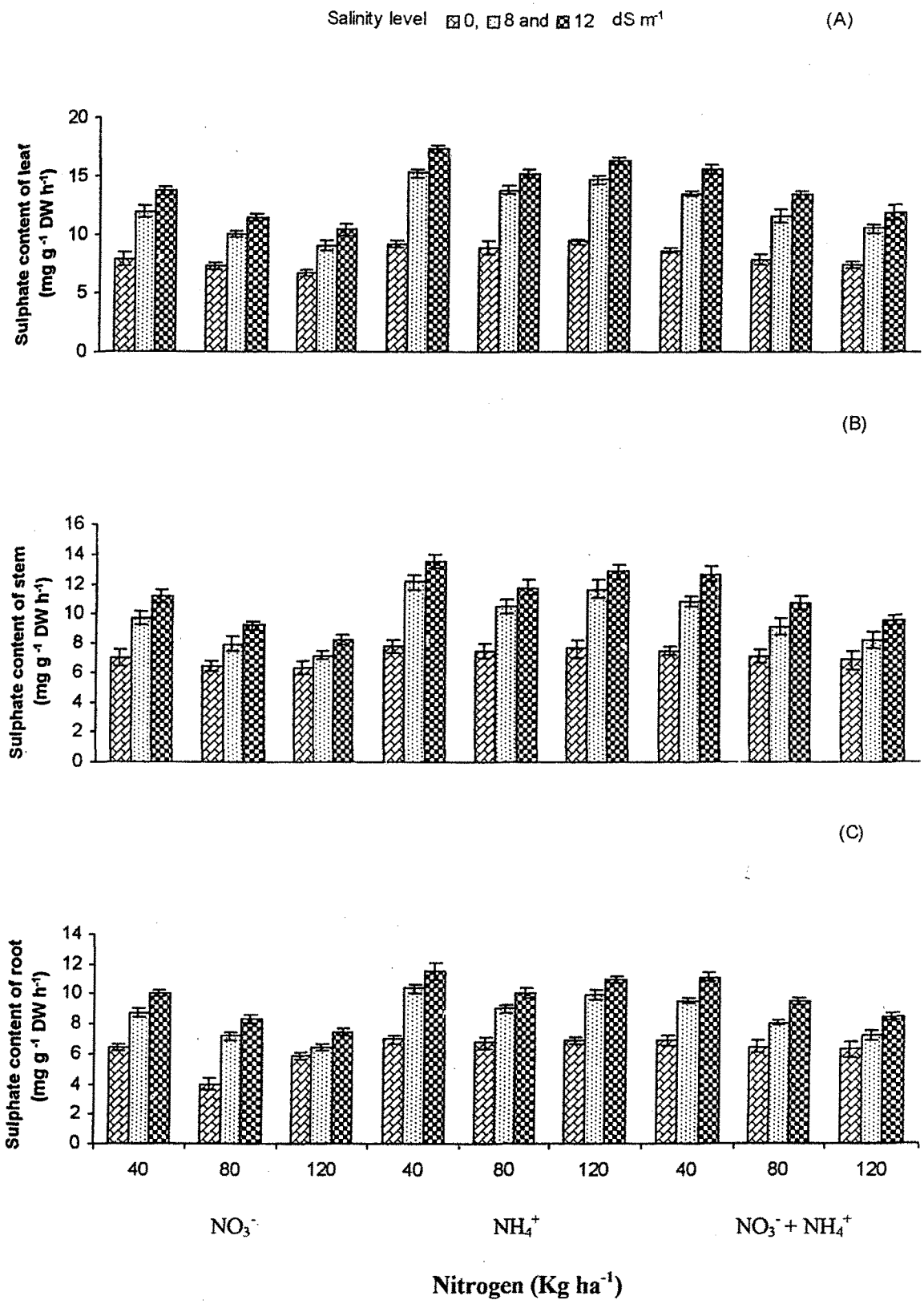

Fig. 16 Effect of nitrogen source, levels and their interaction with salinity on sulphate content of (A) leaf, (B) stem and (C) root in *Brassica juncea* cv. RH-30 at harvest.

Under saline condition, the treatment with ammonical form of nitrogen resulted in maximum per cent increase (49.67 and 66.44) over control in sulphate content of stem. However, the minimum per cent increased (25.41 and 45.05) was observed with nitrate form of nitrogen.

The treatment with nitrate form of nitrogen (120 kg ha^{-1}) exhibited highest alleviation on sulphate content in stem at higher level of salinity 8 and 12 dSm^{-1} as compared to non-saline plants. But minimum alleviation was observed with ammonical form of nitrogen. Nitrate form of nitrogen (120 kg ha^{-1}) treatment showed the minimum increase (11.74 and 30.47%) at 8 and 12 dSm^{-1} of salinity levels. Such increased was highest (38.25 and 60%) at lower level of fertilizer application i.e. 40 kg ha^{-1}.

Sulphate content of root

Plants treated with ammonical form had been found to contain maximum (23.81%) sulphate content over nitrate form of nitrogen (Fig. 16C).

Under saline condition (8 and 12 dSm^{-1}) sulphate concentration increased corresponding to salinity enhancement over control plants irrespective of nitrogen source applied. The per cent upsurge (42.62 and 58.24%) in sulphate accumulation was highest in ammonical form and the lowest (22.49 and 41.37%) nitrate form as compare to non-saline plants.

The nitrate form of nitrogen with a application of 120 kg ha^{-1} resulted in relatively less accumulation of sulphate content in root under saline condition as compare to control plants. While the highest accumulation was observed with ammonical form of nitrogen. Besides this, higher level of nitrogen (120 kg ha^{-1}) in the nitrate form exhibited the minimum per cent increase (10.25 and 27.17%) in sulphate concentration at a salinity

level of 8 and 12 dSm^{-1}, respectively as compared to non-saline plants. Such increases were noticed maximum (35.19 and 56.43%) at lower level of fertilizer application i.e. 40 kg ha^{-1}.

YIELD AND YIELD ATTRIBUTES

Number of pods per plant

A close examination of data in Table 19 revealed that plant treated with combined form of nitrogen and exposed to salinity at 35 days after sowing (Stage I) caused maximum number of pods per plant at harvest as compared to ammonical form.

Under saline condition, the number of pods per plant decreased significantly with every increment in salinity as compared to non-saline conditions irrespective of nitrogen source used. The plant treated with ammonical form of nitrogen exhibited maximum per cent reduction (30.31 and 51.81) and minimum per cent reduction (17.13 and 41.44) of number of pods per plant with combined form as compared to non-saline plants.

With increasing level of nitrogen to 80 and 120 kg ha^{-1} irrespective of source of nitrogen showed significant increase in number of pods per plant as compared to lower level of nitrogen application i.e. 40 kg ha^{-1}. In addition to this, the increase was maximum (29.01 and 47.79%) with application of nitrogen 80 and 120 kg ha^{-1}, respectively in combined form as compared to lower level of nitrogen application. The application of 120 kg ha^{-1} nitrogen in combined form resulted maximum mitigated the adverse effect of salinity as compared to lower level of nitrogen dose i.e. 40 kg ha^{-1}. However, minimum alleviation was observed with ammonical form of nitrogen.

Table 19. **Effect of nitrogen source, levels and their interaction with salinity on number of pods per plant in *Brassica juncea* cv. RH-30 at harvest**

Nitrogen sources	Nitrogen levels (kg ha^{-1})	Salinity levels (dSm^{-1})							
		0	8	12	**Mean**	0	8	12	**Mean**
		Stage-I				Stage-II			
Nitrate form (NO_3^-)	40	43.33	34.33	21.00	**32.88**	42.33	31.66	15.00	**29.66**
	80	51.33	42.33	28.00	**40.55**	48.33	39.00	25.00	**37.44**
	120	60.66	50.33	35.33	**48.77**	56.33	46.33	31.33	**44.66**
	Mean	**51.77**	**42.33**	**28.11**	**40.74**	**49.00**	**39.00**	**23.77**	**37.25**
Ammonical form (NH_4^+)	40	43.33	32.33	20.00	**31.88**	39.00	27.00	14.00	**26.66**
	80	48.33	31.33	22.00	**33.88**	44.33	33.33	19.00	**32.22**
	120	37.00	26.00	20.00	**27.66**	33.33	21.00	13.00	**22.44**
	Mean	**42.88**	**29.88**	**20.66**	**31.14**	**38.88**	**27.11**	**15.33**	**27.11**
Combined form (NO_3^-+NH_4^+)	40	46.33	35.33	23.00	**34.88**	44.33	34.33	19.00	**32.55**
	80	53.33	45.33	36.33	**45.00**	50.33	42.33	31.33	**41.33**
	120	63.66	54.66	36.33	**51.55**	61.66	52.33	41.33	**51.77**
	Mean	**54.44**	**45.11**	**31.88**	**43.81**	**52.11**	**43.00**	**30.55**	**41.88**
Overall mean		**49.70**	**39.11**	**26.88**		**46.66**	**36.37**	**23.22**	

C.D. (P<0.05) for Ns/Nl/Sl = 2.062
C.D. (P<0.05) for NsxNl, Nl x Sl & Ns x Sl = 3.571
C.D. (P<0.05) for Ns x Nl x Sl = 6.294

C.D. (P<0.05) for Ns/Nl/Sl = 1.884
C.D. (P<0.05) for NsxNl, Nl x Sl & Ns x Sl = 3.263
C.D. (P<0.05) for Ns x Nl x Sl = 5.749

Ns = Nitrogen source; Nl = Nitrogen level; Sl =Salinity level

A study of data Table 19 that the highest number of pods per plant at harvest was observe, when plant treated with combined form of nitrogen and exposed to salinity at 55 DAS (Stage II) as compared to ammonical form.

With increase in salinity level from 8 to 12 dSm^{-1} a correspondingly reduction in number of pods per plant was notice in different sources of nitrogen as compared to non-saline plants. Such reductions under salinity were maximum (30.27 and 60.57%) and minimum (17.48 and 41.37%) with ammonical and combined forms, respectively as compared to control plants.

The increasing level of nitrogen (80 and 120 kg ha^{-1}) irrespective of source of nitrogen caused increase in number of pods per plant as compared to lower level of nitrogen application i.e. 40 kg ha^{-1}. Beside this, the plant treated with combined form of nitrogen (80 and 120 kg ha^{-1}) exhibited maximum per cent increase (26.97 and 59.04) as compared to a dose of 40 kg ha^{-1}. The application of higher level of nitrogen (120 kg ha^{-1}) in combined form exhibited maximum alleviation the deleterious effect of salinity as compared to lower level of nitrogen application (40 kg ha^{-1}). However, minimum alleviation was observed with ammonical form of nitrogen.

The plant treated with basal dose of nitrogen fertilizer and saline water application at 35 and 55 DAS (Stage I and II) showed similar trend in number of pods per plant. However, the magnitude was relatively high in plant treated at 35 DAS (Stage I).

Grain yield per plant

Table 20 showed that grain yield per plant at harvest was maximum, when plant treated with combined form of nitrogen and exposed to salinity at 35 days after sowing (Stage I) as compared to ammonical form.

Under saline condition, the grain yield per plant decreased significantly with every increment in salinity as compared to non-saline conditions irrespective of nitrogen source used. The plant treated with ammonical form of nitrogen resulted in maximum per cent reduction (34.55 and 45.66) and minimum per cent reduction (17.31 and 40.95) in combined form of nitrogen as compared to non-saline plants.

Nitrogen in combined form (120 kg ha^{-1}) resulted in relatively higher grain yield per plant under saline condition over control. However, minimum grain yield was notice with ammonical form. Beside this, the higher level of nitrogen (120 kg ha^{-1}) in combined form exhibited minimum per cent reduction (9.94 and 18.28) in grain yield at a salinity level of 8 and 12 dSm^{-1}, respectively as compared to control plants. Such reduction was observed maximum (28.93 and 39.92%) at lower level of fertilizer application (40 kg ha^{-1}). The combined form of nitrogen (120 kg ha^{-1}) showed maximum alleviation on deleterious effect of salinity. However, minimum alleviation was observed with ammonical form.

A study of data in Table 20 revealed that plant treated with combined form of nitrogen and exposed to salinity at 55 DAS contained highest grain yield per plant at harvest as compared to ammonical form.

With increase in salinity level from 8 to 12 dSm^{-1} a corresponding reduction in grain yield per plant was notice as compared to non-saline plant. These reduction under salinity were maximum (25.03 and 33.24%)

Table 20. **Effect of nitrogen source, levels and their interaction with salinity on grain yield (g plant^{-1}) of *Brassica juncea* cv. variety RH-30 at harvest**

Nitrogen sources	Nitrogen levels (kg ha^{-1})	Salinity levels (dSm^{-1})							
		0	8	12	**Mean**	0	8	12	**Mean**
		Stage-I				Stage-II			
Nitrate form (NO_3^-)	40	1.964	1.364	1.113	**1.480**	1.831	1.192	0.927	**1.316**
	80	2.421	1.953	1.629	**2.001**	2.084	1.492	1.306	**1.627**
	120	2.732	2.395	2.176	**2.434**	2.550	2.111	1.925	**2.195**
	Mean	**2.372**	**1.904**	**1.639**	**1.972**	**2.155**	**1.598**	**1.386**	**1.713**
Ammonical form (NH_4^+)	40	1.182	0.722	0.637	**0.847**	0.965	0.557	0.413	**0.645**
	80	1.365	0.956	0.820	**1.047**	1.242	0.753	0.657	**0.884**
	120	1.292	0.835	0.629	**0.918**	0.921	0.467	0.414	**0.600**
	Mean	**1.279**	**0.837**	**0.695**	**0.937**	**1.043**	**0.592**	**0.494**	**0.710**
Combined form (NO_3^-+NH_4^+)	40	1.956	1.390	1.175	**1.507**	1.889	1.218	0.968	**1.358**
	80	2.552	2.127	0.835	**1.838**	2.135	1.577	1.416	**1.709**
	120	2.876	2.590	2.350	**2.605**	2.593	2.165	1.997	**2.251**
	Mean	**2.461**	**2.035**	**1.453**	**1.983**	**2.205**	**1.652**	**1.460**	**1.773**
Overall mean		**2.038**	**1.591**	**1.263**		**1.801**	**1.281**	**1.113**	

C.D. (P<0.05) for Ns/Nl/Sl	=	0.064	C.D. (P<0.05) for Ns/Nl/Sl = 0.057
C.D. (P<0.05) for NsxNl, Nl x Sl & Ns x Sl	=	0.097	C.D. (P<0.05) for NsxNl, Nl x Sl & Ns x Sl = 0.083
C.D. (P<0.05) for Ns x Nl x Sl	=	0.153	C.D. (P<0.05) for Ns x Nl x Sl = 0.139

Ns = Nitrogen source; Nl = Nitrogen level; Sl =Salinity level

and minimum (25.03 and 33.78%) with ammonical and combined form of nitrogen as compared to control plants.

Plant treated with nitrogen in combined form @ 120 kg ha^{-1} resulted in minimum reduction in grain yield as compared to control plants under saline condition. However, maximum reduction was observed with ammonical form of nitrogen. In addition to this, the higher level of nitrogen (120 kg ha^{-1}) in combined form resulted in minimum per cent reduction (16.50 and 22.98) in grain yield at a salinity level of 8 and 12 dSm^{-1} respectively as compared to control plants. Such reduction was observe maximum (35.52 and 48.75%) at lower level of fertilizer application i.e. 40 kg ha^{-1}. The combined form of nitrogen (120 kg ha^{-1}) resulted maximum mitigated the adverse effect of salinity. However, minimum alleviation was observed with ammonical form of nitrogen.

The plant treated with basal dose of nitrogen fertilizer and saline water application at 35 and 55 DAS (Stage I and II) resulted in similar trend in grain yield per plant. However, the magnitude was relatively higher in plant treated at 35 DAS (Stage I).

QUALITY CHARACTERS

Oil content

Plant treated with combined form of nitrogen and exposed to salinity at 35 DAS to contained highest oil content in seed at harvest as compared to two other forms i.e. nitrate and ammonical (Fig. 17A).

Under saline condition, the oil content in seed decrease with every increment in salinity as compared to non-saline conditions irrespective to nitrogen source used. These reduction under salinity were maximum (11.64 and 14.33%) in ammonical form and minimum (9.86 and 12.44%) with combined form.

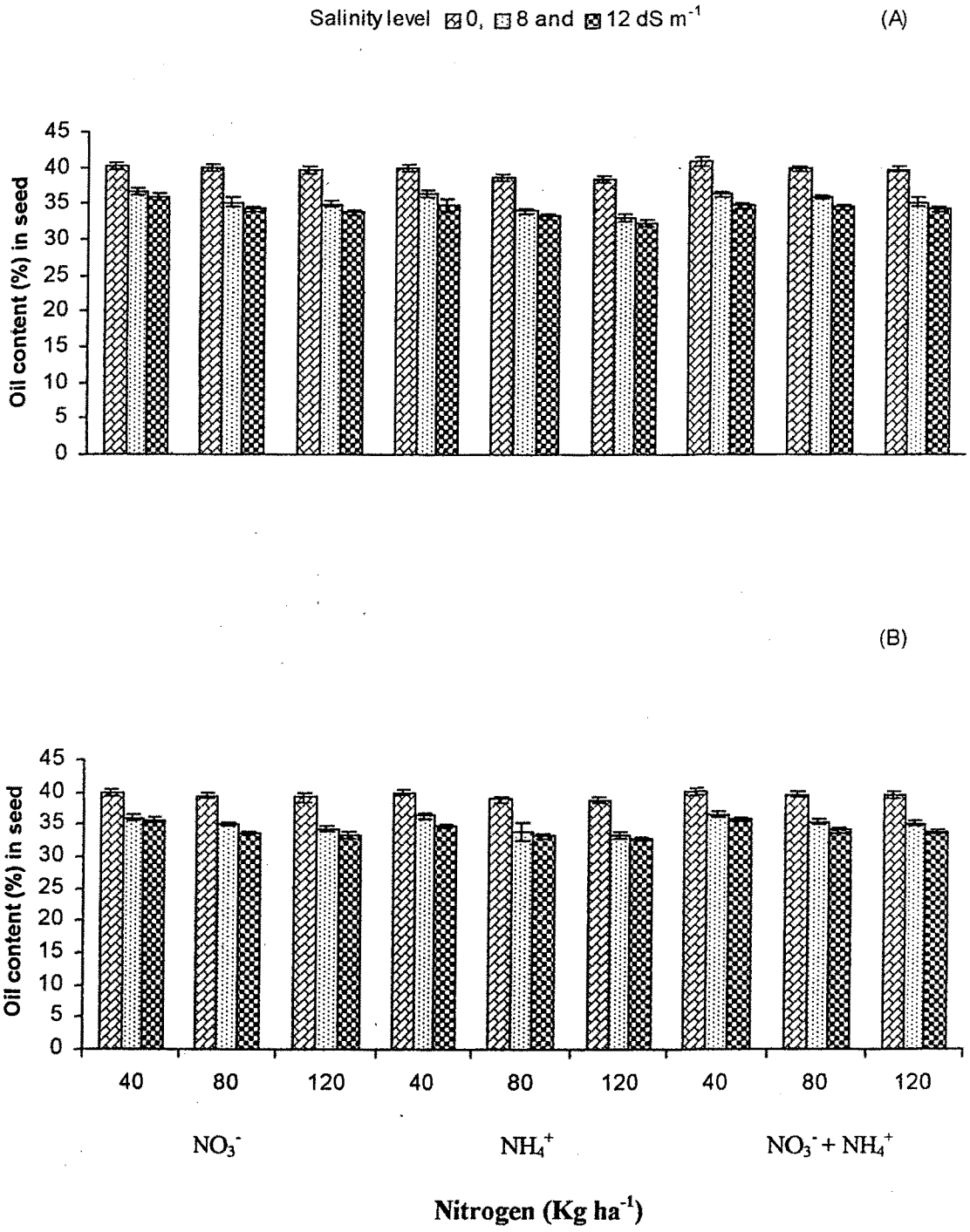

Fig. 17 Effect of nitrogen source, levels and their interaction with salinity on oil content in the seed of *Brassica juncea* cv. RH-30 at harvest (A) stage I and (B) stage II.

The increasing level of nitrogen (80 and 120 kg ha^{-1}) irrespective of source of nitrogen was reduced in oil content of seed as compared to lower level of nitrogen application i.e. 40 kg ha^{-1}. Plant treated with ammonical form (120 kg/ha) exhibited maximum reduction in oil content as compared to a dose of 40 kg ha^{-1} application. However, combined form of nitrogen (120 kg ha^{-1}) resulted minimum reduction in oil content as compared to lower level of nitrogen application. The interactive effects between salinity and different levels of nitrogen source had been found to be non significant.

Fig. 17B represents that the oil content in seed at harvest was observed highest when plant treated with combined form of nitrogen and exposed to salinity at 55 DAS (Stage II) as compared to nitrate and ammonical forms.

Under saline condition, the maximum and minimum reduction was observed with ammonical and combined form respectively as compared to non-saline plants. Plant treated with combined and ammonical form resulted in lowest and highest reduction respectively oil content of seed at nitrogen level of 80 and 120 kg ha^{-1} over 40 kg ha^{-1}. The interactive effect between salinity and different level of nitrogen source was found non-significant.

The plant treated with basal dose of nitrogen fertilizer and saline water application at 35 and 55 DAS (Stage I and II) showed similar trend in oil content. However the magnitude was relatively higher in plant treated at 55 DAS (Stage II).

Protein content

Fig. 18A shows that plant treated with combined form of nitrogen and exposed to salinity at 35 DAS (Stage I) contained highest protein

content in the seed at harvest as compared to two other forms i.e. nitrate and ammonical.

With increase in salinity level from 8 and 12 dSm^{-1} a corresponding reduction in protein content was observed as compared to non-saline plants. These reduction under salinity were maximum (9.75 and 10.47%) and minimum (5.28 and 6.51%) with application of ammonical and combined form of nitrogen, respectively as compared to control plants.

Plant treated with nitrate and ammonical form resulted in highest (5.29 and 9.11%) and lowest (6.64 and 6.73%) protein content, respectively under different level of nitrogen i.e. 80 and 120 kg ha^{-1} as compared to lower nitrogen application i.e.40 kg ha^{-1}. The interactive effect between salinity and different level of nitrogen sources had been found to be non-significant.

Fig. 18B represents that protein content in seed at harvest was observed highest when plant treated with combined form of nitrogen and exposed salinity at 55 DAS (Stage II) as compared to nitrate and ammonical form.

With increase in salinity level (8 and 12 dSm^{-1}) a corresponding reduction in protein content in seed was observed as compared to non-saline plants. These reduction under salinity were maximum (10.15 and 11.38%) in ammonical form and minimum (4.62 and 7.62%) in combined form over control plants.

Different source of nitrogen at the rate of 80 and 120 kg ha^{-1} resulted in significant increase of protein content in seed as compared to 40 kg/ha of nitrogen application. Beside this, the plant treated with nitrated form (120 and/ha) showed maximum per cent increase (10.14) as compared to

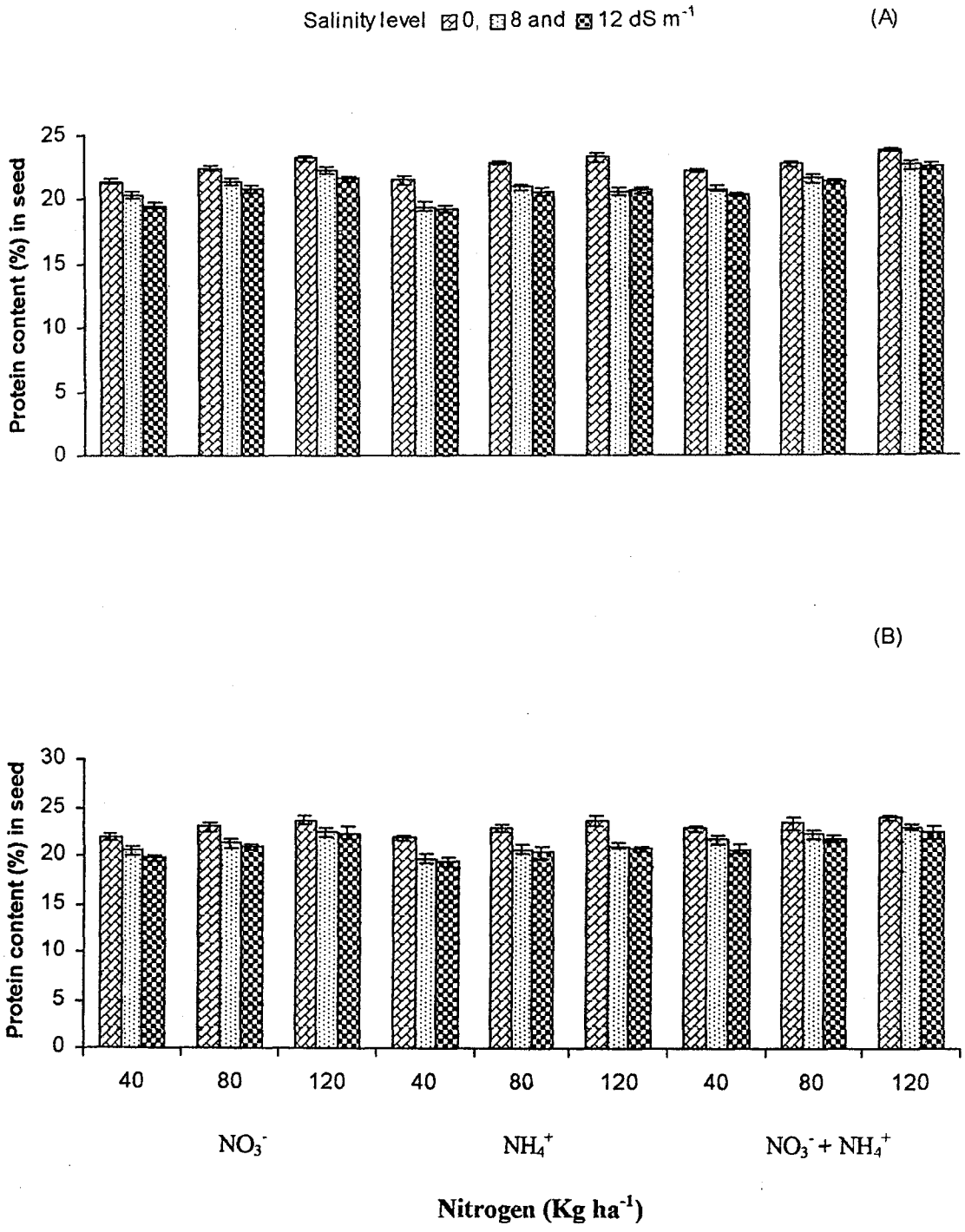

Fig. 18 Effect of nitrogen source, levels and their interaction with salinity on protein content in the seed of *Brassica juncea* cv. RH-30 at harvest (A) stage I and (B) stage II.

lower level of nitrogen application i.e. 40 kg ha^{-1}. The interaction between salinity and different level of nitrogen sources had been found to be non significant.

Protein content in seed showed that similar trend when plant treated with basal dose of nitrogen fertilizer and saline water application at 35 and 55 DAS. However, the magnitude was relatively slightly higher in plant treated at 55 DAS (Stage II).

Oleic acid

Fig. 19A shows that plant treated with combined form of nitrogen and exposed to salinity at 35 days after sowing (Stage I) to contained highest accumulation of oleic acid in seed at harvest as compared to nitrate form.

Under saline condition, the plant treated with combined form of nitrogen exhibited highest accumulation (7.60 and 19.70%) of oleic acid content and ammonical form cause lesser accumulation (7.56 and 8.61%) as compared to non-saline plants.

Nitrogen in combined form (120 kg ha^{-1}) resulted in the relatively higher accumulation of oleic acid in seeds under saline condition over control. However, minimum accumulation was noticed with ammonical form of nitrogen. Beside this, the higher level of nitrogen (120 kg ha^{-1}) in combined form exhibited maximum per cent increase (9.73 and 22.25%) in oleic acid at a salinity level of 8 and 12 dSm^{-1}, respectively as compared to control plants. Such increase was observed minimum (4.94 and 17.05%) at lower level of fertilizer application.

Fig. 19B represents that the highest accumulation of oleic acid in seeds at harvest was observed when plant treated with combined form of

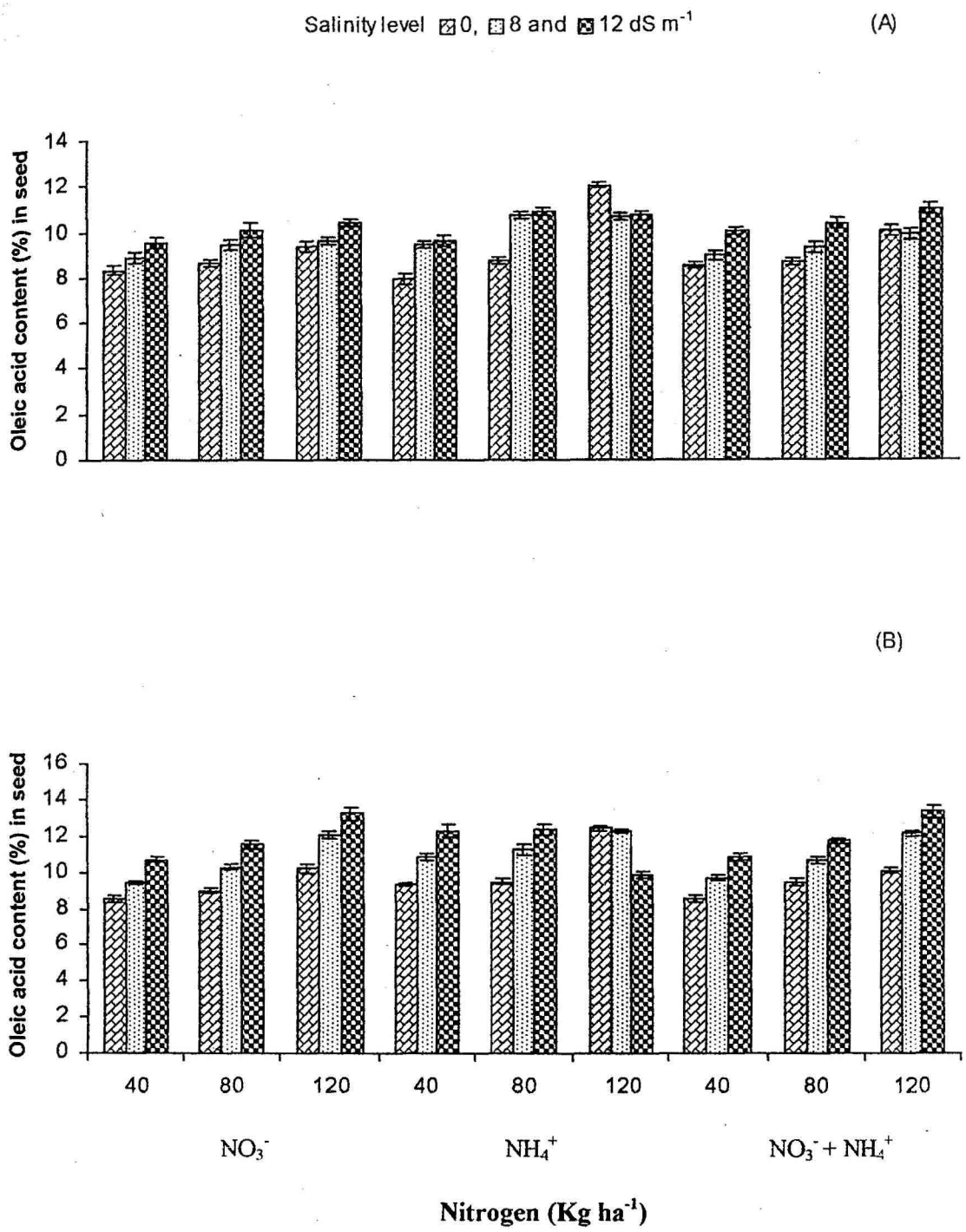

Fig. 19 Effect of nitrogen source, levels and their interaction with salinity on oleic acid content in the seed of *Brassica juncea* cv. RH-30 at harvest (A) stage I and (B) stage II.

nitrogen and exposed salinity at 55 days after sowing (Stage II), as compared to ammonical form.

With increase in salinity level from 8 to 12 dSm^{-1} a progressive icnrease in oleic acid in seed was noticed as compared to non-saline plants. These increases under salinity were maximum (17.18 and 29.72%) and minimum (10.07 and 10.55%) with combined and ammonical forms, respectively as compared to control plants.

The extent of accumulation of oleic acid in seed under saline condition was relatively high with application of nitrogen in combined form (120 kg ha^{-1}) as compared to control plants. However, it was lowest with ammonical form of nitrogen. In addition to this, higher level of nitrogen (120 kg ha^{-1}) in combined form resulted in maximum accumulation (20.51 and 32.21%) in oleic acid at salinity level of 8 and 12 dSm^{-1}, respectively as compared to non-saline plants. Such accumulateion was noticed minimum (13.43 and 27.33%) at lower level of fertilizer application i.e. 40 kg ha^{-1}.

The plants treated with basal dose of nitrogen fertilizer and saline water application at 35 and 55 DAS (Stage I and stage II) showed similar trend in oleic acid content in seeds. However, the magnitude was relativley higher in plant treated at 55 DAS (Stage II).

Linoleic acid

Fig. 20A shows that plant treated with combined form of nitrogen and exposed to salinity at 35 days after sowing (Stage I) contained highest accumulation of linoleic acid in seed at harvest as compared to two other forms i.e. nitrate and ammonical.

Under saline condition, the plant treated with combined form of nitrogen exhibited highest accumulation (13.49 and 15.81%) of linoleic acid content and ammonical form cause lesser accumulation (2.49 and 4.92%) as compared to non-saline plants. Nitrogen in combined form (120 kg ha^{-1}) resulted in the relatively higher accumulation of linoleic acid in seeds under saline condition over control. However, minimum accumulation was noticed with ammonical form of nitrogen. Beside this, the higher level of nitrogen (120 kg ha^{-1}) in combined form exhibited maximum per cent increase (16.53 and 18.40) in linoleic acid at a salinity level of 8 and 12 dSm^{-1}, respectively as compared to control plants. Such increase was observed minimum (9.20 and 10.89%) at lower level of fertilizer application.

Fig. 20B represents that the highest accumulation of linoleic acid in seed at harvest was observed when plant treated with combined form of nitrogen and exposed salinity at 55 days after sowing, as compared to nitrate and ammonical forms. With increase in salinity level from 8 and 12 dSm^{-1} a progressive increase in linoleic acid in seed was notified as compare to non-saline plants. These increase under salinity were maximum (13.52 and 20.27%) and minimum (7.64 and 13.29%) with combined and ammonical form, respectively as compared to control plants.

The extent under saline condition was relatively high with the application of nitrogen in combined form (120 kg ha^{-1}) as compared to control plants. However, it was lowest with ammonical form of nitrogen. In addition to this, highest level of nitrogen (120 kg ha^{-1}) in combined form resulted in maximum accumulation (15.55 and 19.80%) in linoleic acid at salinity level of 8 and 12 dSm^{-1}, respectively as compared to non-

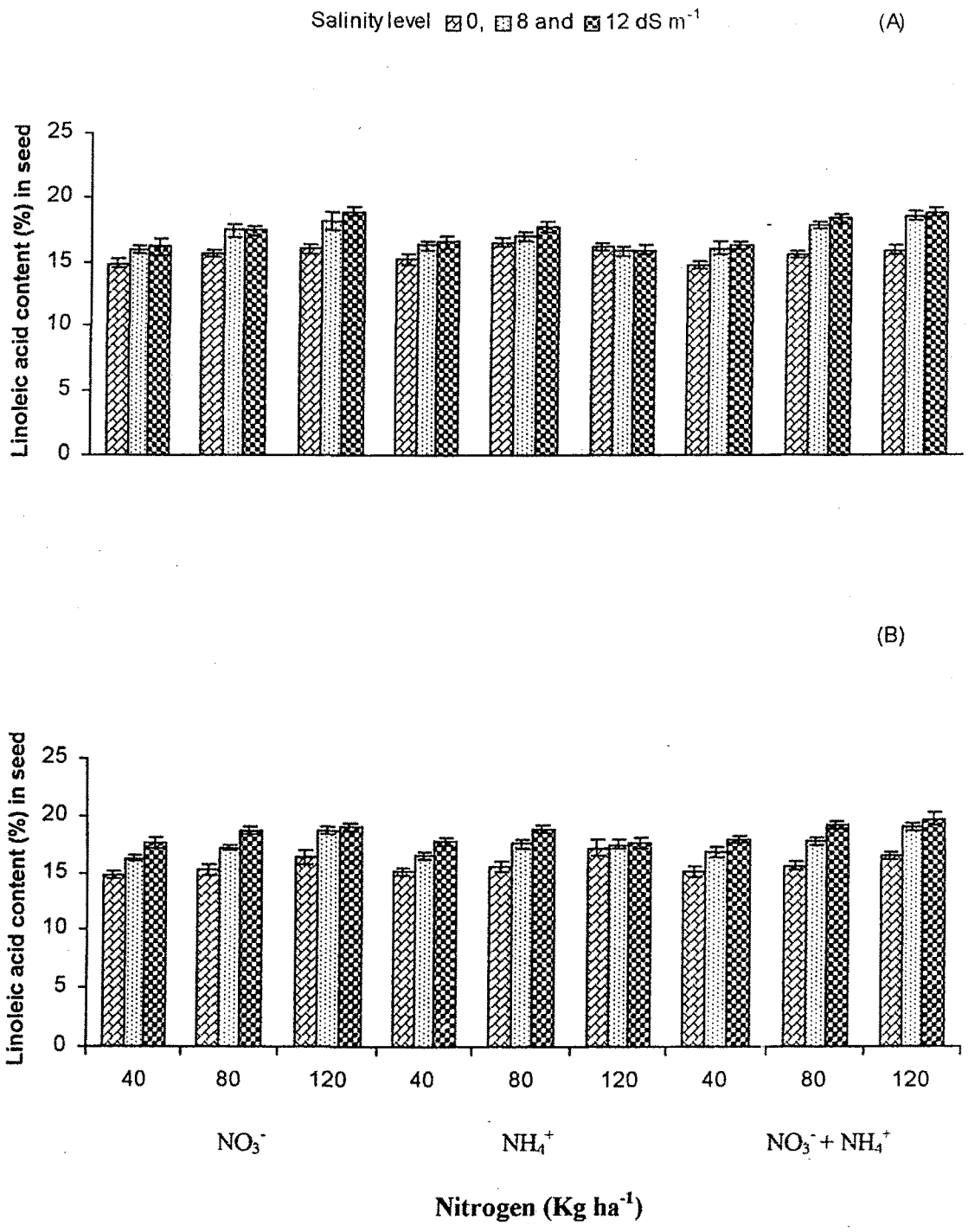

Fig. 20 Effect of nitrogen source, levels and their interaction with salinity on linoleic acid content in the seed of *Brassica juncea* cv. RH-30 at harvest (A) stage I and (B) stage II.

saline plants. Such accumulation was noticed minimum (11.01 and 18.46%) at lower level of fertilizer application i.e. 40 kg ha^{-1}.

The plants treated with basal dose of nitrogen fertilizer and saline water application at 35 and 55 days after sowing (Stage I and II) showed similar trend in linoleic acid content in seed. However, the magnitude was relatively higher in plants treated at 55 days after sowing (Stage II).

Linolenic acid

Fig. 21A shows that plant treated with combined form of nitrogen and exposed to salinity at 35 DAS (Stage I) contained highest accumulation of linolenic acid content in seed at harvest as compared to two other forms i.e. nitrate and ammonical form.

With increase in salinity level from 8 and 12 dSm^{-1} corresponding increase in linolenic acid in seed was observed as compared to non-saline plants. These increases under salinity were maximum and minimum with combined and nitrate form, respectively as compared to control plants. The interactive effect of salinity and different level of nitrogen on linolenic acid content was found to be non-significant.

Fig. 19B represents that the maximum accumulation of linolenic acid in seed at harvest was observed, when plant treated with combined form of nitrogen and imposed salinity at 55 DAS (Stage II) as compared to nitrate and ammonical forms.

Under saline condition, when plant treated with combined form of nitrogen showed highest accumulation (10.77 and 17.37%) of linolenic acid content in seed and ammonical form cause lesser extent accumulation (5.95 and 10.87%) as compared to non-saline plants.

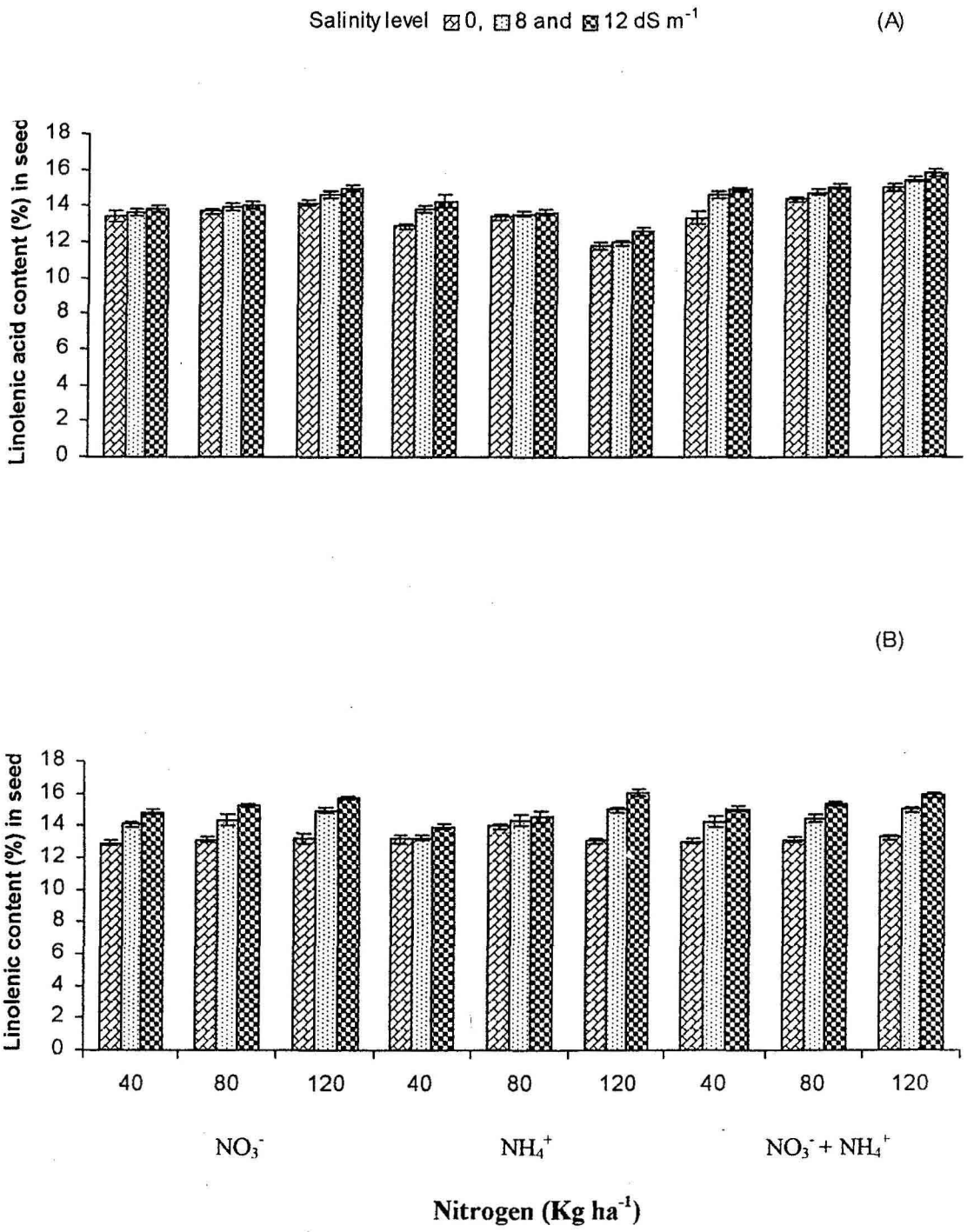

Fig. 21 Effect of nitrogen source, levels and their interaction with salinity on linolenic acid content in the seed of *Brassica juncea* cv. RH-30 at harvest (A) stage I and (B) stage II.

Nitrogen in combined form (120 kg ha^{-1}) resulted in the relatively higher accumulation of linolenic acid in seed under saline condition over control plants. However, minimum accumulation was noticed with ammonical form of nitrogen. Beside this, the higher level of nitrogen (120 kg ha^{-1}) in combined form exhibited maximum increase (12.77 and 19.60%) in linolenic acid at a salinity level of 8 and 12 dSm^{-1} respectively as compared to control plants. Such increase was observed minimum (9.34 and 15.69%) at lower level of fertilizer application.

The plants treated with basal dose of nitrogen fertilizer and saline water application at 35 and 55 DAS (Stage I and II) showed similar trend in linolenic acid content. However, the magnitude was relatively higher in plants treated at 55 DAS (Stage II).

Erucic acid

Fig. 22A shows that erucic acid content in seed at harvest was decreased when plant treated with different nitrogen source and exposed salinity at 35 DAS (Stage I). The reduction was greater with combined form of nitrogen as compared to nitrate and ammonical form.

With increase in salinity level (8 and 12 dSm^{-1}) a corresponding reduction in erucic acid content of seed was observed as compare to non-saline plants. These reduction under salinity were maximum in combined form and minimum with ammonical form as compared to control plants.

The increasing level of nitrogen (80 and 120 kg ha^{-1}) irrespective of source of nitrogen significantly reduction in erucic acid content of seed as compared to lower level of nitrogen application i.e 40 kg ha^{-1}. Plant treated with combined form (120 kg ha^{-1}) resulted in greater reduction in erucic acid content as compared to lower level of nitrogen (40 kg ha^{-1}).

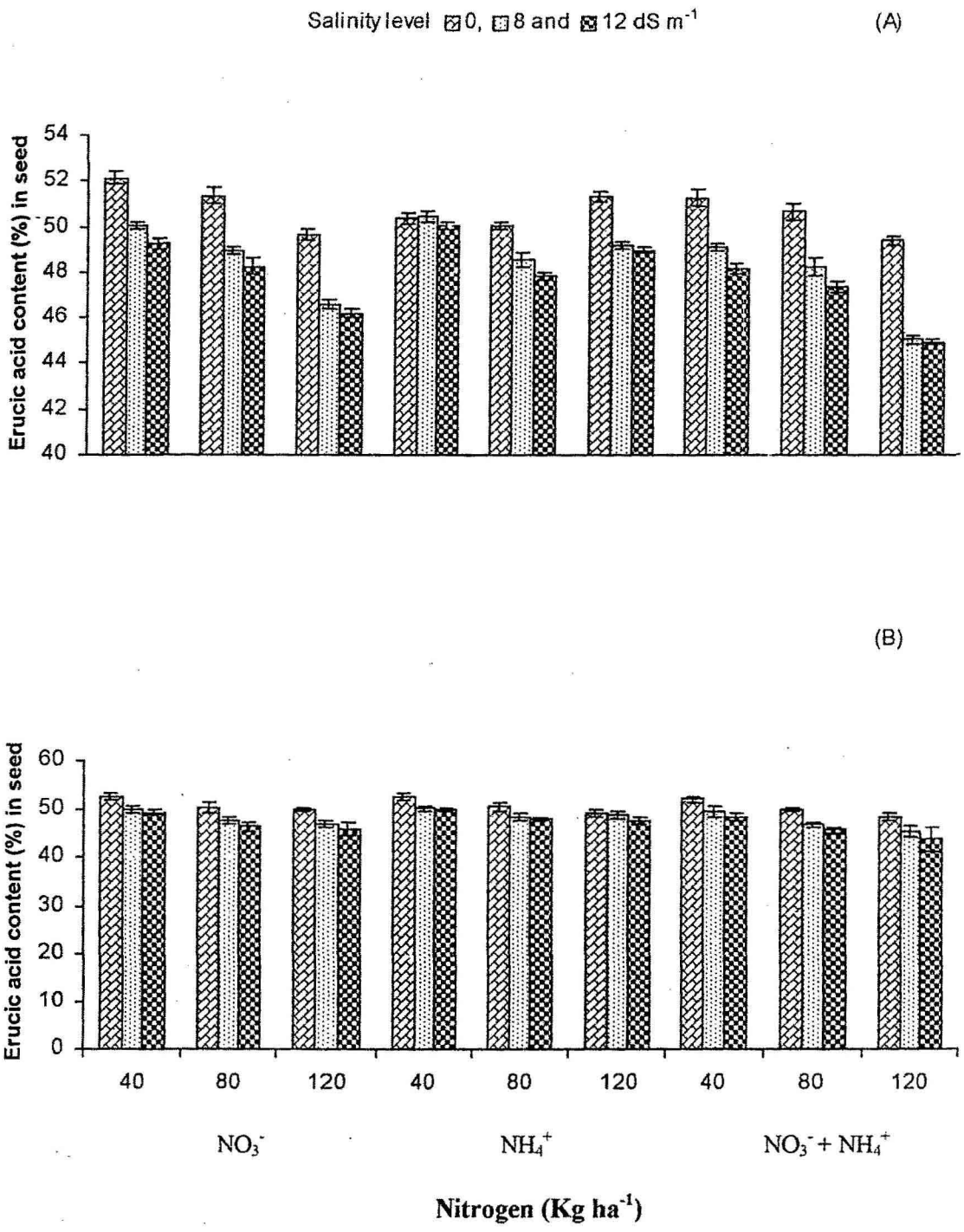

Fig. 22 Effect of nitrogen source, levels and their interaction with salinity on erucic acid content in the seed of *Brassica juncea* cv. RH-30 at harvest (A) stage I and (B) stage II.

However, minimum reduction was observed with ammonical form of nitrogen. The interactive effects between salinity and different levels of nitrogen source had been found to be non-significant.

The erucic acid content in seed at harvest was decreased when plant treated with different nitrogen source and exposed salinity at 55 DAS (Stage II). The reduction was maximum with combined form of nitrogen as compared to nitrate and ammonical form (Fig. 22B).

Under saline condition, the erucic acid content in seed decreased with every increment in salinity as compared to non-saline conditions irrespective of nitrogen source used. The plant treated with combined form of nitrogen exhibited maximum reduction (5.81 and 8.43%) and minimum reduction (3.22 and 4.38%) was observed with ammonical form as compared to non-saline plants.

Nitrogen in combined form (120 kg ha^{-1}) resulted in the relatively lower accumulation of erucic acid content in seed under saline condition over control. However, maximum accumulation was noticed with ammonical form of nitrogen. Beside this, the higher level of nitrogen (120 kg ha^{-1}) in combined form exhibited maximum per cent reduction (6.52 and 9.60%) in erucic acid at a salinity level of 8 and 12 dsm^{-1} respectively as compared to control plants. Such reduction was observed less (4.87 and 7.14%) at lower level of fertilizer application.

The plant treated with based dose of nitrogen fertilizer and saline water application at 55 and 55 DAS (Stage I and II) resulted in similar trend in erucic acid content. However, the magnitude was slightly higher in plant treated at 55 DAS (Stage II).

Palmitic acid

Fig. 23A shows that plant treated with combined form of nitrogen and exposed salinity at 35 DAS (Stage I) had been found to contain lowest accumulation of palmitic acid in seed at harvest as compared to two other forms i.e. ammonical and nitrate. Under saline condition the treatment with combined form of nitrogen resulted in maximum per cent reduction (23.18 and 32.52) over control in palmitic acid of seed. However, the minimum per cent reduction (14.96 and 18.61) was observed with ammonical form.

The increasing level of nitrogen (80 and 120 kg ha^{-1}) irrespective of source nitrogen significantly reduced in plamitic acid content of seed as compared to lower level of nitrogen application i.e. 40 kg ha^{-1}. In addition to this, the reduction in palmitic acid was maximum with application (120 kg ha^{-1}) in combined form of nitrogen as compared to lower level nitrogen. The interactive effect between salinity and different level of nitrogen source had been to be found non-significant.

Fig. 23B represents that plant treated with combined form of nitrogen and exposed of salinity at 55 DAS (Stage II) to contained lowest accumulation of palmitic acid in seed at harvest as compared to ammonical and nitrate forms. With increase in salinity level from 8 and 12 dSm^{-1} a corresponding reduction in palmitic acid in seed was noticed as compared to non-saline plants. These reduction under salinity were maximum (19.59 and 32.43%) and minimum (19.36 and 25.0%) with combined and ammonical forms, respectively compared to control plants.

Plant treated with 120 kg/ha nitrogen in combined form resulted in maximum reduction in palmitic acid as compared to 40 kg ha^{-1} nitrogen.

However, such reduction was observed minimum in two other forms i.e. nitrate and ammonical. The interactive effect between salinity and different level of nitrogen source had been found to be non-significant.

The plant treated with basal dose of nitrogen fertilizer and saline water application at 35 and 55 DAS (Stage I and II) showed similar trends in palmitic acid content. However, the magnitude slightly higher in plant treated at 35 DAS (Stage I) except combined form of nitrogen.

Glucosinolate content

The glucosinolate content in seed at harvest was lowest in plant treated with combined form of nitrogen and exposed salinity at 35 DAS (Stage I) as compared to ammonical and nitrate form (Fig. 24A).

Under saline condition, the treatment with ammonical form of nitrogen resulted maximum per cent increase (10.68 and 22.51) in glucosinolate content of seed over control. However, per cent increase was minimum (9.41 and 16.97) with combined form of nitrogen application.

The treatment with ammonical form of nitrogen (120 kg ha^{-1}) exhibited highest accumulation of glucosinolate content in seed under saline condition, as compared to non-saline plants. But minimum accumulation was noticed with combined form of nitrogen. Beside this, treatment with 120 kg ha^{-1} of nitrogen in ammonical form showed maximum accumulation of glucosinolate content at salinity level 8 and 12 dSm^{-1} as compared to control plants. Such accumulation was lowest at 40 kg ha^{-1} nitrogen application.

Fig. 24B shows that when plant treated with combined form of nitrogen and exposed to salinity at 55 DAS (Stage II) contained lowest

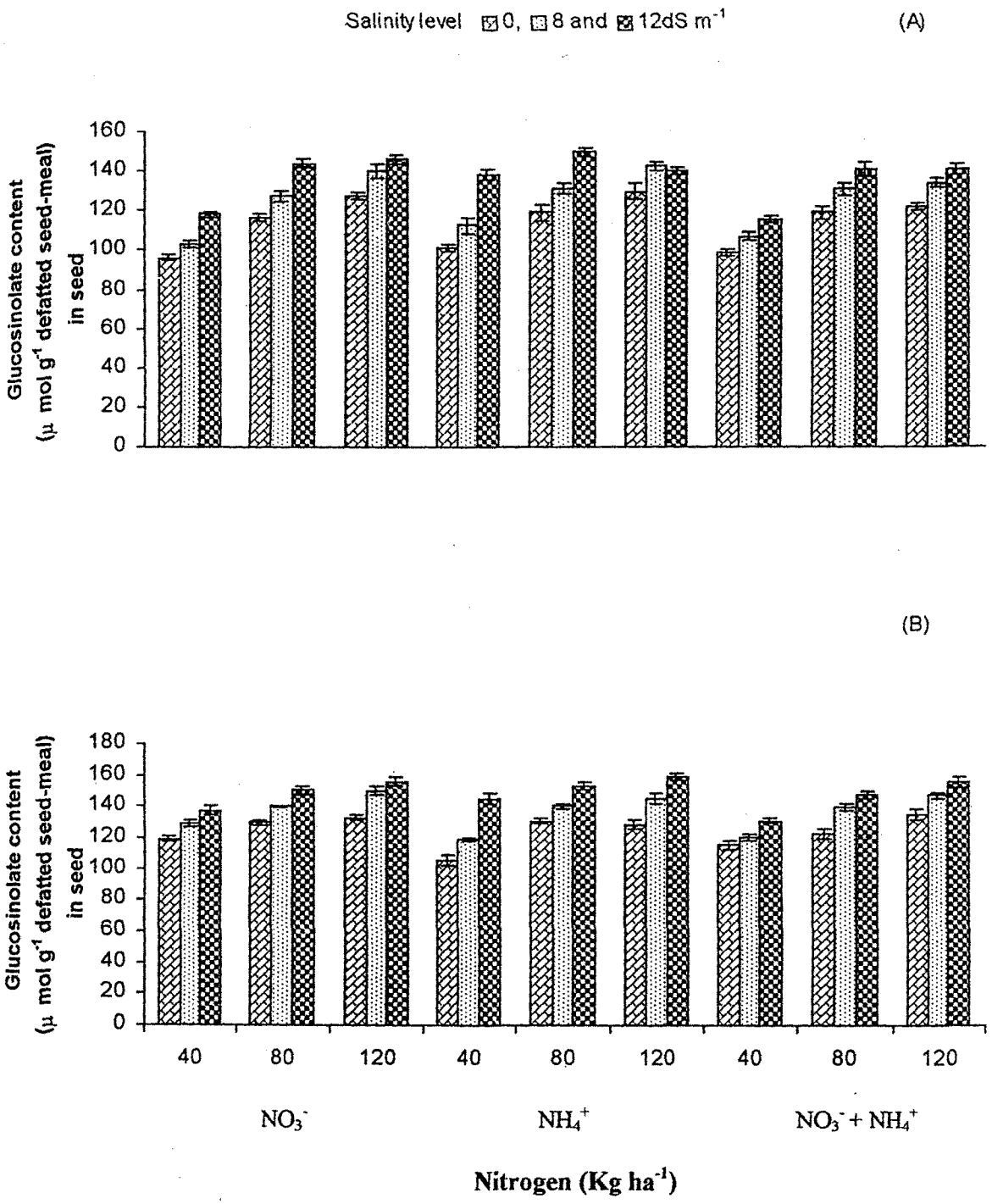

Fig. 24 Effect of nitrogen source, levels and their interaction with salinity on glucosinolate content in the seed of *Brassica juncea* cv. RH-30 at harvest (A) stage I and (B) stage II.

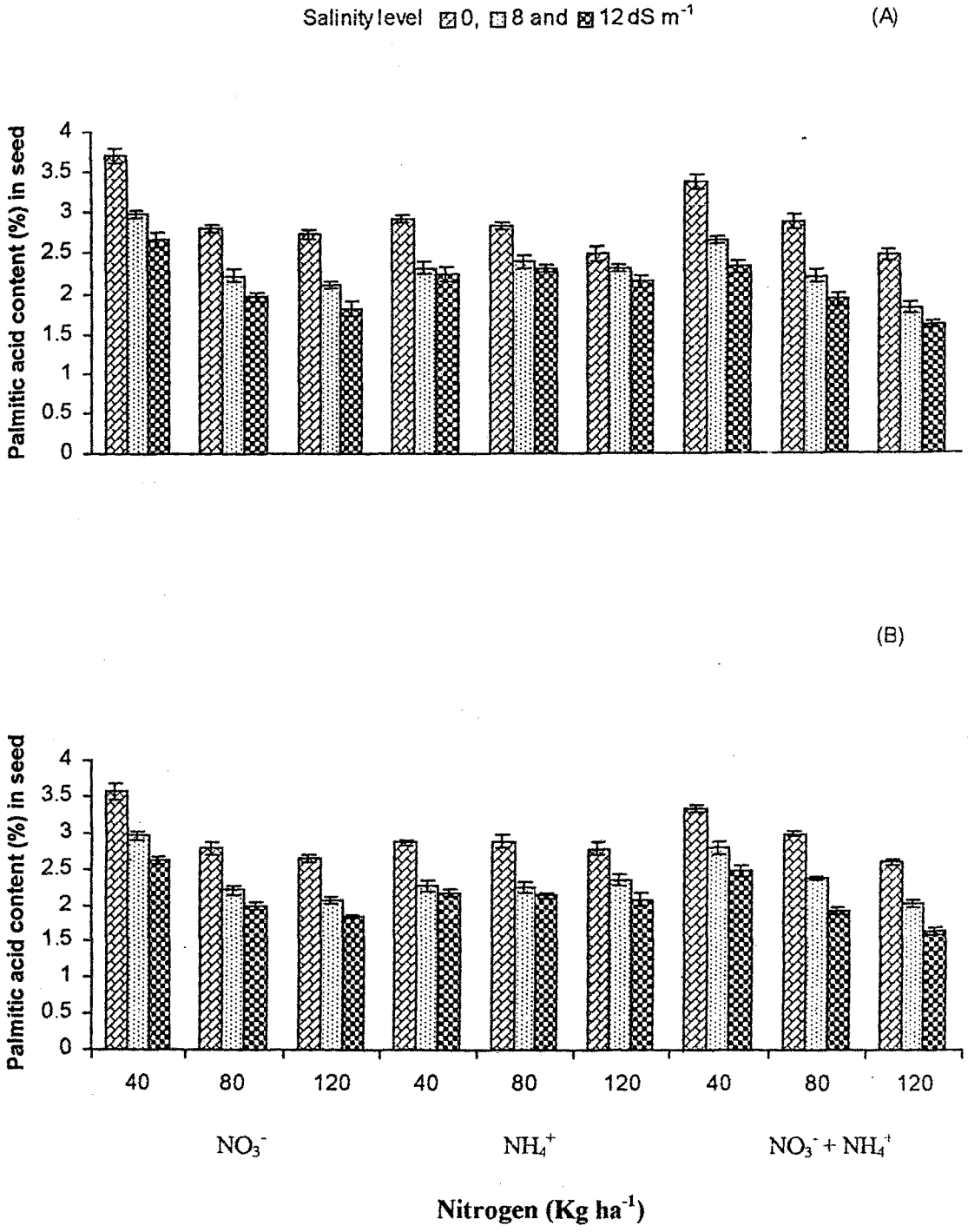

Fig. 23 Effect of nitrogen source, levels and their interaction with salinity on palmitic acid content in the seed of *Brassica juncea* cv. RH-30 at harvest (A) stage I and (B) stage II.

accumulation of glucosinolate content in seed at harvest as compared to ammonical form of nitrogen.

With increase in salinity, a corrosponding increase in glucosinolate content in seed was observed as compared to non-saline plants. These increase under salinity was maximum (10.99 and 25.57%) and minimum (9.13 and 19.39%) with ammonical and combined form of nitrogen, respectively as compare to control plants.

Application of nitrogen in ammonical form (120 kg ha^{-1}) had been found to cause maximum accumulation of glucosinolate content in seed under saline condition as compared to non-saline plants. However, minimum accumulation was observed with combined form of nitrogen. In addition to this, nitrogen at rate of 120 kg ha^{-1} in the ammonical form resulted in maximum accumulation of glucosinolate content as compared to 40 kg ha^{-1} nitrogen application under saline condition.

The glucosinolate content in seed exhibited similar trend when plant treated with basal dose of nitrogen fertilizer and saline water application at 35 and 55 DAS. However, the magnitude way relatively higher in plant treated with 55 DAS (Stage II).

CHAPTER - 5

DISCUSSION

Salinity poses several problems for plant growth and development by inducing physiological disfunctioning (Shannon *et al.*, 1994). Saline environment is generally deficient in nitrogen (Amonkar and Karmarkar, 1995) and in addition salinity interfers with NO_3^- uptake in many plant species which decreases NO_3^- content (Khan and Srivastava, 1998). The reduction in NO_3^- uptake could be due to high Cl^- content in saline soil. Addition of N to plants subjected to salinity improved their growth and yield and thus their salt tolerance (Dubey and Pessarakli, 1995).

The present investigation was carried out on *Brassica juncea* cv. RH-30 to interactive effects of salinity and fertilizers to probe into some morpho-physiological, biochemical, nitrogen metabolism, nutrients status and quality changes at various growth stages. Before sowing of seeds the pots were supplied with nitrogen through different nitrogen sources i.e. Nitrate, ammonical and combined forms having three levels (40, 80 and 120 kg ha^{-1}). The desired salinity levels (ECe 0, 8 and 12 dSm^{-1}) were obtained by adding Cl and SO_4 salts of Na, Ca and Mg alongwith non-saline control. The salinity solutions were applied to plants at 35 and 55 DAS (which corresponds to stage I and stage II). Subsequently, sampling was done at 10 days after applying saline water irrigation.

Growth and development

Plant height at maturity and dry weight of different plant parts (leaf and stem) at both sampling stages (45 and 65 DAS) decreased significantly under high salinity (Tables 1, 2, 3 and 8). Similar inhibitory effect of salinity have been reported by different workers (Ashraf and Yousaf, 1998; Kurban *et al.*, 1998). Chauhan (1991) and Cho Jin woong and Kim Choongsoo (1998) have also reported decrease in fresh and dry weight of various plant parts with increased salinization. Numerous studies have shown that plant height, fresh and dry weight of plant decreased under salt stress (Kuhad *et al.*, 1989; Minhas *et al.*, 1990; Leidi *et al.*, 1991; Delzoppo *et al.*, 1999). The degree of inhibition in plant height and dry weight of different plant parts were related to the N-source used under salinity. A maximum inhibition of plant height and dry weight of different plant parts occurred in plant treated with ammonical forms. However, minimum reduction was observed when nitrogen supplied through combined form (Tables 1, 2, 3 and 8). Growth inhibition in NH_4^+-fed plants was observed in several species, such as cucumber, castor bean and bean (Schenk and Wehrmann, 1979; Allen *et al.*, 1985; Chaillou *et al.*, 1986). The reduction in growth was noticed maximum in ammonical fed plants as compared to nitrate fed plants. Similarly, Magalhaes and Huber (1991) also reported that fresh and dry matter accumulation of tomato shoots and root decreased maximum in ammonical fed plants as compared to nitrate fed plants.

Increasing the salinity levels, as would be normally expected, resulted in a progressive decline in growth parameter. However, nitrogen application through different sources partially alleviated these decline. But the magnitude of the decline in plant height and dry weight of different parts with increasing salinity was markedly less under the higher nitrogen level used (120 kg ha^{-1}) as compared to lower dose of nitrogen (40 kg ha^{-1}) irrespective of the nitrogen source except ammonical form (120 kg ha^{-1}) where the effect was more detrimental. The combined form of nitrogen (120 kg ha^{-1}) significantly alleviated the adverse effects of salt stress as compared to lower level of nitrogen which poorly ameliorated the adverse effect of salinity upto 80 kg ha^{-1}. It is interesting to note again that the per cent reduction in different growth parameters due to salt stress was relatively less with combined form of nitrogen as compared to two other forms (Tables 1, 2, 3 and 8). Reduction in plant growth under salinity is attributed to reduced water absorption due to osmotic effect, nutritional deficiency on account of ionic imbalance and decrease in many metabolic activities. However, supplementation of nitrogen to plant has been acceded in delaying senescence. Nitrogen being requisite for plant growth and development is required either in form of nitrate or ammonium (Maduff and Jackson, 1991). Salinity induced reduction in growth, due to decrease in critical water level, ionic imbalance (Flower *et al.*, 1986) and seedling vigour as well metabolites in root and leaf tissues, may be modulated by nitrate or ammonium supply either by replenishing the ions or through increased metabolites (Mishra *et al.*, 1996). The fertilized induced

improvement in growth and development under salinity stress has been reported in different crops by Garg *et al.* (1982, 1993) and Khan *et al.* (1994).

Growth analysis is fundamental to the characterization of a plant response to an environmental stress. It provides useful information as to when stress effects occur, allowing for a critical analysis of the series of events leading to growth inhibition or stress symptoms. In addition, growth analysis can provide useful information regarding the nature of the stress effect on growth. Changes in the relative growth rate (RGR) with time can be detected and correlated with two variables that influence RGR (i) the net assimilation rate (NAR) and (ii) the leaf area ratio (LAR) (He and Cramer, 1993).

Growth parameters like leaf area, AGR, RGR and NAR in plants declined significantly with increasing levels of salinity (8 and 12 dSm^{-1}) at both the sampling stages i.e. 45 and 65 DAS. The magnitude of reduction was highest in ammonical fed plants and lowest in combined form of nitrogen. By contrasts, N fertilization brought about an enhancement of growth parameters in control and salinized plants. The increasing levels of nitrogen irrespective of its source except ammonical form (120 kg ha^{-1}) significantly increased the growth rate as compared to non-saline plants. The percentage of decline in growth parameters with increasing salinity was less in the higher level of nitrogen application as compared to the lower level of nitrogen. The higher nitrogen application (120 kg/ha) in combined form of nitrogen exhibited the maximum alleviation in the

adverse effect of salinity on growth parameters. But ammonical form of nitrogen upto the level of 80 kg ha^{-1} showed minimum alleviation on the adverse effect of salinity and at 120 kg ha^{-1} further inhibition was noticed in growth parameters (Tables 4, 5, 6 and 7).

Numerous studies have shown that plant growth get reduced under salinity (Singh and Darra, 1971; Narayana and Rao, 1987). This may be due to adverse effect of salinity through osmotic effect or ionic imbalance and on metabolic processes (Greenway and Munns, 1980; Levitt, 1980). The reduced LAR under the saline conditions reflects a reduced 'leafiness' of the plant, probably due to an inhibition of leaf expansion relative to other plant parts (the number of leaves remain unaffected by salinity). Leaf expansion rate is sensitive to leaf turgor (Bradford and Hsiao, 1982), but turgor does not appear to limit the growth of salt stressed plants (Cramer and Bowman, 1991). Salt stress can also reduce hydraulic conductivity (Munns and Passioura, 1984), decrease the extensibility and increase the yield threshold of the cell wall (Cramer, 1992). These changes in the cell growth parameters may be responsible for the decreased expansion growth of plants under salt stress. A decrease of NAR reflects a decrease in the rate of photosynthesis (Rawson *et al.*, 1988) or an increase in respiration (Schwarz and Gale, 1981). Munns *et al.* (1995) also indicated that the salt within the plant reduces growth by causing premature senescence of old leaves and hence a reduced supply of assimilates to the growing regions. They indicated that most changes in metabolism or gene expression leading to growth reduction during the first phase related to

the osmotic effect of salinity, rather than salt specific effect. The adverse effect of salinity might be due to adverse effect on translocation and partitioning of assimilates towards sink and metabolic processes (Narayana and Rao, 1987; Paek *et al.*, 1988; Taneja, 1988). Termaat and Munns (1986) concluded that changes in cereal leaf expansion would be determined by changes in cell wall properties. On the other hand, Neumann *et al.* (1988) indicated that salinity affects growth rate of bean leaves by changes in turgor. One may also speculate that leaf expansion under saline conditions may be retrieved by N fertilization. A steady supply of N to peanut plants inhibited the reduction in growth caused by NaCl, since the uptake of N by the plant was renewed which allowed for the full expansion of leaves as well as the production of new ones (Silberbush and Lips, 1988).

Water relations and gas exchange

The availability of water for its biological roles as solvent and transport medium, as electron donor in the hill reaction, and as evaporative coolant has been impaired by environmental conditions. The relative water content, water potential and osmotic potential had been found was maintained high with combined form of nitrogen as compared to ammonical form. Under saline condition, these parameters exhibited significant decline. Application of nitrogen through combined form (120 kg ha^{-1}) considerably improved the water status in term of water potential and RWC. Salinity induced osmotic effect influence the stomatal regulation and thus affecting the gas exchange processes like photosynthesis and

transpiration. Salinity generally reduced stomatal aperture, which, interfers with CO_2 diffusion, and ultimately reducing photosynthesis whenever CO_2 become a limiting factor, i.e. under highlight intensity and unimpaired light reactions and biochemical pathway of photosynthesis. Stomata often partially close in plant exposed to salinity, even when fully adjusted the internal osmotic potential and turgor is high. When a sensitive plant species is exposed to high salinity then the photosynthesis is disrupted even more. The disruption is initially manifested by leaf chlorosis and then necrosis and reduction in photosynthesis per unit leaf area (Awada *et al.*, 1995).

Photosynthetic rate, transpiration rate and stomatal conductance got affected at various nitrogen and salinity levels in control as well as stress plants at both the sampling stages i.e. 45 and 65 DAS. It is evident from the data that under saline condition, plant exhibited a decline in photosynthetic rate, transpiration rate and stomatal conductance as compared to control plants. However, the maximum and minimum reduction were noticed in ammonical and nitrate form of nitrogen, respectively. The reduction in photosynthetic rate was relatively more in ammonium than in nitrate fed plants. While transpiration rate was relatively lower in nitrate fed plants grown either with or without saline water irrigation as compared to ammonical form. A decrease in stomatal conductance was noted with increasing salinity level, maximum with ammonical form while minimum with nitrate form (Figs. 1, 2 and 3).

Leaf diffusive conductance, photosynthesis and gaseous exchange under salinity have been studied by many workers (Greenway and Munns,

1980; Pasternak, 1987; Boari *et al.*, 1997). Salinity can affect photosynthesis at the stomatal/mesophyll level, depending on types of salinity, duration of treatment, species and plant age (Kleinkopf *et al.*, 1976). However, there are many other factors which may also contribute to this difference in relation to growth stage. Photosynthetic tolerance to salinity may be due to the adjustment of the photosynthetic machinery to salinity at the early growth stage (Plaut *et al.*, 1989). Limitations to photosynthesis by salinity may be attributed to two major factors viz. (i) limitation due to decreased stomatal conductance, thus decreased availability of carbon dioxide for reduction to leaf cells (Walker *et al.*, 1982; West *et al.*, 1986), (ii) disturbance in biochemical processes of photosynthesis (Dowton and Loveys, 1981; Longstreth *et al.*, 1984; Seeman and Critchely, 1985). The reduction in photosynthesis in glycophytes may also occur in the following ways: (i) Low diffusion of CO_2 in the chloroplasts, (ii) alteration in structure and function of organelles responsible for photosynthesis, (iii) changes in photosynthetic reaction i.e. light and dark reaction (iv) effects on transport of assimilated products and intermediary compounds. Parallel response of photosynthesis and transpiration to salinity associated with unchanged intracellular CO_2 pressure (Longstreth *et al.*, 1984; Yeo *et al.*, 1995). Thus, suggest a similar quantitative response of stomata and biochemical capacity of chloroplasts (Leoyd *et al.*, 1987). NH_4^+ fed plants transpired more per unit leaf area and had smaller leaf mass than NO_3^- fed plants. Both facts suggest some sort of regulation of water economy when leaf area seems diminished by a water stress like problem and stomata continue losing water at higher

rates. This phenomenon could be related to the importance of K^+ and its recirculation for N uptake and assimilation (Lips *et al., 1987;* Van-Beusichem *et al., 1988)* and hence, growth response and the protective effect of K^+ on photosynthesis against inhibitory effects of water stress (Pier and Berkowitz, 1987).

The study of impact of improved soil fertility on the photosynthetic rate, transpiration rate and stomatal conductance firmly established the importance of a salinity-fertility interaction at both sampling stages (45 and 65 DAS). It was evident from the data that under saline condition, plants exhibited a decrease in photosynthetic rate. The maximum and minimum photosynthetic rate was observed with nitrate and ammonical form of nitrogen respectively. However, the plants when treated with nitrogen, preferably nitrate-N, showed a more pronounced moderation of adverse effects of salinity. It was also noted that the inhibition of photosynthetic rate by saline irrigation water was consistent at both sampling stages with slight difference in the extent of the effect. Nitrogen in nitrate form (120 kg ha^{-1}) resulted in maximum increase in photosynthetic rate in leaves under saline condition as compared to lower level of nitrogen application (40 kg ha^{-1}). However, ammonical form of nitrogen found promotive up to a dose of 80 kg ha^{-1} and subsequently higher dose (120 kg ha^{-1}) proved deleterious (Figs. 1,2 and 3).

Transpiration and stomatal conductance decreased with increasing levels of salinity at both the sampling stages i.e. 45 and 65 DAS. This decreasing trend was consistent when plants were treated with either forms

of nitrogen. Among different nitrogen forms, transpiration rate and stomatal conductance in plants increased more by ammonium-N than nitrate-N (Figs. 1, 2, and 3). According to Hsio and Lauchli (1986), plant showed relatively higher transpiration rate under ammonium-N which can be related to the depletion of other cations by the presence of an excessive ammonium (NH_4^+) ion concentration. This view was further strengthened at the higher ammonium-N treatment, where plants exhibited a sharp increase in their transpiration rate (Khan *et al.*, 1994). Nitrate form of nitrogen proved promotive irrespective of levels of nitrogen application while ammonical form was promotive upto 80 kg ha^{-1} and became inhibitory at 120 kg ha^{-1}. Beside this, the highest level of nitrogen (120 kg ha^{-1}) in nitrate form showed minimum per cent reduction in transpiration rate and stomatal conductance under saline condition as compared to non-saline plants.

The relationship between photosynthesis and N has been widely studied because of the importance of photosynthesis to plant productivity and the status of N as a limiting element (Chapin, 1980; Novoa and Loomis, 1981). On the other hand, N-nutrition brought about a considerable moderation in salt induced inhibition of photosynthetic rate (Seeman and Sharkey, 1986).

Khan *et al.* (1994) also reported similar results that salinity caused a substantial reduction in carbon assimilation rate, stomatal conductance and transpiration rate. However, salinity effects were considerably moderated by additional N supply, varied with form, concentration and

stage of plant growth. The photosynthesis was reduced more in ammonium than in nitrate fed plants, while the transpiration rate was relatively lower in nitrate fed plants grown either with or without NaCl. The plants also responded differentially to salinity and N levels. The promotive effect of N on photosynthesis and other parameters in saline as well as in non-saline conditions, may be attributed to enhanced synthesis and availability of carbon assimilatory enzymes and cofactors required for optimal photosynthesis. It has been reported that under decreased N-concentration, photosynthetic activity get reduced (Powell and Ryle, 1978), while under increased nitrogen levels, photosynthesis get enhanced (Nichiporovich and Slobosdoskova, 1972). The possible reason is that nitrogen is a major constituent of enzymes responsible for photosynthic carbon reduction and the components of the pigments including chlorophyll which generate ATP and NADPH (Edward and Walker, 1983). Thus, a low nitrogen supply results in suppressed photosynthetic rate and lowering of the carbohydrate supply for growth. In fact, the effect of N-nutrition on photosynthesis reflects both N-availability as well as the partitioning of N in plants (Khan *et al.*, 1994). The present results are in accordance with other reports in which reduction of photosynthesis was observed in plants grown in presence of NaCl and attributed to stomatal resistance or due to the reduction in capacity of the photosynthetic machinery (Seeman and Critchley, 1985; Seeman and Sharkey, 1986; Khan *et al.*, 1994).

Biochemical parameters

Harsh environmental situation interfere with normal metabolism of plants, which respond by different type of biochemical adjustments.

An increase in salinity progressively decreased the levels of chloroplastic pigments (Total chlorophyll and carotenoid content in leaves) at both stages (45 and 65 DAS) of *Brassica juncea* cv RH-30 (Tables 13 and 14). Several other worker's also reported such decrease under salinity in number of arid zone crop's such as pearlmillet, clusterbean, mungbean, wheat and Indian mustard (Garg and Lahiri, 1986). The reduction in chlorophyll content may be because of interference of salt ions with the *de novo* synthesis of proteins which constitute the structural component of chloroplast (Sudhakar *et al.*, 1991) or the enhanced activity of chlorophylase was as suggested by Rao and Rao (1981) in pigeonpea.

Under saline condition, maximum and minimum per cent reduction in chloroplastic pigment was observed with ammonical and combined form of nitrogen, respectively at both the sampling stages (45 and 65 DAS). It was in conformity with the earlier report by Mishra *et al.* (1996) in *Brassica juncea.*

With increase in levels of nitrogen through different sources, significant increase in chloroplastic pigments in leaves were noticed at both the sampling stages. However, the increase was highest with combined form of nitrogen as compared to ammonical form. It suggest that the adverse effect of salinity on chloroplastic pigments was partially mitigated with nitrogen application. Higher dose of nitrogen (120 kg ha^{-1}) in combined form resulted maximum alleviation in the adverse effect of salinity on chloroplastic pigments. However, it was minimum with ammonical form (Tables 13 and 14).

In conformity with this Neiman and Paulson (1971) reported that enhanced level of pigmentation due to nitrogen supplement under salinity stress. Alleviation of adverse effect on carotenoid level by either from nitrogen indicated structural repairment of chloroplastic which might be deformed under salinity. Garg *et al.* (1990) also reported that the levels of total chlorophyll declined under salt stress but nutritional improvement under both normal and saline conditions led to higher concentration of chlorophyll. HF condition increased the chlorophyll content as compared to LF condition under salt stress.

From the accumulation pattern of chloroplastic pigments, it appeared that inhibitory effects of saline waters irrigation were partially alleviated with application of increasing levels of nitrogen through different sources (nitrate, ammonical and combined form). However, combined and nitrate form was proved more effective as compared to ammonical form of nitrogen (Tables 13 and 14).

An increase in salinity progressively decreased the levels of starch content in leaves along with increased levels of total soluble carbohydrates, proline and free amino acids in leaves at both the sampling stages i.e. 45 and 65 DAS. The reduction in starch content and increased in total soluble carbohydrates, proline and free amino acids were more pronounced at 12 dSm^{-1} (Tables 15, 16, 17 and 18). Such decrease in the starch content and increase in the total soluble carbohydrate content of plants under salinity has been reported (Lahiri *et al.*, 1987; Garg *et al.*, 1993; Hamdia and El-Komy, 1997; Kuznetsov and Schevyakova, 1997; Verma *et al.*,1997;

Gadallah, 1999). This might be due to inhibitory effects of salinity stress on the translocation from source site (Poloneko *et al.*, 1983; Hendrix and Huber, 1986). Weimberg (1987) reported that increased level of sucrose under salinity might be due to increased hydrolysis by enhanced activity of α-amylase under salinity conditions as reported by El-Fouly and Jung (1972). It has been shown earlier that proline accumulation may be due to increased proteolysis or due to decreased protein synthesis. Proline synthesis take place via feedback mechanism. The key enzyme of proline biosynthesis is pyrroline-5-carboxylate synthetase (P5CS) controlled by proline concentrations. Under salt stress this feedback inhibition is disturbed and hence proline accumulates (Delauney and Verma,1993; Gzik,1996). Madan *et al.* (1995) reported that higher levels of proline synthesizing enzymes and proline concentrations under salinity in *Brassica* species. The higher concentrations of proline under stress are helpful to plant as they maintain osmotic potential of leaf. Leaves are main site where proline accumulates (Chandler and Thorpe, 1987). Proline accumulates in cytoplasm and contributes to adjustment in osmotic status of plant. Raghav Ram and Murray (1985) reported that proline may be related to maintenance of conformation characteristics of enzymes. Proline disappeared in the leaves when salinity stress relieved with simultaneously increase in leaf water potential. Nandwal (1989) suggested that accumulated proline could also serve as a readily available energy and nitrogen source for the relief of the plant under stress condition.

Under salinity, accumulation of proline was more conspicuous than other organic solutes. The accumulation of proline and free amino acids

under salt stress has been reported in mustard and other crops by several workers. Metabolic alterations in Indian mustard and related species have been studied by Kumar (1984), Borodina (1991), Jain *et al.* (1991), Alia *et al.* (1993), Das *et al.* (1994), Madan *et al.* (1994) and Garg *et al.* (1997). This increase in free amino acid concentrations might be due to reduced protein synthesis and enhanced hydrolysis under salinity (Udovenko *et al.*, 1972). Salt induced protein degradation may be essential in providing amino acids for synthesis of new proteins required for growth on survival under the modified conditions and also substrate for energy metabolism (Raymond *et al.*, 1994).

Under saline condition, the maximum and minimum reduction in starch content of leaves were noticed with ammonical and combined form of nitrogen, respectively at both sampling stages. However, with each increment in salinity level (8 and 12 dSm^{-1}) the maximum and minimum accumulation of total soluble carbohydrates, proline and free amino acids were observed with combined and ammonical form of nitrogen respectively at both the sampling stages (45 and 65 DAS). The improved nutritional status (nitrogen application) of plant under salt stress, which ultimately, led to a better crop performance, was obviously mediated through an improved metabolic efficiency. This contention was verified from ascertaining the levels of certain metabolites in plant under different treatments at 45 and 65 DAS. The salinity-fertility interaction was significant in all the cases. Declined level of starch content under salinity was restored partially by application of nitrogen dose of 120 kg ha^{-1} using

different form of nitrogen. Increasing level of nitrogen irrespective of source improved the reduced level of starch nd it remained markedly higher with 120 kg ha^{-1} nitrogen application. Likewise, total soluble carbohydrates, prolines, free amino acids also increased and remained highest at 120 kg ha^{-1} nitrogen application. The aforesaid trends suggest that nitrogen dose of 120 kg ha^{-1} imparted a relatively better metabolic efficiency to plant under salt stress as compared to 40 kg/ha. Again, it appears that the magnitude of metabolic dearrangement under salt stress were ameliorated more by use of combined form as compared to nitrate and ammonical form of nitrogen (Tables 15, 16, 17 and 18). These observations are similar to those found in other crops (Rathert, 1983; Lahiri, 1987; Garg *et al.*, 1990; 1993).

Alia *et al.* (1993) opined that proline accumulation has adaptive significance as it lowers the generation of free radicals and thus, reduces lipid peroxidation linked membrane deterioration under salt stress. The adaptive role of proline is related to survival rather than maintenance of growth (Greenway and Munns, 1980). Proline can also stabilize membrane (Mansour, 1998; Gadallah, 1999). Mansour (1998) provides direct evidence that proline can protect cell membrane against salt injury. NaCl induced cellular aberrations in onion epidermis, resulting from cell membrane disruption, were mitigated by proline application. A decrease in shoot Cl^- and Na^+ accumulation and thus enhanced growth in saline environment in response to endogenous proline application was proposed to the effect of proline on membrane stabilization (Lone *et al.*, 1987).

Enzymatic studies

Disruption of nitrogen metabolism by salinity has been attributed: (a) decreased nitrogen uptake, (b) decreased nitrate reductase activity, (c) altered amino acids synthesis and enhanced hydrolysis of proteins, (e) slow synthesis of nucleic acid, and (f) increased activity of hydrolysing enzymes such as RNase, DNase, protease and several others leading to degradation of macromolecule.

An increase in salinity levels from 8 to 12 dSm^{-1} inhibited the activity of nitrate and ammonia assimilation enzymes, such as nitrate reductase, nitrite reductase, glutamine synthetase, glutamate synthase and glutamate dehydrogenase as compared to non-saline plants at both sampling stages. The activity of nitrate reductase in leaves proved more sensitive with each increment in salinity levels (8 and 12 dSm^{-1}) as compared to nitrite reductase activity at both the sampling stage i.e. 45 and 65 DAS (Figs. 4, 5, 6, 7 and 8). Nitrate reductase activity (NRA) is generally reported to be inhibited by salinity in a number of crop plants such as wheat, pearlmillet, sorghum, mustard, pea, cluster bean and tomato (Plaut, 1974; Garg *et al.*, 1990, 1993; Lahiri *et al.*, 1996). The reduction in NRA has been attributed to the inhibition of NO_3^- uptake by Cl^- in many investigations. A progressive decline in NRA with increasing salinity points toward the disturbed nitrogen metabolism in a number of other studies (Lahiri *et al.*, 1987; Garg *et al.*, 1993). The activity of nitrite reductase was either inhibited or unaffected in many crop species and halophytes since this enzyme is generally less sensitive to salinity than nitrate reductase (Amonkar and Karmarkar 1995; Dubey, 1997).

Ammonia either directly absorbed by plant roots or as a result of reduction of NO_3^- is further assimilated and incorporated into amide group of glutamine by the action of the enzyme glutamine synthetase (GS) and subsequently into glutamic acid by glutamate synthase. Increasing salinity levels caused significant decline in the activity of GS and GOGAT over control plants. But the activities, as such, were relatively higher at the 65 DAS as compared to 45 DAS (Figs. 6, 7 and 8). Similar results has also been reported by Dubey *et al.* (1991), Garg *et al.* (1993), Singh and Dubey (1994). An increase in salinity also led to decreased activities of glutamate dehydrogenase at both the growth stages. However, the decline was sharp upto 8 dSm^{-1} salinity and marginal at the 12 dSm^{-1}. Among the stages, the activity of GDH was more pronounced at second sampling stage. Less decline in activity of GDH under salinity could be a measure to use accumulated ammonia under high salt stress when the GS-GOGAT system is less efficient. The inhibitory effect of salt stress on the level of activity of enzymes have also been reported in mny plants by Sharma *et al.* (1989), in Brassica, Garg *et al.* (1993) and Lal and Bhardwaj (1987). High salinity enhanced the activity of glutamate dehydrogenase showing that these plants have the potential to assimilate NH_3 by this enzyme in saline environment (Amonkar and Karmarkar, 1995; Dubey, 1997). Under saline condition, the maximum and minimum inhibition in NRA and NiR was noticed with ammonical and nitrate form of nitrogen, respectively as compared to non-saline plants (Figs. 4 and 5). With each increment in salinity levels (8 and 12 dSm^{-1}), the ammonia assimilation enzymes : glutamine synthetase (GS),

glutamate synthase (GOGAT) and glutamate dehydrogenase exhibited maximum and minimum inhibition with nitrate and ammonical form of nitrogen respectively as compared to non-saline plants at both the sampling stages (Figs. 6, 7 and 8). The highest activity of ammonium assimilating enzymes under saline condition was observed in plants treated with ammonical form of nitrogen as compared to combined and nitrate. These results are in conformity with the earlier report in different crops i.e. rice and tomato (Magalhaes and Huber, 1991). This view was supported by Srivastava and Singh (1987) in higher plants suggesting that GDH activity in root and shoots was generally greater in NH_4^+ than NO_3^- treated plants. In contrast to this, the activity of GS and GDH in roots of tomato and corn enhanced linearly in response to increasing NH_4^+ concentrations. This suggests that high GDH activity may correspond to a high incorporation of NH_4^+ into amino acid. However, GDH could be functioning as a deaminating enzyme to provide carbon skeletons to plants with low photosynthetic rate due to NH_4^+ toxicity. Likewise, Magalhaes and Huber (1991) also reported much higher GS and GDH activity in rice, tomato and corn with NH_4^+ nutrition. Differential response with respect to species and organ has also been observed. GS activity in rice increased as the level of NH_4^+, increased indicating that GS activity is a key factor in the detoxification and metabolism of NH_4^+ in green tissues of efficient plant species.

We have noticed the impact of improved soil fertility on the activity of enzymes related to N-metabolism and firmly established the importance

of salinity-fertility interaction. The data indicated that a progressive increase in salinity reduced the activity of NRA and NiR with increase dose of nitrogen irrespective of source. But the plant treated with 120 kg ha^{-1} nitrogen in nitrate form generally maintained a higher NRA and NiR activity as compared to lower level of nitrogen application (40 kg/ha^{-1}) at the both sampling stages (45 and 65 DAS). Similar results were also observed in wheat (Garg *et al.*, 1982) and suggested that the higher N availability in the substrate (arising from higher N uptake) under the IF (Intensive fertilizer) condition possibly induced a greater efficiency for NR enzyme despite the salt stress upto a certain level of salinity. Mishra *et al.* (1996) also reported that nitrate and ammonium supplementation to the seedling enhanced the level of protein content and nitrate reductase in leaf as well as root tissues of *Brassica juncea* under high salinity. The protection of enzyme activity by nitrogen under salinity has been recorded in cultivars of *Brassica campestris* (Mishra *et al.*, 1994).

An increase in salinity led to decreased activities of GS, GOGAT and GDH. But the activities were higher at 65 DAS as compared to 45 DAS. Again, the higher level of nitrogen (120 kg ha^{-1}) irrespective of nitrogen source displayed significantly higher activities for these enzymes as compared to plants treated with lower level of nitrogen (40 kg ha^{-1}) under salinity. However, the activities of ammonia assimilating enzymes were highest in plant treated with ammonical form of nitrogen as compared to combined and nitrate form (Figs. 6, 7 and 8). Similar results have been obtained with Indian mustard (Sharma *et al.*, 1989; Garg *et al.*, 1993)

where improvement in soil fertility significantly enhanced the activity of NRA as well as those of ammonia assimilating enzymes such as GS, GOGAT and GDH.

Mineral composition

The normal mineral nutrition of the plant is disturbed under saline conditions, which may be partly responsible for various metabolic disturbances in plant leading to general suppression of growth and reduction in yield under saline condition. Salinity disturbs the supply of nutrients from the media by two means, either due to presence of excess salts and thus some of the essential elements are made non-available or by changing the membrane phospholipids composition due to which permeability of the membranes to various nutrients get changed. Under such conditions uptake of essential elements decreased or accumulation of toxic ions increased. Reduced uptake of essential elements creates mineral deficiency, whereas, accumulation of toxic ions disturbed the plant metabolism in adverse ways leading to reduced growth and development of the plant (Sharma *et al.*, 1994; Porcelli *et al.*, 1995; Garg and Gupta, 1998). In the present study, different nutrient elements such as nitrogen, phosphorus, potassium, magnesium got decrease under saline conditions in different plant parts (leaves, stem and root) while calcium, sodium, chloride and sulphate increased at harvest. Salinity had affected the mineral composition of stem, leaves and roots. Accumulation of sodium, chloride and sulphate was maximum at high salinity level of 12 dSm^{-1} over control in different plant parts. However, sodium and chloride

concentrations were highest in root but sulphate in leaves under salinity. Leaves exhibited highest level of N, Mg and Ca while P and K content were highest in stem under saline conditions (Figs. 9 to 16). Similar results were obtained by Sagi *et al.* (1997). Earlier reports also indicated that salinity reduces N accumulation in plants (Feigin, 1985; Garg *et al.*, 1990, 1993). Salinity causes a decrease in uptake of N and that growth of plants is linked to N uptake. Increasing N concentration in the nutrient media under saline conditions results frequently in renewal of growth (Silberbush and Lips, 1988). Van Beusichem *et al.* (1988) observed smaller concentrations of K^+,Ca^{2+} and Mg^{2+} in *Ricinus communis* plants fed with NH_4^+ as compared to NO_3^- fed plants. Cox and Reisenhauer (1973) reported that ammonium increases anion uptake, whereas nitrate increases cation concentration in wheat plants. Changes in the pH of the nutrient medium and type of N supplied, affected ion uptake (Kirkby and Mengel, 1967). Leidi *et al.* (1991) also reported that ammonium grown plant showed lower concentrations of N, K^+, Ca^{2+} than nitrate grown plants under saline conditions. While Na^+ and Cl^- concentrations was observed highest in ammonical form as compared to nitrate fed plants. Reduced uptake in ammonium fed plants has been frequently reported (Gashaw and Mugwira, 1981; Blacquiere *et al.*,1987). Ammonium nutrition would affect cation uptake as a consequence of the intracellular buffering mechanism which maintains electron neutrality by a net efflux of H ions and reduced intake of K or Ca ions (Van Beusichem *et al.*, 1988).

Salsac *et al.* (1987) reported that NH_4^+ nutrition could lead to insufficient water uptake because of the reduced intake of mineral ions (mainly K^+) and organic anions that can operate as osmotic. Thus, the larger growth inhibition caused by salinity in NH_4^+ - fed plants may be the result of a combination of several factors ranging from effects on water status and carbon partitioning to inorganic ion (K^+ and Ca^{2+}) induced deficiencies by NH_4^+ and Na^+. Leidi *et al.*(1991) reported that salinity and nitrogen form had different effects in the levels of N, K, Na and Cl in the shoots of different species. NH_4^+ fed plants had been found to contain lower level of N,K and high level of Na and Cl under saline conditions as compared to nitrate fed plants. The form of N nutrition may exert a considerable influence on the mineral composition of plants (Kirkby and Mangel, 1967; Kurvits and Kirkby, 1980; Allen *et al.*, 1985).

As far as P content is concerned, salinity may decrease, have no effect or increased phosphorus accumulation in plants depending upon the plant and environmental conditions (Champagnol, 1979; Feigin, 1985). With increased salinity, Na content increased with a corresponding decline in K content. This can be explained on the basis of earlier reports which suggests reduced uptake of K^+ and Na^+ accumulate in plant cell (Bhatt and Appututtan, 1971; Garg and Garg, 1980). Although Cl^- and $H_2PO_4^-$ ions may not be competitive in terms of uptake and accumulation by increasing Cl^- in tomato shoot (Papadopoulos and Rendig,1983) and by both Cl^- and SO_4^{2-} in barley and sunflower (Zhukovskaya, 1973). Garg *et al.* (1982, 1993) also reported decreased P uptake by increasing salinity

in wheat and mustard although P concentration was marginally affected. The reduced P uptake may thus be due to decreased plant growth in such cases.

Many studies have shown that K^+ concentration in plant tissues get reduced as Na salinity or Na^+/Ca^{2+} ratio in the root media is increased (Lahiri *et al.*,1987; Subbarao *et al.*, 1990). Reduction in K^+ uptake in plants by Na^+ is a competitive process and occurs regardless of whether the solution is dominated by Na^+ salts of Cl^- or SO_4^{2+}. The effect of Na^+ on K^+ uptake is two-fold. At low concentrations, Na^+ may actually increase K^+ uptake, though decreasing it at higher concentrations (Nimbalkar and Joshi, 1975). But in most of the studies Na salts decreased dry matter production and the leaf content of K as well as its uptake (Garg and Garg, 1980). Salinity results in accumulation of Na and decline of K. As a result of this K/Na ratio also decreased. P level in different plant parts decreased irrespective of nitrogen source used with each increment of salinity levels (8 and 12 dSm^{-1}) but the decrease was more with ammonical form of nitrogen as compared to two other forms i.e. combined and nitrate forms. Plant treated with combined form of nitrogen had better K/Na ratio than the ammonical form. Thus, the plant treated with different forms of nitrogen showed marked difference in uptake of ions by the roots, and also in the proportion of the absorbed ions being translocated to the leaves. The difference in ion accumulation presumably reflect differences in selectivity at plasma membrane during ions uptake, in phloem transport of ions to the apex, and in ion compartmentation (Sharma, 1991). In our

study, the gradual decrease in potassium with salinity in different plant organs (stem, leaves, roots) was accompanied with more Na^+ uptake and thus consequently decreased K/Na ratio. Flowers and Lauchli (1983) also reported such decreased in K/Na ratio under salinity stress. The increased levels of sodium and less of potassium was also reported in barley (Delane *et al.*, 1982), chickpea (Pandey and Ganapathy, 1984), cotton (Rathert, 1983) and wheat (Kumar, 1997). Subsequent studies by Hu and Schmidhalter (1998) revealed that Na^+, Cl^- concentrations increased under salinity were more in the elongated leaves of wheat. The higher concentrations of Na^+ and Cl^- may cause ion imbalance but probably did not result in ion toxicity in growing leaves. Higher Na concentration in roots than in shoots found in annual rye grass grown with or without salinity may have been the result of the plant ability to avoid Na toxicity by reducing the transport to the shoots where it may disturb ion charge balance and cause specific ion toxicity (Green and Munns, 1980; Cramer *et al.*, 1989).

In the present study, plants treated with NH_4 form as N-source had a lower concentrations of N, P, K, Mg, Ca in different plant parts (stem, leaves and roots) as compared with other two form i.e. combined or nitrate form. Plant treated with combined form had the highest N, P, K, Mg and Ca as compared to other two forms. While ammonical treated plants had highest concentrations of Na^+, Cl^- and SO_4^-. Plants fed with combined form were found to have the highest concentration of N, Ca, Mg in leaves while P and K concentration were observed highest in stem. Beside this,

ammonical fed plants had highest accumulation of sodium and chloride in roots while sulphate concentration was maximum in leaves (Figs. 9 to 16).

Among divalent cation, Ca and Mg play important role in growth and development of plant. Magnesium concentration in different plant parts declined with each increment in salinity levels (8 and 12 dSm^{-1}). However, the maximum and minimum decline in magnesium concentrations were observed with ammonical and combined form of nitrogen, respectively. In contrast to magnesium, concentration of calcium increased with increasing salinity. The increase was more in plants treated with combined form of nitrogen than ammonical form (Figs. 12 and 13). Singh (1992) also reported increase of calcium in *Brassica* species under salinity. Generally, calcium content decreased under salinity as reported by various workers (Khan and Ashraf, 1988; Cramer *et al.*,1991; Gorham and Bridges, 1995). Our findings on Ca are in contrast to the above quoted work as we used NaCl, $CaCl_2$, $MgCl_2$ and $MgSO_4$ salts for salinity. Calcium is known to suppress Na^+ accumulation and Ca^{2+} activity decreased by Na^+ in a solution (Lynch and Lauchli, 1988; Cramer *et al.*, 1991). It showed antagonism between Na and Ca. The increase in Ca concentration with salinity may have resulted in an increased accumulation of carbohydrates in cell vacuoles without increasing the osmotic pressure (Osmond, 1967) while balancing the excess of cation over anions. Changes in Mg^{+2} level depends on type of salinity used in the experiment. All the three possibility for Mg level have been reported under salt stress i.e. increase, decrease and no change. The increased Mg^{2+} contents under salt stress was reported

in sorghum by Khan and Ashraf (1988), *Brassica* (He and Cramer, 1993a), wheat (Kumar, 1997). These results are different from our findings, the difference may be attributed to the use of different salinity source in the experiment. Decreased Mg^{2+} concentration under salinity stress has been reported in *Brassica* species (Paek *et al.*, 1988; Singh, 1992). The response of Mg^{2+} varied considerably with increase in external $CaCl_2$ concentrations. But there was no evidence that high Ca^{2+} caused deficiency of Mg^{2+} as reported in cotton by Gorham and Bridges (1995).

In the present experiment, anion level (Cl and SO_4) increased under saline conditions. The maximum and minimum increase were observed with ammonical and combined form of nitrogen, respectively under salinity.

Plant treated with combined form had been found to absorb less Cl than ammonical form. However, plant treated with ammonical form had higher SO_4^2 concentrations than plants fed with combined form (Figs 15 and 16). The increased chloride concentrations under salinity was observe in lentil (Tal *et al.*,1979), sorghum (Sinhá *et al.*, 1986; Yang *et al.*, 1990), *Brassica* (He and Cramer, 1993c), wheat (Sharma, 1996; Kumar, 1997). The higher chloride concentration may cause ion imbalance (Hu and Schmidhalter, 1978). The increased sulphate levels with salinity has also been reported by Sharma *et al.* (1994) and Kumar (1997) in wheat. Fertilizer induced salt tolerance may possibly be achieved through consistent maintenance of higher concentrations of N, P, K, Mg and Ca and lower concentrations of Na,Cl and SO_4 in different plant parts. In the

present study, the concentrations of N, P, K, Mg and Ca were consistently more with higher dose of nitrogen application (120 kg ha^{-1}) as compared to lower dose of nitrogen application (40 kg ha^{-1}) irrespective of nitrogen source (nitrate, ammonical and combined forms). The plant treated with combined form of nitrogen (120 kg ha^{-1}) had been found to contain highest concentrations of N, Mg, Ca in leaves while P content was maximum in stem as compared to other two forms i.e. nitrate and ammonical form. However, nitrogen application (120 kg ha^{-1}) in combined form also exhibited the reduced Na,Cl and SO_4 concentrations in stem, leaves and roots as compared to nitrate and ammonical forms under saline condition.

The combined form of nitrogen (120 kg ha^{-1}) showed maximum alleviation in the adverse effect of salinity on N, P, K, Ca and Mg as compared to other two forms of nitrogen. Morever, the higher dose of nitrogen (120 kg ha^{-1}) in combined form also maintained lower concentrations of Na, Cl and SO_4 in different plant parts as compared to nitrate and ammonical form of nitrogen (Figs. 9 to 16).

The interaction between nitrogen and salinity has been studied in several plant species, such as peanut, wheat and maize (Silberbush and Lips, 1988; Lewis *et al.*, 1989). The detrimental effects of salinity were more pronounced in NH_4^+ fed plants than in NO_3^- fed plants (Lewis *et al.*, 1989).

As salinity may induce nutrition deficiencies or imbalances, improvement in soil nutritional status through supplemental fertilization has been shown to overcome such salt induced adverse effects, particularly

in susceptible crops. Garg *et al.* (1993) demonstrated that an improvement in soil fertility could favourably influence the concentration and absolute quantity of NPK and reduce the concentration of Na in the mustard shoots under salinity of irrigation water. Similar results also reported by Garg *et al.* (1982) in wheat that improved fertility has been found to be associated with an increased in the concentration and uptake of NPK, particularly of N, decreased tissue concentration of sodium and chloride and a decrease in the Na:K ratio. It is noteworthy that reported data indicate that increased NO_3^- in the substrates decreased Cl^- uptake and accumulation (Garg *et al.*, 1982,1990; Feigin *et al.*, 1987). The decrease in Cl^- concentration has been found to be associated with decreased Na^+ concentrations also.

Yield and yield attribute

At harvest, number of siliquae and seed yield per plant were recorded under different treatments i.e. salinity and fertilizer. Siliquae and seed yield were adversely affected with different levels of salinity at both sampling stages i.e. stage I and stage II. The maximum and minimum reduction was observed with ammonical and combined form of nitrogen, respectively with each increment in salinity levels (8 and 12 dSm^{-1}). The saline water induced salt stress reduced the number of siliquae and yield per plant and this adverse effect was mitigated by application of increasing levels of nitrogen through different source of nitrogen. However, the combined form of nitrogen has overcome the adverse effect of salinity to a greater extent as compared to the other two forms i.e.

nitrate and ammonical forms, respectively. Beside this, higher level of nitrogen application (120 kg ha^{-1}) in combined form significantly alleviated the adverse effects of salt stress as compared to lower nitrogen dose (40 kg ha^{-1}). Ammonical form of nitrogen showed minimum alleviation in the adverse effect of salt stress on yield characters (Tables 19 and 20). This points to the nutritionally induced tolerance to salt stress. It has also been observed in number of other crops (Lips *et al.*,1990; Garg *et al.*, 1990, 1993).

Adverse effect of salinity on crop yield mediated through metabolic processes has been reported in different crops (Kumar and Malik, 1983; Dhawan *et al.*,1987; Chauhan *et al.*,1988; Sharma *et al.*,1990; Sinha, 1991; Mishra, 1994; Sharma and Gill, 1994; Qadar,1994; Sharma and Manchanda, 1997; Yadav, 1998). Sharma *et al.* (1990) found that threshold values of soil salinity (ECe) were 4, 6 and 7 dSm^{-1} for wheat, mustard and barley crops, respectively. The relationship also indicated the yield decrease per unit increase in ECe for different crop and yield benefit to be expected from a salinity control programme. Work carried out at CSSRI, Karnal has shown that economic yield of Indian mustard can be obtained upto pH 9.2 and ECe 6.5 dSm^{-1} (Mishra, 1994).

The nutritionally-induced tolerance to salt stress has also been observed in number of different crops reported by Lips *et al.* (1990), Garg *et al.* (1990, 1993). Shaviv *et al.* (1990) found that wheat grown in soil salinized with NaCl was more tolerant in terms of grain yield under a combination NH_4^+ and NO_3^-. In number of other reports, increased dose

of fertilizers have been advocated to alleviate the growth and yield inhibition by salinity. Chauhan *et al.* (1988) observed 37 and 80 per cent improvement in seed yield of Indian mustard with 60 and 90 kg N ha^{-1} application, respectively in comparison with 30 kg N ha-1 following irrigation with saline water. Yield enhancement of Indian mustard upto 120 kg/ha observed on sodic soils at Karnal further corroborates this phenomenon (Anonymous, 1988).

Field experiments on the combined effects of saline water irrigation and nitrogen levels on the yield, water use and NUE of Indian mustard indicated that (a) use of saline water can boost the growth and yield of dry land mustard, and (b) within certain limits a non-saline water supply can be substituted by applying nitrogen and saline water (Dayal *et al.*, 1995). It was suggested that fertilizer N dose should be adjusted in relation to the supply of water and its predicted salinity. Garg *et al.* (1982) also reported that fertilizer induced improvement of growth and yield of wheat under salinity stress. Garg *et al.* (1990, 1993) also reported that increasing salinity of irrigation water progressively decreased grain yield of wheat and Indian mustard but improved soil fertility significantly enhanced the yields as well as imparted high salt tolerance to fertilized plants. Salinity induces a decrease in the number of grains produced by wheat plants regardless of their nitrogen source. However, the translocation of assimilates from the leaves to the grains was larger in nitrate-fed plants. This phenomenon was evident on the production of larger individual grains by nitrate-fed plants (Leidi and Lips, 1990). The improved development

of plants receiving combined form (mixed ammonium-nitrate) may be due to a nitrate-enhanced supply of carbohydrates to the roots (Lips *et al.*, 1987). This indicated that plants in mixed ammonium nitrate readily absorbed ammonium and the nitrate. This is evident by following uptake of these inorganic nitrogen species from the nutrient medium. The medium pH drops initially, indicating preferential uptake of ammonium and rises later with the subsequent uptake of nitrate (Lips *et al.*, 1990).

At harvest, siliquae/plant and seed yield were recorded under different treatments i.e. salinity and fertilizer. Siliqua and seed yield were affected adversely with different levels of salinity at both sampling stages (Stage I and Stage II). The saline water induced salt stress reduced the number of siliquae and yield per plant and thus adverse effect was ameliorated by application of nitrogen through different source. But combined form of nitrogen had overcome the adverse effect of salinity to a greater extent as compared to the other two forms i.e. nitrate and ammonical form. However, higher level of nitrogen application (120 kg ha^{-1}) in combined form significantly mitigated the adverse effect of salt stress as compared to lower nitrogen dose (40 kg ha^{-1}). Ammonical form of nitrogen showed minimum alleviation of the adverse effect of salt stress on yield characters (Tables 19 and 20). This points to nutritionally induced tolerance to salt stress as has been observed in number of other crops (Lips *et al.*, 1990; Garg *et al.*, 1990, 1993).

Quality Test

Oil and protein content

In the seeds of Indian mustard, the oil and protein level are 29.0-45.5 and 18.7 to 24.9 per cent, respectively. Rapeseed-mustard extraction is a protein rich material for compound for animals feed. The protein concentrate can be utilized in manufacture of biscuits, high protein candies and weaning foods containing mixtures of cereals and pulses. The oil and protein content in seed at harvest decreased with every increment in salinity as compared to non-saline condition at both stages i.e. Stage-I and Stage-II (Figs. 17 and 18). These reduction under salinity were maximum in ammonical form and minimum with combined form. Gupta (1982) observed that salt and moisture stress reduced oil filing by affecting the rate of photosynthesis in the leaves, transport of metabolites from the leaves to the seeds and synthesis of fatty acids at the peak period of oil filing.

In Indian mustard decrease in oil content with salinity has been studied by many workers, but the quality aspects of mustard oil in relation to salinity has received scant attention. Oil synthesis in developing seeds of *Brassica campestris*, *B. napus* and *B. juncea* was adversely affected by moisture stress and salinity (Munshi *et al.*, 1986; Subramanian and Maheshwari, 1991). Similarly, in rape and linseed (Deo and Ruhal,1971) and sunflower (Raju and Ranganaya Kulu, 1978) reported decreased per cent oil under salinity. But the quality of the oil remained unaffected in sunflower.

The increasing level of nitrogen irrespective of nitrogen source attributed to the increased percentage of protein and decrease in the oil concentration in seed at both sample stage (Stage-I and Stage-II). Application of combined form nitrogen at the rate of 120 kg ha^{-1} resulted in minimum reduction of oil content as compared to lower level of nitrogen. However, maximum reduction was observed in ammonical form. The increasing level of nitrogen irrespective of different source resulted in increase in the protein content. However, maximum and minimum increase in protein content of seed using 120 kg ha^{-1} nitrogen was noticed with nitrate and ammonical form, respectively as compared to lower dose of nitrogen i.e. 40 kg ha^{-1} (Figs. 17 and 18). It pointed to a reciprocal relationship between protein concentration and oil concentration of *Brassica juncea* seed with increasing nitrogen supply. These results are similar to those of other workers (Smith *et al.*,1988; Wright *et al.*, 1988; Taylor *et al.*,1991; Zhao *et al.*, 1993).

Adequate use of N fertilizers is required for optimum economic yields and oil production (Mason and Brennan, 1998). However, high amounts of N fertilizer decreased the oil concentration and increased the protein concentration in canola seeds. There was a negative correlation between oil concentration and the protein concentration of the canola seed (Wright *et al.*, 1988). Ridley (1973) suggested that the oil concentration plus the protein concentration for rapeseed was approximately fixed, so that as either the protein or oil percentage increased, the other decreased.

Dhindsa and Gupta (1974) suggested that the protein yield was significantly higher with high dose of N. Nitrogen is a basic constituent of protein and with increase in the rate of nitrogen application, the nitrogen availability increased which resulted in increase protein content in seeds.

From our studies on fertilizer x salinity interactions it suggested that the quality parameter like protein get improved however the per cent oil content declined. Seed protein content increased and oil content decreased with increase in nitrogen rate was reported by several workers on oil seed crops (Gupta and Friend, 1975; Voltan and Mosco, 1982; Szukalska-Golab, 1985; Seo *et al.*, 1986).

Fatty acid composition

The quality and usage of rapeseed-mustard oil is determined by the presence of saturated and unsaturated fatty acids. The quantity of different fatty acids in oil determines its shelf life, usage as edible oil or for industrial utilization. Rapeseed-mustard oil has substantial amount of unsaturated fatty acids and the lowest concentration (around 7%) of saturated fatty acids. Of the total fatty acids in oil, the erucic acid is predominant in the Indian cultivars, with more than 50 per cent. The nutritional quality of oil and seed meal is determined by the quantity and quality of fatty acids, proteins and essential amino acids. The health hazard of saturated fatty acids relates to the evidence linking high intake to coronary heart disease. A recent report suggested the reduction in intake of saturated fatty acids and trans fatty acids to reduce the incidence of coronary disease. High oleic and low linolenic content reduce the formation

of trans fatty acids. Continuing aim of rapeseed plant breeding research is to improve the quality of rapeseed oil. Research in this direction has resulted in decreasing the level of undesirable long chain fatty acids viz., eicosenoic and erucic acid (Stefansson *et al.*, 1961, Downey, 1964). Further, desirable change in fatty acid composition would be an increase in linoleic acid to 35 per cent and decrease in linolenic acid to 1 per cent. High oil content with proper proportion of desirable fatty acids has greater importance for human nutrition.

The fatty acids composition of mustard seeds at harvest showed sharp changes with salinity treatment at both stages i.e. Stage -I and Stage -II (Figs. 17 to 21). Although the trend remained almost similar at both sampling stages. However, there were qualitative differences in individual fatty acids at both the stages. With increasing salinity levels (8 and 12 dSm^{-1}), oleic, linoleic, linolenic acids got increased while palmitic and erucic acids exhibited significant decrease at both sampling stages.

Erucic acid which constitute the major fatty acid of mustard affected markedly by salinity treatments at both sampling stages (Fig. 22). High level of salinity (8 and 12 dSm^{-1}) resulted considerably decrease in erucic acid content of seeds. Similar results have been reported by Ahuja *et al.* (1987); Gupta *et al.* (1991) and Gaur and Gupta (1994). Under saline condition, the plant treated with combined form of nitrogen exhibited maximum accumulation of oleic, linoleic and linolenic acids as compared to non-saline plants. While minimum accumulation was observed with ammonical form of nitrogen. However, the accumulation of palmitic and

erucic acids content was maximum and minimum with ammonical and combined form of nitrogen, respectively (Figs. 19 to 23).

Sukhija *et al.* (1983) reported that under stress conditions, polyunsaturated fatty acids generally increase as these are related to membrane lipids which might have been altered to maintain integrity of the cell. In yet another adaptive measure of the cell, polar lipids which are membrane lipids. Generally increase under the stress as the cell restore polarity by increasing synthesis of polar lipids (Demel, 1987; Gaur and Gupta, 1994). In the sugarbeet also, salinity induced changes on the composition of fatty acids of plasma membrane were reported by Yahya (1998).

The increasing levels of (80 and 120 kg ha^{-1}) nitrogen irrespective of source significantly increased the oleic, linoleic and linolenic acids while palmitic and erucic acids exhibited significant decrease as compared to lower level of nitrogen (40 kg ha^{-1}). Application of nitrogen in combined form (120 kg ha^{-1}) under salt stress resulted in relatively higher accumulation of oleic, linoleic and linolenic acids while palmitic and erucic acids showed further decline as compared to 40 kg ha^{-1} nitrogen application. However, increasing level of ammonical form showed maximum reduction of oleic, linoleic and linolenic acids while reduction was minimum of palmitic and erucic acids as compared to combined form of nitrogen. Salinity fertility interaction showed that treatment with combined and nitrate form of nitrogen accumulated more unsaturated fatty acids (oleic, linoleic, linolenic) in mature seed at both sampling. However, reduction of palmitic and erucic acids was relatively more with combined

and nitrate form as compared to ammonical form. Low content of erucic and palmitic acids while high content of unsaturated fatty acids (oleic, linoleic and linolenic acids) are considered to be beneficial for quality characters of seeds. In our study, application of nitrogen in combined forms proved more beneficial in facilitating the improvement in desirable characters under salinity stress.

Votan and Mosco (1982) also reported in oilseed rape that increasing level of nitrogen increased the oleic acid linoleic acid and decreased the palmitic acid content, while linolenic content remain unaffected. Increasing the dose of fertilizer, decreased the oil content but increased the linolenic and linoleic acid (Zhao *et al.*, 1991).

Glucosinolate

Glucosinolates are the main secondary metabolites of cruciferous plants and the composition of the glucosinolate profile is species-specific (Louda and Mole,1991). The profile is further influenced by development stage, tissue specificity and biological or physical stress of the plant. The biological effects of glucosinolates and their breakdown products have been studied for quite some time. The importance of glucosinolates in plant interactions with insects and herbivores has also been frequently reported (Blau *et al.*, 1978; Roessingh *et al.*, 1992). Furthermore, induction of certain glucosinolates in response to defence related substances such as salicyclic acid and jasmonate (Kiddle *et al.*, 1994; Doughty *et al.*, 1995) also indicates a role in plant defence. The toxicity of glucosinolates in themselves is minor, but upon tissue disruption they are hydrolyzed by

the plant enzyme myrosinase to form toxic compounds such as isothiocyanates, thiocyanates and nitrites (Gil and Macleod ,1980), which impart pungency to oil and seed meal. Further, the degradation products render seed meal unpalatable and harmful especially for non-ruminants (poultry and piggery). The seed meal of rapeseed-mustard cultivars grown in India have very high glucosinolate content (upto 200 micro moles/g defatted seed meal), whereas, the internationally accepted norm is less than 30 micromoles glucosinolate g^{-1} defatted seed-meal.

With increase in salinity level from 8 to 12 dSm^{-1} the glucosinolate content in seed at harvest increased over control plants irrespective of nitrogen source used at both the sampling stages i.e. State I and Stage II. Its content was highest and lowest with ammonical and combined form of nitrogen, respectively under saline condition (Fig. 24). Since salinity stress induces both osmotic and ionic effect. Higher glucosinolate contents have been associated with water stress (Malier and Cornish, 1987), high temperatures during seed growth and disease (Salisbury *et al.*,1987).

Miler and Cornish (1987) working on *Brassica napus* and *Brassica rapa* reported that water stress increased glucosinolate concentrations in both cultivars whether the stress was applied throughout or only after flowering. Seeds from plants which were stressed throughout had higher concentrations of glucosinolates than those after flowering.

When plants were stressed for water only after flowering their seeds appeared to contain as much glucosinolate as did in plants stressed from soon after sowing. This pointed to the accumulation of the metabolites

responsible for glucosinolate synthesis occur primarily after flowering and that this is the sensitive period in which water stress cause an increase of glucosinalates in the seeds (Mailer and Cornish,1987). Robbelen and Thies (1980) also observed progressive accumulation of glucosinolates in the seed of rape during their development and reached maximum at maturity. The water stress applied after flowering also had similar effects on the oil content as did stress applied throughout. It appears that conditions prevailing during the synthesis of seed material determines their oil and glucosinolate.

Application of nitrogen in ammonical form (120 kg ha^{-1}) had been found to cause maximum accumulation of glucosinolate content in mature seed under saline condition. However, minimum accumulation was observed with combined and nitrate form at both sampling stages. The glucosinolate content in seed exhibited similar trend when treated with basal dose of nitrogen fertilizer and saline water irrigation at both stages, i.e. stage I and stage II. However, the magnitude of accumulation was relatively higher when plants were salinized at stage II (Fig. 24). Increased level of glucosinolate under fertilizer and salinity interaction favour resistance of plants to insects, pests and diseases. Increasing N supply resulted in increase of glucosinolate levels in oilseed crops have been reported by several workers (Forster, 1978; Bilsborrow *et al.*,1993; Zhao *et al.*, 1993).

Under condition of sufficient S supply, increasing the N supply enhances the synthesis of amino acids, particularly S-containing amino

acids which are the precursors for glucosinolate synthesis (Underhill, 1980). Therefore, given a sufficient supply of sulphate, increasing the N supply can lead to an enhanced synthesis of both protein and glucosinolates.

CHAPTER - 6

SUMMARY AND CONCLUSION

The present investigation was carried out on *Brassica juncea* cv. RH-30 under screen house conditions, with the following objectives: (i) To investigate the influence of nitrogen source on growth and development, nutriention and quality characters in *Brassica* under saline irrigation, (ii) To probe into the biochemical basis of nitrogen metabolism under combined effects on nitrogen fertilizer and saline irrigation in Brassica. Before sowing of seeds the pots were supplied with nitrogen through different nitrogen sources i.e. nitrate, ammonical and combined forms having three levels (40, 80 and 120 kg ha^{-1}). The desired salinity levels (ECe 0, 8 and 12 dSm^{-1}) were obtained by adding Cl and SO_4 salts of Na, Ca and Mg alongwith non-saline control. The salinity solutions were applied to plants at 35 and 55 DAS (which corresponds to stage I and stage II). Subsequently, sampling was done at 10 days after applying saline water irrigation. Under saline condition, different growth parameter's such as dry weight of plant, AGR, RGR and NAR exhibited significant decline over non-saline plants. However, maximum and minimum reduction was observed with ammonical and combined form of nitrogen, respectively. Application of higher dose of nitrogen irrespective of source significantly

improve the different growth parameters as compared to lower level of nitrogen. In addition to this, application of nitrogen (120 kg ha^{-1}) in combined form exhibited maximum alleviation in the adverse effect of salinity. However, minimum alleviation was noticed with ammonical form. Water relation and gas exchange with salt stress (8 and 12 dSm^{-1}) showed significant reduction in plant water status in terms of relative water content, water potential and osmotic potential at both sampling stages. The plant treated with ammonical and combined form exhibited maximum and minimum reduction, respectively as compared to non-saline plants. The higher level (120 kg ha^{-1}) of nitrogen in combined form maintained relatively high water status as compared to lower level of nitrogen (40 kg ha^{-1}) under saline condition. However, response was extremely poor with ammonical form.

Reduced plant water status under saline condition, markedly decline photosynthetic rate, transpiration rate and stomatal conductance as compared to non-saline plants. However, the maximum and minimum reduction were noticed in ammonical and nitrate form of nitrogen respectively at both sampling stages i.e. 45 and 65 DAS. The reduction in gas exchange parameters (photosynthesis, transpiration and stomatal conductance) was relatively more in ammonical than in nitrate fed plants. The inhibition in these parameters under salinity was partially restored with use of different nitrogen sources. Nitrate form of nitrogen (120 kg ha^{-1}) proved better over other two forms in partially reviving the gas exchange parameters.

Salinity caused decline in starch, total chlorophyll content and carotenoids. However, the maximum and minimum reduction were noticed with ammonical and combined form of nitrogen, respectively at both sampling stages. Total soluble carbohydrates, proline and total amino acids got accumulated. But the maximum and minimum accumulation were noticed with combined and ammonical form of nitrogen, respectively at both sampling stages (45 and 65 DAS). The improved nutritional status (nitrogen application) of plant under salt stress, which ultimately, led to a better crop performance, was obviously mediated through an improved metabolic efficiency. Declined level of starch, total chlorophyll and carotenoids under salinity was restored partially by application of different form of nitrogen (120 kg ha^{-1}). The maximum and minimum alleviation of adverse effect of salinity was observed with combined and ammonical form of nitrogen respectively. Likewise, accumulation of total soluble carbohydrates, proline, free amino acids also remained highest at 120 kg ha^{-1} nitrogen application. However, minimum and maximum per cent accumulation was observed with higher level of nitrogen in combined and ammonical form, respectively. The aforesaid trends suggest that nitrogen dose of 120 kg ha^{-1} imparted a relatively better metabolic efficiency to plant under salt stress as compared to 40 kg ha^{-1}. Again, it appears that magnitude of metabolic dearrangement under salt stress were ameliorated better by use of combined form of nitrogen as compared to nitrate and ammonical form of nitrogen.

Along with biochemical changes, enzymes of nitrogen metabolism were influenced drastically with salinity treatment. Activity of nitrate assimilation enzymes i.e. nitrate reductase and nitrite reductase got significantly inhibited under salinity as compared to non-saline plants at both sampling stages. The maximum and minimum inhibition in NR and NiR was noticed with ammonical and nitrate form of nitrogen, respectively. The higher nitrogen dose irrespective of source used, induced a greater efficiency for nitrate assimilation enzymes. The application of nitrate at the rate of 120 kg ha^{-1} enhanced maximum activities of NR and NiR as compared to ammonical forms. Besides enzymes of nitrate metabolism, the activities of ammonia assimilation enzymes (glutamine synthetase, glutamate synthase and glutamate dehydogenase) also exhibited maximum and minimum inhibition with nitrate and ammonical form of nitrogen, respectively in saline plants at both sampling stages. But the higher level of nitrogen (120 kg ha^{-1}) irrespective of nitrogen source displayed significantly higher activities of these enzymes as compared to plant treated with lower level of nitrogen (40 kg ha^{-1}). It is pertinent to mention here that activity of ammonia assimilating enzymes were highest in plant treated with ammonical form of nitrogen as compared to nitrate form of nitrogen.

Salinity has been known to affect the uptake and assimilation of various essential nutrients required for normal growth and development. With salinity treatment, different nutrient elements such as nitrogen, phosphorus, potassium, magnesium got decreased in different plant parts.

The maximum and minimum reduction was observed with ammonical and combined form of nitrogen, respectively while reverse was true for calcium, sodium chloride and sulphate. Nitrogen application (120 kg ha^{-1}) in combined form had been found to maintain highest concentrations of nitrogen, phosphorus, magnesium and calcium, along with reduced concentration of sodium, chloride and sulphate. However, reverse was true with ammonical form of nitrogen.

Siliquae and seed yield were adversely affected with different levels of salinity at both sampling stage. The maximum and minimum reduction was observed with ammonical and combined form of nitrogen, respectively. Induced salt stress reduced the number of siliquae and yield per plant and this adverse effect was mitigated by application of nitrogen through different sources. However, the higher level of nitrogen (120 kg ha^{-1}) in combined form mitigated the deleterious effect to a maximum extent over lower level of nitrogen (40 kg ha^{-1}). Beside this, application of ammonical form showed minimum alleviation in the adverse effect of salt stress on yield characters.

The oil and protein content in seed at harvest decreased with every increment in salinity as compared to non-saline condition at both the stages. Reduction under salinity were maximum in ammonical form and minimum with combined form. The increasing level of nitrogen irrespective of nitrogen source attributed to the increased percentage of protein accompanied with decrease in the oil content . Application of nitrogen (120 kg ha^{-1}) in combined form resulted in minimum reduction of oil

content as compared to lower level of nitrogen. However, maximum reduction was observed in ammonical form. The maximum and minimum increase in protein content in seed was observed using 120 kg ha^{-1} nitrogen with nitrate and ammonical form, respectively as compared to lower level of nitrogen (40 kg ha^{-1}). The fatty acids composition of mustard seed at harvest showed sharp changes with salinity treatment at both sampling stages. With increasing salinity levels (8 and 12 dSm^{-1}), oleic, linoleic, linolenic acids got increased while palmitic and erucic acids exhibited significant decrease at both stages. Under saline condition, the plant treated with combined form of nitrogen exhibited maximum accumulation of oleic, linoleic and linolenic as compared to non-saline plants, while minimum accumulation was observed with ammonical form of nitrogen. However, the accumulation of palmitic and erucic acids content was maximum and minimum with ammonical and combined form of nitrogen, respectively. Application of higher level of (120 kg ha^{-1}) nitrogen in combined form under salt stress brought higher accumulation of oleic, linoleic and linolenic acids while plamitic and erucic acids showed further declined as compared to nitrogen dose of 40 kg ha^{-1}. However, ammonical form showed maximum accumulation while reduction in palmitic and erucic acids was relatively less.

With increase in salinity level from 8 to 12 dSm^{-1} the glucosinolate content in seed at harvest increased over control plants at both sampling stages. Its content was highest and lowest with ammonical and combined form of nitrogen, respectively under saline condition. The application of

nitrogen in ammonical form (120 kg ha^{-1}) had been found to cause maximum accumulation of glucosinolate content in seed under salinity. But, minimum accumulation was observed with combined form of nitrogen.

From the above information, following conclusions were drawn:

1. Under saline condition, different growth parameters, water relation and gas exchange, biochemical, nitrogen metabolism, yield and quality parameters got adversely affected.
2. The higher level of nitrogen (120 kg ha^{-1}) in combined form showed maximum alleviation in the detrimental effect of salinity on growth parameters, water relation and biochemical, yield and quality characters.
3. (a) The application of nitrate form at the rate of 120 kg ha^{-1} exhibited a more pronounced moderation of adverse effects of salinity on gas exchange and nitrate assimilation enzymes (NR and NiR). However, minimum by ammonical form of nitrogen.

   (b) Treatment with ammonical form (120 kg ha^{-1}) resulted in maximum activity of ammonium assimilation enzymes (GS, GOGAT and GDH). However, it was minimum with nitrate form.
4. Combined form of nitrogen (120 kg ha^{-1}) exhibited the increase in N, P, K, Mg and Ca along with decrease in Na, Cl and SO_4.
5. Combined form of nitrogen (120 kg ha^{-1}) partially mitigated the decrease in yield and yield attribute. However, minimum alleviation was noticed with ammonical form of nitrogen.

6. The application of nitrogen in nitrate form (120 kg ha^{-1}) significantly increased the protein content with corresponding decrease in oil content. The unsaturated fatty acids (oleic, linoleic and linolenic acids) got enhanced with combined form (120 kg ha^{-1}) with simultaneously declined level of erucic and palmitic acids. The maximum and minimum accumulation of glucosinolate was observed with higher level (120 kg ha^{-1}) of ammonical and combined form of nitrogen.

7. Different levels of nitrogen sources (nitrate, ammonical and combined forms) were partially responsible for alleviation of the harmful effects of salts. However, combined form was more effective in mitigation of stress.

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ABSTRACT

a)	Title of Dissertation	:	Physiological basis of yield variation in *Brassica juncea* L. under the influence of nitrogen source and saline water irrigation
b)	Full name of degree holder	:	Narayan Singh Nathawat
c)	Title of degree	:	Doctor of Philosophy
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The present investigation was carried out on *Brassica juncea* cv RH-30 under screen house conditions to evaluate the interactive effect of salinity and nitrogen fertilizer on morpho-physiological, nitrogenm metabolism and quality characters. Before sowing of seeds the pots were supplied with nitrogen through different nitrogen sources i.e. nitrate, ammonical and combined forms having three levels (40, 80 and 120 kg ha^{-1}). The desired salinity levels (ECe 0, 8 and 12 dSm^{-1}) were obtained by adding Cl and SO_4 salts of Na, Ca and Mg alongwith non-saline control. Salinity solutions were applied to plants at 35 and 55 DAS which correspondsto stage I and stage II. Subsequently sampling was done at 10 days after applying saline water irrigation. All growth parameters viz., dry weight of different plant parts, leaf area, relative growth rate and net assimilation rate decline under salinity at both stages. The combined and ammonical form of N exhibited minimum and maximum reduction under salinity levels (8 and 12 dSm^{-1}). The application of higher level (120 kg ha^{-1}) of nitrogen in combined and ammonical form resulted in maximum and minimum alleviation of the detrimental effect of salinity. Water relation parameters viz., relative water content, leaf water potential and leaf osmotic potential decreased at both sampling stages under saline condition. The maximum and minimum reduction was observed with combined and ammonical form of nitrogen, respectively. However, the application of 120 kg ha^{-1} combined and ammonical forms helped in maintaining maximum and minimum plant water status. Reduced water status under salinity resulted in decline photosynthetic rate, transpiration and stomatal conductance. The plants treated with nitrate and ammonical form of nitrogen showed minimum and maximum reduction salinity (8 and 12 dSm^{-1}). The nitrate form of nitrogen (120 kg ha^{-1}) proved better over other two forms in partial moderation of the gas exchange parameters. Biochemical concentration got altered by salinity at both stages. Total chlorophyll, carotenoid and starch declined significantly under salinity. However, maximum and minimum reduction were noticed with ammonical and combined form of nitrogen, respectively. Total soluble carbohydrates, proline and

total free amino acids got accumulated. In contrst to the above effect, combined and ammonical form of nitrogen exhibited maximum and minimum accumulation under saline condition. Alleviation in the decline of starch, total chlorophyll and carotenoids under salinity was maximum and minimum with combined and ammonical form of nitrogen, respectively. Likewise, minimum and maximum per cent accumulation of total soluble carbohydrates, proline and free amino acids were observedwas observed with higher level of nitrogen in combined and ammonical of form of nitrogen, respectively. Alongwith biochemical changes, enzymes of nitrogen metabolism (NR, NiR, GS, GOGAT and GDH) were inhibited with salinity treatment. The maximum and minimum inhibition in nitrate assimilation enzymes (NR and NiR) were noticed with ammonical and nitrate forms, respectively. The higher level of nitrate and ammonical forms of nitrogen (120 kg ha^{-1}) resulted highest and lowest activities of NR and NiR. Besides enzymes of nitrate assimilation, the activities of ammonia assimilation enzymes (GS, GOGAT and GDH) also exhibited maximum and minimum inhibition with nitrate and ammonical forms, respectively. But the higher level of nitrogen (120 kg ha^{-1}) significantly gave maximum and minimum activity with ammonical and nitrate forms of nitrogen. With saline treatment, different nutrient elements such as N, P, K, Mg got decreased in different plant parts. The maximum and minimum reduction were observed with ammonical and combined form of nitrogen, respectively while reverse was true for Ca, Na, Cl and SO_4. Nitrogen application (120 kg ha^{-1}) in combined form had been found to maintain highest concentrations of N, P, K, Mg and Ca, along with reduced conc. of Na, Cl and SO_4. Siliquae and seed yield adversely affected with different levels of salinity at both sampling stages. The maximum and minimum reduction were observed with ammonical and combined forms of nitrogen, respectively. Induced salt stress reduced the number of siliquae and yield per plant and this adverse effect was mitigated to the extent of maximum and minimum by higher level of nitrogen in combined and ammonical forms, respectively. The oil and protein content at harvest decreased with every increment in salinity at both stages. Reduction under salinity were maximum in ammonical form and minimum with combined form. The maximum and minimum increase in protein content in seed was observed using 120 kg ha^{-1} nitrogen with nitrate and ammonical form. The fatty acids composition of mustard seed at harvest showed sharp changes with salinity treatment at both sampling stages. With increasing salinity levels (8 and 12 dSm^{-1}), oleic, linoleic, linolenic acids got increased while palmitic and erucic acids decreased. Under saline condition, maximum accumulation of oleic, linoleic and linolenic acids with combined form while minimum accumulation with ammonical form of nitrogen were observed. However, the accumulation of palmitic and erucic acids contents was maximum and minimum with ammonical and combined form of nitrogen, respectively. Application of higher level of (120 kg ha^{-1}) nitrogen in combined form under salt stress brought highest accumulation of oleic, linoleic and linolenic acids while palmitic and erucic acids showed further declined. Under salinity, the glucosinolate content in seed increased at both sampling stages. Its content was highest and lowest with ammonical and combined form of nitrogen. The maximum and minimum accumulation of glucosinolate content under salinity was observed with higher level of nitrogen, respectively. Mostly combined form of nitrogen (120 kg ha^{-1}) had edge over other two form in partially alleviating the deleterious effect of salinity on morpho-physiological, biochemical, yield and quality characters in *Brassica juncea*.

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