

THE EFFECT OF SALINITY ON GRAIN FILLING IN WHEAT

by

Kazem Poustini

Thesis submitted for the degree of
Doctor of Philosophy of the
University of London

Wye College

March, 1990



**In the Name of Allah the Most Compassionate
and Merciful**

To begin with, it is appropriate to praise God, the Master of the world, to whom all beings belong, and from whom every living creature receives his life and entity. Praise be to God who created a world so disciplined with such a precise and wonderful relationship between all the events which take place therein.

In this world who could observe a minor phenomenon and not seek its cause? Or which free and noble-minded person could see all these beauties and splendid complexities and not recognise the hand of the magnificent Creator? When an archeologist discovers a broken jug beneath the earth, we conclude the presence of a past civilization. Similarly, man with such an amazing and astonishing world around him must also recognise the work of its Creator and pay respect to Him.

Man, in order to acquire salvation in this life, and to prepare for the heavenly life after death must be fully prepared. It is his deeds, in addition to belief, which are essential for making spiritual connections with almighty God, rather than subscribing to the void actionless words and medieval superstitions which can give a wrongful sense of anti-science to religious beliefs. The teachings of the prophet Mohammad and other prophets of God (Peace Be Upon Them All), are the only means of man's salvation and the only guide for him to lead a truly God-loving and heavenly life, both in this world and the one hereafter. Man must seek a life free from sin. He must be fully obedient to God, understanding towards others and acquire knowledge to establish one harmonious society. This is the principal task for which the great prophets were sent by God.

May Allah help us towards a better understanding of His message, and the fulfillment of His commands.

ACKNOWLEDGEMENTS

I am very grateful to the government of the Islamic Republic of Iran for providing me with a scholarship for these studies during the period when the country was fighting a war.

I wish to express my sincere thanks to my supervisor, Professor D.A. Baker, for his guidance, useful discussions and suggestions. I appreciate his constant encouragement throughout this period of research.

The author wishes to extend his grateful thanks to Miss Shelagh Reardon for her expert and generous help with the electron microscope. His thanks are also due to Mrs. Sue Briant for typing this thesis, to the staff of the Biological Sciences Department for their help and to all his friends for their encouragement.

And finally the author is indebted to his mother for her many kindnesses and also to his wife for her patience and understanding throughout this period of time.

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ABSTRACT

Some physiological responses of wheat plants (*Triticum aestivum* L. cvs. Hobbit, Inia 66 and Sholeh) to salinity were investigated. These included photosynthesis, partitioning of phosoassimilates and grain filling, with particular emphasis on the latter.

The sap from the cavity and endosperm of developing grains was collected while they were attached to the plants and analysed for sucrose, potassium and sodium content. Marked changes in the concentrations of these solutes were observed in salt-treated plants. Potassium increased by 95%, sodium by 152% and sucrose by 50% in the cavity sap of salt-treated plants. In the endosperm fluid K^+ decreased by 22%, Na^+ increased by 42% and sucrose decreased by 42%. These observed changes indicate an inhibitory effect of salinity on sucrose transport from the cavity to the endosperm. Together with observations of a low pH in the cavity and a high pH in the endosperm, which were unchanged by salinity, the results lend support to the hypothesis that a proton co-transport of sucrose is involved in the transport of sucrose from cavity to endosperm. This can be affected by a decreased ratio of K^+/Na^+ in the cavity fluid under salt stress conditions. Electron microscope studies on the cells of the aleurone layer showed some anatomical changes, e.g. suberized cell wall thickenings that may block the apoplastic pathway for assimilate transfer into the endosperm.

Salinity reduced the photosynthetic rate of both cultivars.

Net assimilation rates were affected and the root/shoot ratio of cultivar Sholeh was increased by salinity. The pattern of carbon partitioning within the plants was affected by salinity to a greater extent in Inia 66 than in Sholeh, resulting in a substantial reduction in the carbon allocation to the ear at the highest level of salt stress.

Although considerable changes were observed in both photosynthesis and the partitioning pattern as a result of salinity, these changes *per se* may not prove to play the crucial role in limiting grain yield. It is concluded that it is sucrose transport into the endosperm which is the major rate limiting factor in grain filling and which is sensitive to salt stress.

1. INTRODUCTION

The proportion of food energy provided by cereals in human diets varies from about 25% in one of the western countries to as much as 80 to 85% in some locations where only small amounts of other foods are available or where incomes are extremely low (see Olson and Fery, 1987). Thus, the grain crop has already become arguably the most significant political weapon in the modern world (Wibberley, 1989).

Among the grain crops wheat is one of the most important species grown in many regions of the world. While making a large contribution to human nutrition, accounting for approximately one quarter of the world's food production (Wyn, Gorham and McDonnell, 1984), wheat can be grown in a whole range of climates. There are cultivars that can be grown in latitudes from 30° north or south to 60° north or south. Wheat is not grown in sub-tropical areas (from the equator to 30° north or south latitude) because the temperatures are too high and grains tend to sprout during preharvest rains. Asia is the world leader in growing wheat followed by Europe and North America. It is the most widely grown of the cereal grains; 22 percent of cereal grain area is wheat compared with 13 percent for rice.

Wheat is a C_3 plant which develops and matures best in cold, moist environments. Water, whether from rainfall or irrigation, is required at 25 to 100 cm annually. The wheat seed is actually a dry

fruit, that is a grain or caryopsis. It germinates between 4°C and 37°C with an optimum of 20° to 25°C (Klein and Klein, 1988).

Salinity, being defined as the presence of excessive concentrations of soluble salts, is a major problem in cultivated lands in some regions of the world, and a potential problem for a number of irrigated lands. Salinization of croplands primarily results from poorly drained irrigation, as after irrigation evaporation and transpiration extract almost pure water, leaving behind the salts. If the salts are not flushed and leached by rainfall or additional irrigation, they can concentrate in and on the surface of the soil (Toenniessen, 1984).

Considerable areas of the earth's surface are affected by salinity. Based on different reports, it has been estimated that between 3.4 million and more than 9 million km² of land are salt affected. About 13% of land under cultivation and 30 to 50% of irrigated land is affected by salt (McWilliam, 1986; Flowers and Yeo, 1988).

More than one third of the salt affected soils of the world can be found in Asia and very few countries in this continent are free from salt affected soils. In many of them soil salinity and alkalinity have developed to such a degree that they constitute cardinal problems for the national economies (see Szabolcs, 1989). In Asia the greatest extension of salt affected soils after the U.S.S.R., China, India and Pakistan, can be found in Iran. The arid conditions of this country contribute to the formation of saline

soils, which are very prevalent in several areas. In Iran the saline soils are closely associated with salt marshes and with their soil cover. The total extension of these kinds of salt affected soils exceeds 15 million ha and occupies nearly 10% of the total surface area of the country (Dewan and Famouri, 1942; cited in Szaboles, 1989).

Excessive sodium concentration is the most common cause of salinity in nature with other ions contributing to salinity including Ca^{++} , Mg^{++} , Cl^- , SO_4^{++} , HCO_3^- and occasionally NO_3^+ and K^+ (Jones, 1985; Flowers and Yeo, 1986). Although sodium is known to be harmful to crop production, there is now good evidence that sodium is an essential micro-element for *Atriplex* and some other plants, notably those showing the C_4 photosynthetic pathway (Brownell and Crossland, 1972; Sutcliffe and Baker, 1981). The different responses of plants to salinity allow them to be classified into two major groups, as either halophytes or glycophytes. Halophytes are those plants which survive to complete their life cycle at salinity concentrations higher than 300 mM, which are not tolerated by glycophytes. Tolerance to high levels of salinity in halophytes is accompanied by a high ion content, while glycophytes respond to salinity basically by ion exclusion (Flowers, Troke and Yeo, 1977).

In glycophytes the stress effects of salinity can be summarized as water stress, salt stress and ion-imbalance stress. Water stress is a general condition and the adaptations of plants to salinity may show features similar to those for drought. Plants adjust osmotically to increasing salinity thus maintaining a gradient

of water potential between the soil and the plant. Three principal mechanisms exist to reduce plant water potential. First, tissue water content may decrease, second tissue inorganic ion concentrations may increase and thirdly non-toxic organic osmotica such as proline and glycinebetaine may increase, primarily in the cytoplasm (Jones, 1985). Salt stress arises from an excessive uptake of salt by the plants, which is an unavoidable consequence of high ion concentrations in the medium. Ion imbalance then results from the disturbed ionic ratios in the cells (Lüttge and Smith, 1984).

Whether ion toxicity or water deficit impose the main limitations to plant growth in saline conditions is a question for debate. From a study carried out on rice it was concluded that salinity damage at 50 mM external concentration is due predominantly to excessive ion entry (Yeo and Flowers, 1984). They found also that there is an overall negative relation between Na^+ uptake and varietal survival in saline conditions. On the other hand, water deficit has been ruled out by Munns and Termaat (1986) as limiting shoot growth at high salinity, at least for non-halophytes. These investigators proposed that salt stress causes damage through an effect of Na^+ or Cl^- on metabolism in the leaves or through the effect of NaCl outside the roots on uptake and subsequent transport of essential nutrients.

The responses of glycophytes to salinity are different, showing a range of tolerance/sensitivity. Bean plants, one of the most sensitive of crops, can tolerate EC's (of soil saturation extract) of not more than 6, while barley, a tolerant plant, can resist EC's of up to 25. In wheat a 70% reduction in relative yield

is expected at EC's of 10 (Shannon, 1984).

One of the most important objectives of recent studies on salinity in plants has been to identify the mechanism(s) employed by plants to tolerate the stress effects of salinity. Yeo and Flowers (1984) proposed that in addition to K^+ - Na^+ selectivity there are numerous other possibilities for mitigating the consequences of excessive initial salt entry. Ions may be extruded from the roots or retained in the cortical vacuoles in preference to long-distance transport. If transported to the xylem, these ions may be reabsorbed anywhere along the water pathway (mature roots, stems, petioles in dicots, leaf veins) and possibly retranslocated back to the medium. Salts entering the shoot may be involved in complex interactions. They may be diluted by high growth rates, or compartmentalised between leaves in both an intercellular and intracellular manner. According to Baker (1978b) univalent cation selectivity in cereal plants appears to be established mainly at three sites and by different strategies: 1) at the plasmalemma, either during influx or by K^+ -dependent sodium efflux; 2) at the tonoplast by Na^+/K^+ selective influx causing sodium to be sequestered in vacuoles, and 3) selectivity during release to the xylem sap or reabsorption of sodium. From the study conducted by Nassery and Baker (1974) on barley it was concluded that the operation of a sodium extrusion pump could be effective at concentrations of sodium chloride above 50 mM when the plant is cultivated in saline soils in the presence of potassium and calcium. Jeschke and Nassery (1981) reached a similar conclusion, stating that the K^+ - Na^+ exchange system across the plasmalemma is highly efficient in wheat when compared with onion and

sunflower. However, in contrast to barley and rye which are regarded as robust crops resistant to salt, bread wheat is generally thought to be less resistant to salt stress (Wyn Jones *et al.*, 1984).

The effects of Na^+ , K^+ and their ratio on plant metabolism have been studied by some authors. Pal *et al.* (1984) found that K^+ and Na^+ increased in the barley grain with the use of saline water, while varieties with higher K/Na ratios performed better. Gill and Dutt (1987) observed that barley had higher concentrations of Na^+ than bread-wheat, and that disturbed ionic ratios hindered plant metabolic functioning and caused greater injury to wheat (susceptible) than barley (tolerant). The study carried out on barley by Greenway *et al.* (1965) showed that treatment with NaCl increased Cl^- and Na^+ concentrations of developing inflorescences and grain over control levels, but K^+ concentrations of inflorescences and developing grain were not affected by the treatment, in marked contrast with all other plant organs. The concentration of K^+ in developing grains was 0.29 m equiv/g dry weight for control plants and 0.23 m equiv/g dry weight for salt treated ones. But the Na^+ concentration of grains was 0.019 m equiv for control and 0.039 m equiv per gram dry weight for salt treated plants. In a salt tolerant barley variety grown in solutions of 1 mM (control) and 125 mM NaCl (see Greenway and Munns, 1980), the grain ion concentrations (g^{-1} DW) were 0.26 and 0.3 for K^+ and 0.03 and 0.014 for Na^+ in the control and treated plants respectively which showed that the K^+/Na^+ ratio had been increased from 9.6 to 17.8 by salt treatment.

Wheat is moderately resistant to saline conditions, while barley is considered as resistant. Among the physiological responses of these plants to salinity, the carbohydrate content of the grains and grain yield are important. It is well known that salt stress reduces the grain yield of these crop plants. Pal, Singh and Singh (1984) found that barley grain yield decreased significantly with an increase in the salinity of irrigation waters. The average grain yield of two years cultivation declined by 12.7, 22.7, 31.8, 43.5, 59.8 and 77.4% with an application of waters with ECs of 4, 8, 12, 16, 20 and 24 mmhos/cm respectively, compared with the yield level of control with EC of 2.2 mmhos/cm. They also observed that starch, nitrogen and phosphorus contents decreased (see also Greenway, 1963). Iyengar, Patolia and Kuriant (1977) showed that saline irrigation reduced the grain and some other attributes of barley plants. The carbohydrate content of the seeds was not markedly affected, although a considerable accumulation of minerals in the shoot took place as a result of sea water irrigation. Based on data of Greenway, Gunn, Pitman and Thomas (1965), the dry weight (g) of the grains per plant of barley grown in 125 mM NaCl was 34.1% of the control in one variety and 50.3% in another. They also observed that grain formation was not affected by treatment with sodium chloride, and that in the inflorescences there were particularly large varietal differences in ion content, i.e. higher Cl^- and Na^+ and lower K^+ concentrations in the sensitive than in the resistant variety.

As wheat is the most important source of human nutrition, the study and ultimate improvement of salt tolerance in this crop deserves the highest priority. The development of crop varieties

that combine high yield potential with a high salinity tolerance will not be an easy task. Salinity tolerance is a complex whole plant characteristic with physiological and biochemical functions controlled by numerous genes. Moreover, environmental and soil factors heavily influence their expression (Maas and Nieman, 1978; Toenniessen, 1984). However, according to Yeo, Yeo and Flowers (1988) who studied rice, there is useful genetic diversity for sodium uptake and hence salt resistance within modern varieties of this crop plant. On the other hand, with wheat plants, there have been signs of a link between ion exclusion as a physiological criterion and salt tolerance in wheat as found by Wyn Jones *et al.* (1984). Thus, the potential for developing varieties more resistant to salinity appears promising. Some progress has already been made towards developing salt tolerant crop plants and there appears to be a potential for further genetic improvement. It is reported that the character for Na^+/K^+ selectivity, which is an important physiological one in salt-tolerance, derives from the D genome. *Aegilops squarosa* which possesses this genome evidences high Na^+/K^+ selectivity (see Yeo and Flowers, 1989).

Photosynthesis, as one of the most important physiological processes, provides 90% of the plant's dry matter and is known to be affected by various environmental factors. Salinity has an adverse effect on photosynthesis of crop plants. The net photosynthesis of rice leaves has been inversely correlated with the sodium concentration in the tissue (Yeo, Capron and Flowers, 1985). The extent of this effect depends on species, varieties and other growing conditions. The effects of salinity on photosynthesis in plants such

as wheat have been studied by several investigators. A reduction of photosynthesis and vegetative growth is a generally accepted effect of salinity, but the causal relationship between these two is in debate.

This decrease in growth includes a reduction in the leaf area of the plant which has been considered by some authors as the cause of photosynthetic depression under salt stress conditions. It is believed to be because of the prevention of normal cell enlargement, arising from a suboptimal turgor pressure that restricts leaf area increase. Consequently, growth may be retarded even if photosynthetic rates per unit leaf area remained unaltered by salinity (Berry and Downton, 1982). The reduction in turgor was attributed by Munns and Passioura (1984) to an ion build-up in the cell walls, when ion contents of cells reach a maximum.

Any reduction in photosynthesis under saline conditions is believed to be merely a secondary effect of reduced growth, brought about by water deficit (Greenway and Munns, 1980). Others believe, however, that reduction in photosynthetic efficiency is something additional to the reduction in leaf area. It is reported that an increased salt concentration is often accompanied by a decrease in leaf area and in net carbon assimilation. One simple interpretation of this observation is that increased vacuolar concentration is accompanied by increased cytoplasmic processes (Pitman, 1988). The same view has been expressed by Beadle, Long, Imbamba, Hall and Olembo (1985), that salinity at sublethal levels reduced productivity by reducing leaf area and leaf photosynthetic rate. The reduced

rates of photosynthesis per leaf area under salt stress have been attributed to different mechanisms such as stomatal resistance and cell resistance to CO_2 flux.

It is known that salt stress does not result in any major decrease in the photosynthetic potential of the spinach leaf. Actual photosynthesis may be reduced by other factors such as decreased stomatal conductance (Robinson, Downton and Millhouse, 1983). Yeo (1983) also reported that photosynthesis is reduced due to increased stomatal resistance. This view is supported by some other workers (Gale, 1975; Kemp and Cunningham, 1981; Beadle *et al.*, 1985). The reduction in stomatal conductance under salt stress is estimated to be substantial with the intercellular CO_2 concentrations reduced by up to 30% (Seemann and Crithley, 1985).

There are some suggestions regarding the mechanism(s) of stomatal response to salinity. This could result from such factors as the failure of guard cells to osmotically adjust, ionic interference with stomatal function or increased endogenous concentrations of abscisic acid (Berry and Downton, 1982). Beadle *et al.* (1985) suggested that salinity-induced deficiencies of K^+ and Mg^{++} in particular could be expected to depress photosynthesis because of their vital roles in stomatal opening and thylakoid-stroma ion gradients respectively. Low K^+ is also reported to reduce net gaseous exchange by an increase in both stomatal and mesophyll resistance (see Baker and Chaudhry, 1986).

Associated with stomatal opening is the process of

transpiration. There are many reports of a depression of transpiration under saline conditions. The transpiration of barley seedlings was reduced by 25% at 60 mM external concentration, while it was independent of that up to 40 mM (Pitman, 1975). The reduction of transpiration and the increase of stomatal resistance were originally ascribed to reduced turgor and subsequent stomatal closure, resulting from a reduction in water uptake (Gale, 1975). Comparatively low transpiration rates, as an adaptive mechanism of the leaves to water and salinity stress, imply not only economic use of water but also a lower supply of salt to the leaves via the transpiration stream (Lüttge, 1984). Transpiration at high levels alters the uptake ratio of Na^+/K^+ by roots. It is known that although the total uptake of Na^+ and K^+ was unaffected, a higher transpiration rate increased Na^+ uptake and decreased K^+ uptake (Pitman, 1965).

Other factors, known to affect photosynthesis in saline conditions, include the mesophyll conductance. According to Greenway and Munns (1980) a primary effect of salinity on the photosynthetic machinery was caused by increases in the mesophyll resistance which could account for two thirds of a 50% reduction in photosynthesis, the rest being due to stomatal resistance (supported by Kingsbury, Epstein and Pearcy, 1984). They also put forward the possibility that increased mesophyll resistance may be the consequence of a reduced carbohydrate utilization; the concept of sink dominance on photosynthesis. Seemann and Crithley (1985) reported that salt stress brought about a reduction in photosynthetic CO_2 fixation in *Phaseolus*, independent of altered diffusional limitations. They

concluded that it might be because of end product inhibition of certain processes of carbon metabolism or a reduction of quantum yield of photosynthesis.

With regard to the balance of photosynthates within the plants under salt stress, another process which should be mentioned as affecting carbon balance and net photosynthesis is salt respiration. Nieman (1962) demonstrated that respiration of leaves of twelve crop species tended to increase in both tolerant and sensitive species under saline conditions. The effect was generally greater and occurred at lower levels of salt in the more sensitive species. Schwarz and Gale (1983) also reported a large increase in maintenance respiration in saline conditions.

It appears that the respiratory loss of carbon under salt or water stress is a balance of its components. As some authors pointed out, while the growth component always decreases in response to stress, the maintenance component should increase if there are indeed additional metabolic costs. Thus, the total rate of respiration may either increase or decrease (McCree, 1986).

The rate of photosynthesis can also be related to the structure of the leaves. As Evans and Dunstone (1970) explained, the fall in photosynthetic rate is more likely to be due to the reduced surface-volume ratio of the larger mesophyll cells. Some anatomical changes in the leaves of bean and cotton induced by salinity partially compensated for the increased resistance to CO₂ uptake (Beadle *et al.*, 1985).

Apart from leaf structure, a cellular component of structural response to salinity is also reported. The decreased photosynthetic role in some varieties of rice under saline conditions has been attributed to a disorganization of cellular structure. Some changes in the integrity of cells and organelles are observed (Flowers, Duque, Hajibagheri, McGonigle and Yeo, 1985).

The effect of salinity on the growth rate of the plant and its relationship with photosynthesis is of importance. The shoot fresh weight of 4 varieties of wheat, as a percentage of low salt controls differed from 15% in 250 mM to 77% in 75 mM NaCl (Wyn Jones *et al.*, 1984). Salinity of 100 mM NaCl reduced the growth rate by 20% when pressure was applied to the roots of wheat and barley (Termaat *et al.*, 1985). They concluded that shoot turgor alone was not regulating the growth of the plants. Kingsbury *et al.* (1984) demonstrated that two wheat lines differed substantially in their relative growth rate and photosynthesis for several weeks following salinization. The sensitive line showed a greater effect of salinity on photosynthesis and growth rate. Findings of this kind have been taken as evidence for a regulatory effect of photosynthesis on growth under salt stress conditions. On the other hand, there is another line of evidence supporting the view that reduction in growth is not the result of photosynthetic depression. It is reported that although high salinity causes a decrease in net photosynthesis at all environmental conditions, the capacity for high light to overcome depressed growth cannot be explained completely by a reduction in the rate of photosynthesis (Kemp and Cunningham, 1981). Munns and

Termaat (1986) found that a salt treatment which reduced growth caused neither a change nor an increase in the total concentration of reserve carbohydrates. Taking these results and all the evidence into account they concluded that under salt stress (and also water stress), carbohydrates are accumulating in the leaves as a result of decreased utilization. Munns *et al.* (1982) showed that NaCl treatments of barley increased the concentration of soluble carbohydrates in the elongating leaves, while the amount of starch did not change. They concluded that photosynthesis was not limiting growth (see also Munns and Weir, 1981). This conclusion is also supported by Poloneko, Dumbroff and Mayfield (1983). Yeo (1983) speculated that the reduction of growth is due to competition for carbohydrates with the vacuolar osmotic adjustment. He observed that in *Triticum* sugars were responsible for 70-100% of the osmotic adjustment at high irradiance. During an initial growth reduction, the decreased carbohydrate consumption for growth was equivalent to the increase in soluble sugars.

The reduction of growth comprises a reduction in both root and/or shoot. Delane, Greenway, Munns and Gibbs (1982) observed that in seedlings of salt tolerant barley the shoot dry weight decreased as NaCl increased. Wyn Jones *et al.* (1984) also found similar results for four cultivars of wheat, as mentioned earlier. The root/shoot ratio of *Phaseolus vulgaris* was also increased by increasing salinity (Seemann and Crithley, 1985).

Favouring assimilate transfer to the harvestable portion of the crop is one of the important ways of improving crop productivity.

Retrospective physiological analyses have demonstrated that shifts in the assimilate partitioning pattern largely account for the evolutionary increase in yield of a range of crop species (Patrick, 1988). Throughout the history of improvement in the genetic yield potential of field crops, it has been the partitioning of photosynthetic product between economic yield and the rest of the plant (harvest index, HI) that has been of primary importance, even though selection was not directed specifically to that end. The observed increase in HI for several improved crop species reflects a shift from excessive vegetative development in traditional varieties to a greater partitioning into the fruits or other sinks for assimilates (Gifford, Thorne, Hitz and Giaquinta, 1984). More photoassimilates than those accumulated in the economic parts of the plant are produced by the source organs, but are allocated to non harvested organs or lost through respiratory processes. In fact, there is a wide range of alternative sinks to which assimilates are partitioned. This partitioning can be between starch storage in the chloroplast (for later mobilization) and immediate export into the mesophyll cell cytoplasm; between source retention in leaf mesophyll and loading into the phloem for export; between retention in the phloem and unloading into growing sinks; between vegetative growth and reproductive growth and among competing alternative sinks such as root and shoot, elongating wheat stem and ear growth, or grains within an ear (Gifford *et al.*, 1984). In addition to all alternative sinks a proportion of fixed carbon is lost through photorespiration estimated to be 15 to 20% in C3 plants, and also true respiration (40 to 50%) used for catabolism (Gifford *et al.*, 1984). Lambers, Simpson, Beilharz and Dalling (1982) reported that of the carbon

translocated to the roots of a 22 day old wheat plant, 32% was respired, while 29% was incorporated into dry matter.

Not only are there many alternatives for partitioning in different levels of assimilate movement, but it cannot be assumed that all assimilates irrespective of their position or origin move freely within the plant. It is well established that individual sinks may derive assimilates from fairly specific groups of leaves. Thus the developing wheat ear obtains most of its assimilates from the uppermost (flag) leaf (Quinlan and Sagar, 1962; Lupton, 1966). Moreover, the partitioning pattern may be changed in different stages of the plant's growth. For instance a very young leaf obtains assimilates required for growth from older leaves, but as it expands it enters a phase when it may be exporting assimilates although still a net importer, and then it becomes a net exporting organ with virtually no import. Since the availability of assimilate does not appear to be a determinant factor in the transition from import to export of assimilates, it has been proposed that the differentiation of vein-loading structure may determine the switch from import to export (Wareing and Patrick, 1975).

Apart from the effect of the developmental stage of the plant there are some other endogenous factors which regulate photosynthate partitioning. The vascular bundle is a factor which may affect the assimilate allocation. The influence of the path of transport on assimilate distribution is associated with variations in the carrying capacity, the ability to transfer metabolites laterally to the adjacent non-conducting tissue, the exchange between individual

vascular elements or bundles and the pattern of vascular connections within the plant (Wardlaw, 1976). It is reported that, among wheat from all stages in the evolution of the crop, the sectional area of phloem in the peduncle has increased in parallel with the peak rate of storage in the ear (Evans *et al.*, 1970). The apparent restrictions on assimilate distribution in wheat are considered to be imposed by the vascular geometry of the plant, but manipulative experiments have demonstrated that these limitations are overcome readily when 'sink' demand is increased (Wardlaw, 1968 cited in Wareing and Patrick, 1975). Gifford and Evans (1981) found that dry weight accumulation in the grain was not reduced by restricting phloem cross section.

The effect of the sink is another factor influencing assimilate distribution. Increased demand by a specific growth centre will change the pattern of assimilate distribution. Wardlaw (1976) found that doubling the rate of import of dry matter by the ear more than doubled the speed of movement of ^{14}C -assimilates through the peduncle. There is an increasing body of evidence suggesting that the hormone content of a growth centre may affect its ability to mobilize assimilates. One possibility (and perhaps the most currently accepted) is that hormones influence assimilate mobilization by regulating sink strength (Wareing and Patrick, 1975).

Environmental factors also affect the photoassimilate distribution. Temperature is one of these factors that changes the direction of carbon movement. Dry weight accumulation in the stem of wheat was, in contrast to the grain, found to be highest at lower

temperatures (Wardlaw, 1970). It was found that at higher temperatures around the whole plant there was greater movement of ^{14}C photosynthate to the ear and a very much reduced storage in the stem, as well as a small effect on movement to the root. Taking all this and other results together, Wardlaw (1976) concluded that the dominant effect of temperature in wheat is directly on grain growth with a consequent change in the availability of dry matter for storage and root growth.

Drought has a marked effect on assimilate distribution in plants. In wheat it was found that the amount of carbon exported from the flag leaf was reduced by about 40 per cent with water stress (Wardlaw, 1967). The amount of assimilated carbon which was transported to the dominant sink, the ear, remained about the same under drought conditions but there was a marked reduction in the amount of carbon translocated to the basal region of the plant (Moorby, 1981). Wardlaw (1967) found that water stress in wheat reduces the ability of the leaf to utilize available energy, resulting in a relatively greater reduction in the transfer of assimilates to the conducting tissue than of photosynthesis. This in turn would result from a turgor-induced stimulation of the transport of sugars into storage compartments (vacuoles) in the leaf mesophyll.

Partitioning can be affected by light, as light exercises both quantitative and qualitative effects on the growth of plants. Wardlaw (1976) reported that in wheat the patterns of ^{14}C -accumulation in two levels of light demonstrated the dominant role of the grain as a sink following anthesis. At the lower light level the

grain level was maintained at the expense of stem storage. There are also some effects from light period on carbon allocation between sucrose and starch in leaves, on C partitioning to root and shoot, on growth of reproductive shoot apices and on the transport process directly. All these suggest that the manipulation of light quality or duration can alter assimilate partitioning patterns to increase crop productivity (Patrick, 1988).

It is also reported that in both cases of partitioning to meristematic and expansion/storage sinks, biomass gain is vulnerable to alterations in the tissue concentration of mineral ions. These concentrations depend on the programmed partitioning of mineral ions from root to shoot and within the shoot. Potassium may play a significant role as an integrator of sugar transfer to and from the leaf apoplast as well as contributing significantly to generation of sieve-tube turgor. This interaction could account for the negative effects of K deficiency on assimilate export and provide a potential locus for K efficiency in C partitioning (Patrick, 1988).

The characteristics of assimilate partitioning in wheat have been studied by some authors. Comprehensive investigations have been carried out by Rawson and Hofstra (1969) on assimilate translocation at different stages of each leaf. They found in a single variety employed that during grain filling the overall pattern of movement to all plant fractions, apart from the stem, did not change greatly from that at ear emergence. At ear emergence leaf 2 was supplying more to the roots (26%) and slightly less to the tillers (20%), leaf 3 was supplying the roots and tillers with equivalent quantities of

substrate (27 and 28%), leaf 4 exported preferentially to the tillers (31%) and much less to the roots (13%), leaf 5 supplied both the tillers and stems (18 and 25% respectively), whilst leaf 6 donated assimilate particularly to the ear (37%) and slightly less to the stem (26%). Throughout the experiment a small percentage of the ^{14}C from each leaf (about 1%) was transported to the older leaves. Following ear emergence the amount of ^{14}C translocated to the stem changed appreciably. Export from the flag leaf to internodes 1 and 2 of the stem was particularly high at anthesis and these internodes were rapidly increasing in dry weight at this time as a result. By early grain filling, shortly after the stem had reached maximum dry weight, the contribution of leaf 6 to the stem was still substantial but by mid to late grain filling this had fallen to a much lower level. It is well known that during the period of grain growth the flag leaf and penultimate leaves are the main supplier to the ear (Evans, 1975). King, Wardlaw and Evans (1967) reported that about 45% of flag leaf assimilates were translocated to the ear, and only 12% to the roots and young shoots. Another report shows that all the moveable assimilates were transported out of the fed leaf during the first 24 hours after labelling and movement was mainly to the main stem ear (50%) and a further 30% to the two internodes (Rawson and Hofstra, 1969).

In addition to the carbon assimilated at the grain filling stage, remobilization of previously fixed carbon from the other plant organs is another source for grain filling. From two internodes of the stem remobilization of reserved assimilates occurs between ear emergence and maturity (Rawson and Hofstra, 1969). Austin, Edrich,

Ford and Blackwell (1977a) reported that only 14.3% of the products of photosynthesis over the stem elongation period were relocated to grains. This relocation accounted for an average of 7 per cent of the final grain weight. It was estimated that during the 18 days following anthesis, photosynthesis contributed 48% of the final grain dry weight. Of this about half was translocated to the grain within 10 days of initial assimilation, the remainder being stored temporarily in the stems and leaves and translocated later to the grains. The assimilates mobilized from stems were equivalent to 2.7-12.2% of the final grain weight in control ears (Rawson and Evans, 1971). Mobilization increased when photosynthesis was reduced. The velocity of assimilate movement can affect the pattern of partitioning and was found to vary between 80 to 100 cm/h acropetally through top internodes and only half of this basipetally (Wardlaw, 1964).

With regard to the effects of salinity in general terms, the diversion of carbon which is normally used in growth or storage is of importance. A portion of this carbohydrate can be used in the extra respiration imposed by salinity, as it is known that when roots of the plants are absorbing salts, the respiration rate rises (Bidwell, 1974). On the other hand, under salt stress, carbohydrates are known to be accumulated and used for osmotic adjustment. The increased carbohydrates accumulated under salinity may have been removed from the pool available for immediate metabolism or growth. For example, storage would include accumulation of non-structural carbon in association with osmotic adjustment and maintenance (Cheesman, 1988).

Assimilates produced in the source organs of plants are transported through the phloem to the ear and grains. The nature of the solutes and their pathway from the site at which the phloem terminates up to the endosperm where starch storage occurs are of great importance in relation to the regulation of grain dry matter and yield. Sites of resistance to solute movement, the effect of chemical conditions and the effect of starch formation rates are all believed to be possible rate-determinant factors for grain dry matter accumulation.

In order to consider the resistance of the tissues to assimilate movement, the first step is to study the structure of the grains. In cereal grains a crease runs the length of the kernel with a single vascular bundle embedded within the pericarp at the bottom of this furrow. Unloading from this bundle occurs along its entire length. Although there is no reticulation of the phloem in the grain bundle, this narrow unloading region is functionally enlarged by tissues linked by plasmodesmata. It has been concluded that a symplastic continuum connects the phloem to large companion cells and, in turn, to vascular parenchyma, the pigment strand and finally the nucellus (Thorne, 1985).

Since the pericarp bundle is the main channel for nutrient transfer to the endosperm cells any discontinuity which exists can impose restrictions on grain filling. Sakri and Shannon (1987) concluded that although the tracheary elements of the pericarp vascular bundle are not in direct continuity with those of the rachilla, there was no anatomical evidence of discontinuity of the

phloem cells between the bundles of the rachilla and the pericarp. Therefore assimilate flow through the sieve elements into the kernel is not restricted. The assimilates then move circumferentially across the entire grain within the interconnected symplasm of the nucellus. Only then do assimilates enter the apoplast of the embryonic aleurone and endosperm (Oparka and Gates, 1981b).

Good evidence exists for symplastic continuity within maternal tissue leading from phloem to the aleurone but as in other species there is no symplastic connection between the generations. In wheat the phloem is continuous along the furrow from the mother plant (Duffus and Cochrane, 1982), but the xylem is discontinuous at several locations within the inflorescence and furrow of the grain. Between the vascular tissue and the inside of the grain lies the pigment strand, containing the placental-chalazal region and the nucellar projection. Extensive development of transfer cells occurs where the nucellus projects into the endosperm cavity, a gap between the maternal and embryonic cells. In the developing caryopsis, solutes appear to be unloaded from the phloem symplastically through the chalaza and then into the apoplast via the transfer cells in the nucellus. In barley, the xylem is discontinuous at the point of kernel attachment to the rachilla, therefore the phloem is the only continuous vascular connection between the kernel and the rest of the plant (Sarah and Chevalier, 1984). In wheat, the aleurone layer is fused to the testa around the whole surface of the grain except at the furrow, where there is a clearly defined endosperm cavity that runs the whole length of the grain. This cavity contains a clear, rather viscous fluid (about 1 μ l can be expressed from most grains).

On one side the cavity is bounded by the aleurone layer and on the other by the nucellar projection (see also Duffus and Cochrane, 1982). The cells of the aleurone layer bounding the cavity are large and appear to have thinner walls than those sandwiched between the endosperm and the testa (Jenner, 1974).

It is important to know the factors regulating the grain filling. Grain filling is thought to be resolvable into two components, rate and duration; both components are variable and display genetic and environmental influences. Both between and, in many studies, within cultivars, the rate rather than the duration of grain filling has been identified as being the more important determinant of grain size (see Jenner, 1985; and Peat, 1985). Jenner and Rathjen (1978) proposed that differences in the rate of grain growth can be ascribed in two independent physiological bases. The first is variation in a determinant of the endosperm's capacity to accumulate starch. The second determinant relates to a kinetic one, expressed in the rate of accumulation of dry matter per unit quantity (or concentration) of sucrose.

The question whether source or sink is the dominant factor regulating the amount of dry matter accumulation has been extensively debated. There is a line of evidence supporting the idea that the supply of assimilates by the leaves is the factor that controls grain size. The influence of assimilates on the growth rate of kernels has been studied by a number of investigators. In many experiments they have attempted to reduce the assimilate availability to the kernels by shading or defoliation or reducing the number of kernels per

spike. Experiments by Simmons and Crookston (1982) showed that both the growth rate of the kernel and the final weight per kernel were increased by a reduction of the kernel number during the linear phase of dry matter accumulation. There was no consistent effect of the treatments on grain filling duration.

There are indications from a large number of reports that the above mentioned interpretation is not acceptable. It is evident that the sink demand is the crucial factor regulating assimilate movements. In the case of wheat and barley it is known that within the caryopsis there are factors limiting the grain filling processes. Different locations within the grain structure of wheat, mentioned earlier, have been considered as sites of resistance to assimilate transport into the endosperm cells. The external supply of assimilates to the developing grains is thought to be a less important determinant of growth and maturation than are limiting factors within the grain itself. These include the rate of sucrose unloading and the capacity for conversion of sucrose to starch in the endosperm (Thorne, 1985). Experiments by Migus and Hunt (1982) showed that the efficiency of carbon dioxide uptake is usually below its maximum potential due to some sink-mediated mechanisms.

Despite the fact that in intact plants there are significant diurnal fluctuations in the level of sucrose in the leaves and culm, it is reported that there is no change throughout the day or night in the amount of sucrose in the grain. Some of their experiments also showed that removing the flag leaf blade had no effect on the level of sucrose in the grain on the second day of defoliation; and

culturing detached ears on solutions of sucrose had no effect on the sucrose content of the grain. It is therefore evident that the level of sucrose in the grain is not limited by the production of assimilates (Jenner and Rathjen, 1972a). Based on the findings of many authors it is well documented that the processes transporting carbohydrates in the final stages of their pathway into the grain impose a limitation on the accumulation of dry matter in the grain (Jenner, 1973). In relation to the effect of structure, Jenner (1976) suggested that the grain can be regarded as possessing a series of conductors for sucrose, each having some properties of resistance (see also Jenner and Rathjen, 1977). Therefore, in wheat there are two sections of the pathway from the phloem into the endosperm; from the phloem to the endosperm cavity and between the free space and the internal volume of the cells. It was also observed by Jenner (1974b) that symplastic sucrose in the wheat grains is at a much lower concentration than apoplastic sucrose in the cavity and he concluded that the movement of sucrose into the endosperm cells from the free space was entirely diffusional. The observations of Lingle and Chevalier (1984) showed that in barley the rate and duration of starch synthesis are limited by processes within the endosperm and not by sucrose availability.

Experimental investigations have shown that in wheat both sucrose and amino acids are transported in the phloem by the same mechanism along the furrow. Metabolism of amino acids occurs before and/or after transport in the phloem (Donovan, Jenner and Lee, 1983). It may also be inferred from the experiments of Fisher and Gifford (1986) that the movement of assimilates from the sieve tubes and

across the crease tissue are important control points in grain filling. They found that the sucrose concentration in the sieve tubes was almost tenfold that in the endosperm cavity sap.

The characteristics of the assimilates entering the endosperm may to some extent be affected by the barriers between this compartment and the sieve tube. Any change in the structural features of these tissues could lead to alterations in the rates or concentrations of the moving solutes.

The ultrastructure of the tissues of wheat grains and leaves has been investigated. Zee and O'Brien (1970) studied the structure of the pigment strand and found that from about 9 days after anthesis the cell walls thicken and lignify. At the same time an electron-dense layer of adcrusting substance begins to form and accumulates initially near to that part of the cell wall which is not traversed by plasmodesmata. The observations of O'Brien and Carr (1970) on the mestome sheath of wheat and oat leaves also showed a suberized lamella which occurred in all walls of the cells. They also found that in maize the walls of the parenchyma sheath ("starch sheath") contain similar suberized lamellae. Oparka and Gates (1981a) observed that the cells of the pigment strand of rice grains were readily identified by a modified inner wall layer which had a lamellar appearance and was made up of alternating layers of electron-opaque and electron-lucent materials. They found large deposits of granular material, frequently at the corners of the cells. Cochrane (1983) working with varieties of barley and wheat observed that towards the end of the linear phase of grain filling,

i.e. 35 days after anthesis, a layer of suberin is laid down outside the plasma membrane and that at about the same time the primary wall becomes lignified. If it is assumed that these processes are also taking place in the endosperm, then it would appear that assimilates continue to reach the endosperm through the symplasm of the chalazal tissue, i.e. the lignified layer.

The significance of these depositions is whether or not this material prevents assimilate movement into the endosperm. Most authors believe that this deposition is a suberin-like material, which can be considered as a barrier for assimilate transfer through the apoplast. However, views differ in relation to the effect of the deposition on symplastic connections. Zee and O'Brien (1970) suggested that the adcrusting substance is suberized material, containing two classes of substance: lipids that resist dehydration and a polyphenolic material (perhaps lignin) that reacts strongly with silver hexamine. They suggested that cell to cell transfer through the plasmodesmata is stopped by the accumulation of the adcrusting substance, and that there is a stage when symplastic transport across these cells becomes impossible. However, O'Brien and Carr (1970) who studied the mestome sheath of wheat and oat leaves concluded that the suberized lamellae which occurred on the cell walls may be restricting solute transfer between the bundle sheath and mesophyll, and that these lamellae are perforated by plasmodesmata at all pit fields they share with living cells. The cell wall lignification of the chalaza of wheat and barley is reported to provide a means whereby, during the later stages of grain filling, water loss from the endosperm can take place without

interrupting the supply of assimilates to the starchy endosperm cells (Cochrane, 1983). A study on rice by Oparka and Gates (1981a) showed that in wall thickenings the possibilities exist for a symplastic transfer of assimilates from the companion cells to the vascular parenchyma elements. They observed that the suberin-like material forms only on the inner walls of the adjacent pigment strand cells and may thus efficiently isolate the apoplastic compartment as well as preventing a loss of solutes from the symplast to the apoplast. It appeared from their study that all cells of the pigment strand were connected by plasmodesmata which traversed both the cellulose region of the wall and also the modified inner wall layer.

It is generally accepted that in cereals sucrose is the principal form in which carbohydrate is transported from photosynthetic tissues to organs where starch is deposited (Jenner, 1970; Ho and Gifford, 1984). It has been suggested that the onset of the declining phase of starch accumulation is caused by a fall in the synthetic capacity of the endosperm and not by a reduction in the supply of sucrose to the site of starch synthesis (Jenner and Rathjen, 1975). Therefore it is known that the normal development pattern of accumulation of dry matter in the endosperm of wheat grains is determined within the endosperm itself rather than through the provision of precursors from the rest of the plant (Kumar and Singh, 1981). However, there is some evidence indicating that the concentration of sucrose in the endosperm regulates the rate of starch synthesis (Jenner, 1970).

Starch synthesis can be affected by a number of factors which

result in different grain sizes and yields. Conversion of sucrose to starch is known to be affected by temperature. Increasing the temperature in the range above 20°C during the grain filling period reduces single grain weight in temperate cereals. Exposure of the ears alone, even to brief periods at these temperatures, is sufficient to elicit this response in wheat. Of the two major components of dry matter accumulation in the endosperm, starch and protein, starch deposition is the more sensitive to temperature. The respiratory loss of carbohydrates is in part a reason for the grain weight reduction. Wardlaw, Sofield and Cartwright (1980) analysing the movement of ^{14}C -labelled photosynthate from the flag leaf to the ear, showed that carbon lost through respiration is a component of the ear demand for photosynthate. Their data indicate that although there is a faster rate of import of photosynthate by the grain at higher temperature, it appears that this increment was balanced by greater respiratory losses.

The duration of grain filling is shortened at high temperatures, probably as a consequence of accelerated development rather than through any lack of assimilate supply. Bhullar and Jenner (1986) who cultured wheat endosperm in solutions of [^{14}C] sucrose showed that increasing the temperature resulted in two opposing effects in the range above 20°C; accelerated increase in the number of sites (cells and amyloplast) for starch synthesis coupled with a reduction in the (starch) synthetic system. They concluded that, combined with a comparatively low temperature optimum for starch deposition, these responses can explain in part the nature of the response of the rate of grain filling to variation in temperature

(see also Wardlaw, 1970). Reduced starch accumulation in barley as a result of elevated temperature has been reported by Macleod and Duffus (1988) who observed a reduction in the activity of the sucrose cleavage enzyme UDP sucrose synthase. Enhanced initial growth rate of the grains, and also the increase of the N/C ratio by warmth has been shown by Spiertz (1977).

Light is known to be effective on both cell number per endosperm and grain dry weight. Similar effects can be seen from the concentration of nutrients in the external medium. Singh and Jenner (1984) and Jenner (1980) showed that the rate of accumulation of dry matter in developing wheat grains is reduced by shading, and this response is accompanied by a reduction in cell number. It was concluded that the reduced cell division is a result of the reduction in the availability of carbohydrates in the endosperm. Another site of light response of the grains is its outer green layer. A suggestion made by Gifford and Bremner (1981b) is that a major function of the green layer may be the provision of oxygen for starch synthesis. In the light CO_2 generation and O_2 consumption occurs by endosperm respiration.

It is evident that water stress reduces the grain yield in wheat. This is not a direct effect on grain filling and it was shown that although senescence was accelerated and photosynthesis reduced by water stress, the supply of assimilates from the uppermost parts of the plant was always in excess of grain requirements. The stress effect on grain size could well result indirectly from the effect of stress on grain set (Wardlaw, 1971). Jenner (1985) concluded that

the non-reduced intercellular concentration of sucrose implies that under water stress other factors beside shortage of assimilate are involved. It has also been shown that although water stress severely reduced the water content of the leaves, stem, and to a smaller extent that of the ear structure, there was little or no effect on the water content of the grain (Wardlaw, 1971).

It is known that in wheat K^+ and Na^+ have a stimulatory effect on starch synthesis. Rijven and Gifford (1983b) found that the alkali cations, except Li^+ , stimulate conversion of sucrose to insoluble matter, with K^+ -stimulation of starch synthesis being the most effective.

Hydrolysis of sucrose during movement from the phloem to the endosperm and before uptake by the endosperm has been investigated by several authors. It is reported that among some tropical cereals sucrose hydrolysis is an integral part of the import mechanism, but in wheat and barley sucrose is not hydrolyzed during transport from the phloem to the endosperm (Thorne, 1985). That hydrolysis is not a prerequisite for sucrose import has been demonstrated by a number of authors (Jenner, 1974a; Rijven and Gifford, 1983a; Donovan, Jenner and Lee, 1983). The report of Chevalier and Lingle (1983) shows that in barley as well as wheat sucrose is not cleaved as it moves from the phloem to the endosperm (see also Sakri and Shannon, 1975). In maize, it is reported that a high proportion of sucrose entering the grain kernels appears to be hydrolysed; and in sugarcane hydrolysis of sucrose appears to be an obligatory step in the absorption of that sugar by cells (Jenner, 1974a).

As the assimilates are transferred from the phloem tissue, the characteristics of the sap of the cavity may reflect the balance between the assimilate unloaded in the cavity and that taken up by the endosperm tissues in different environmental conditions. It is known that under normal environmental conditions there is a downhill gradient of sucrose concentration from the phloem towards the endosperm. Jenner (1974b) found that a steep gradient in concentration exists between the vascular bundle and the endosperm cavity and another between the free space and the endosperm cells. The concentration of sucrose in the vascular bundle, whole endosperm and the cavity liquid were found to be about 70, 20 and 30 mg ml⁻¹ respectively. Gifford and Bremner (1981a) who studied starch formation in detached grains of wheat observed that the sucrose concentration in the medium giving the highest rate of starch synthesis, corresponded closely with a sucrose concentration of about 200 mM ascertained in the endosperm cavity. Ho and Gifford (1984) who collected sap from the grain cavity by centrifugation, found that the cavity sap contained 10-50 mM sucrose, a small amount of hexose but a high concentration of oligosaccharides (up to 9 times that of sucrose). In comparing two cultivars of wheat they found that a high growth rate was associated with high cavity sap sucrose concentration. They also found that the sucrose concentrations in the cavity sap of the two cultivars were identical.

The mechanism by which sucrose passes across the plasma membrane in wheat grain has been studied by some authors. Ho and Gifford (1984) observed that PCMBs reduced the linear rate of uptake

of sucrose 5-fold and the total uptake by 66% in 6 hours. They found support for the idea of Rijven and Gifford (1983a) that the transfer of sucrose across the plasma membrane may not be entirely diffusional. Therefore they deduced that the mechanism of sucrose uptake by endosperm cells may be better described as a "facilitated diffusion" (involving a saturable carrier), as opposed to active transport against a concentration gradient.

pH is another factor that is known to affect sucrose uptake and/or conversion. Rijven and Gifford (1983a) found that the uptake and conversion of sucrose were stimulated by lowering the pH, and by fusicoccin, a promoter of proton extrusion. The investigation of Jenner (1974) on isolated grains showed some increase in uptake of sucrose and formation of starch by lowering the pH.

The characteristics of wheat grain filling and some related processes within the plant have been reviewed here. Wheat appears to have a potential for an increased yield production, which may be restricted by some intrinsic and/or environmental limitations. An understanding of the dominant factors regulating this potential will provide information which could help plant breeders in the production of new cultivars with a higher yield capacity, particularly under conditions of environmental stress. In order to have a better understanding of the physiological factors which limit grain yield under both normal and salt stress conditions, the effect of salinity on grain filling in this crop has been investigated in the current study. The transport of solutes, such as sugars within the grain and the response of the plants to salt stress in terms of photosynthesis

and photoassimilate partitioning have been determined and the results of these studies presented here.

2. MATERIALS AND METHODS

2.1 Plant materials

The study was conducted on three cultivars of wheat (*Triticum aestivum* L.). Cultivar Hobbit, a British winter variety, was used in the preliminary morphological, anatomical and photosynthetic studies. The other two cultivars were Iranian spring varieties, Inia 66 and Sholeh, known respectively to be sensitive and resistant to salinity. Inia 66 was used in all other experiments and Sholeh only in the studies on photosynthesis and assimilate partitioning.

2.2 Growing conditions

Seeds were germinated on moist towel paper in 2.5 cm diameter glass tubes enclosed by a black plastic film. Five days after soaking the seedlings were transferred to 15 cm plastic pots (2 plants per pot) with a plastic saucer underneath. The pots were filled with standard compost (Appendix I). The plants were maintained in a glasshouse under specific conditions which were 16 hours daylight, using high pressure sodium lamps. Day temperature was 21°C and night 17°C. Fertilizer was applied once a week, starting three weeks after germination, through watering as 0.5 g l⁻¹ from an equal mixture of Libfeed 311 and Libfeed 214 (Appendix II). As required, some weeks after germination the treated plants were subjected to salinity. This was for three weeks with the preliminary experiments, two weeks with the experiments on photosynthesis and assimilate partitioning and thirty days with the plants used for

grain filling and E.M. studies. The plants were subjected to salinity gradually, starting with 1 g l^{-1} NaCl in water and increased by 1 g l^{-1} over 4 day intervals up to 5 g l^{-1} with the preliminary experiments. With the plants for photosynthesis and assimilate partitioning experiments, in addition to the control (ordinary water), there were only two steps of salinization, i.e. 2.5 g l^{-1} and 5 g l^{-1} applied gradually over 4 day intervals and for grain filling and E.M. studies there was only one salt treatment of 4 g l^{-1} in addition to the control.

The preliminary experiment was conducted in a completely randomized block design with 6 treatments (0 to 5 g l^{-1} NaCl) and 5 replicates. The plants did not elongate since the temperature requirements of vernalization for the variety employed were not met. The experiments for studying photosynthesis, growth parameters and assimilate partitioning were carried out with a completely randomized block design and factorial treatments. Two levels of salinity, 2.5 g l^{-1} and 5 g l^{-1} NaCl and ordinary tap water (control) were used combined with two cultivars of the plants (Inia 66 and Sholeh).

2.3 Determination of photosynthetic parameters

Thirty-five days after sowing the net photosynthetic rates and the stomatal conductance were measured using an LCA-2 infra red carbon dioxide analyser (ADC). Parts of the flag leaves between 7 to 12.6 cm from the tip were used for these measurements, and the readings recorded 30 seconds after inserting the leaves into the chamber. The data for both photosynthesis and stomatal conductance

used for statistical analysis were the average of 4 readings. Transpiration rates were calculated using the formula of Long and Hallgren (1985) from the data obtained from the leaf chamber analyser.

2.4 Determination of growth parameters

Harvesting for growth analysis was carried out 7 and 23 days after salinization. Harvested plants were divided into roots and shoots, and leaf areas (leaf blades) measured with a Delta devices area meter. Roots were washed and, together with shoots, dried in an oven at 60°C for 48 hours. The dried samples were then weighed and from the values obtained growth parameters, including relative growth rate, net assimilation rate (on the basis of the 16 day period) and root/shoot ratio, were calculated (Harper, 1983). The values obtained were then statistically analysed. Leaf length and tiller number of the plants were also recorded.

2.5 $^{14}\text{C}\text{O}_2$ incorporation

To study the assimilate distribution pattern within the plant, $^{14}\text{C}\text{O}_2$ application was employed in a factorial experiment. Fourteen to 16 days after anthesis 3 plants from each plot were labelled by feeding the flag leaf with $^{14}\text{C}\text{O}_2$ released from 2.5 μl of sodium bicarbonate containing 5 μCi of ^{14}C dried on a paper disc. Individual flag leaves were enclosed in plastic bags containing radioactive paper disc to which a few drops of 15% lactic acid were applied to release $^{14}\text{C}\text{O}_2$. Plants were then harvested 1, 6 and 24

hours after feeding and divided into flag leaves (fed organ), ears, other leaves, tillers and roots. Harvested materials were washed, dried in an oven at 60°C and then weighed and finely ground. When necessary samples were made up to 300 mg by adding ordinary wheat flour. They were then combusted in an OX-400 oxidizer, setting the combustion zone at 900°C, catalyst zone at 680°C and assay time on 4 minutes. Trapped $^{14}\text{CO}_2$ from each sample was counted on a 1211 Rackbeta liquid scintillation counter (LKB Wallac). The scintillation cocktail used was 46% NE233, 27% phenylethylamine and 27% industrial methylated spirit. The values given by the counter were corrected for concentration and analysed statistically.

2.6 Light microscopy

Pieces of leaf from 1.0 to 1.5 cm above the ligules were taken and placed immediately into vials of FAA (formalin, 50% glacial acetic acid and alcohol, 5:5:90 v/v). The technique of Johansen (1940) was used for preparation of the samples. After fixation the samples were passed through two series of acetone solutions for dehydration, and infiltration followed by embedding, sectioning and staining with crystal violet and erythrosin. They were then mounted in D.P.X. and examined under a microscope.

2.7 Electron microscopy

To study the ultrastructure of aleurone cells grain samples were used from the plants of Inia 66 grown under both normal and saline conditions (subjected to 4 g l^{-1} NaCl for more than 3 weeks).

Samples were taken at the same stage of grain growth as the cavity sap was collected, i.e. 12-16 days after anthesis (Section 3.4). Grains were sliced transversely into 2 mm sections. The sections were fixed overnight in 2.5% gluteraldehyde in 0.1 M cacodylate buffer, pH 7. After washing in CAB, they were post-fixed for 1 h with 1% OsO_4 in the same buffer. They were then passed through an acetone dehydration gradient and embedded in epon-araldite resin. Silver-gold sections were cut on a Reichert Ultracut-E-ultramicrotome, some of them were stained in toluidine blue for light microscopy and some stained in 5% uranyl acetate in 1% acetic acid for 1 h at room temperature and Reynold's lead citrate for 10 min. They were then examined on an Hitachi H-700 TEM.

Other sections from the same sample were cut to investigate the nature of the wall thickenings. The staining solution was silver hexamine (45 ml hexamine 3%, 5 ml silver nitrate, 5 ml 0.05 M disodium tetraborate). The sections were stained in this solution at 60°C in the dark for 30 min. After washing in distilled water they were passed through 3% sodium thiosulphate for 5 min and then two changes of distilled water.

A scanning electron microscopy study was conducted on leaves of the plants. Fifty seven days after sowing the seeds, leaf samples from the plants were taken, using pieces of flag leaves, 0.5-1.0 cm from the ligules. The samples were fixed in 2.5% gluteraldehyde in 0.05 M phosphate buffer pH 7. The fixative was then replaced by 0.05 M phosphate pH 7. After 20 minutes the samples were passed through a series of different concentrations of ethanol followed by drying in

an Emscope CPD 750 critical point drier. They were then glued on stubs and, after being cut with a sharp razor, coated with gold for 4 minutes using a sputter coater. They were then examined on an Hitachi S430 SEM fitted with a Hexland cryo stage.

2.8 Collection of grain fluids

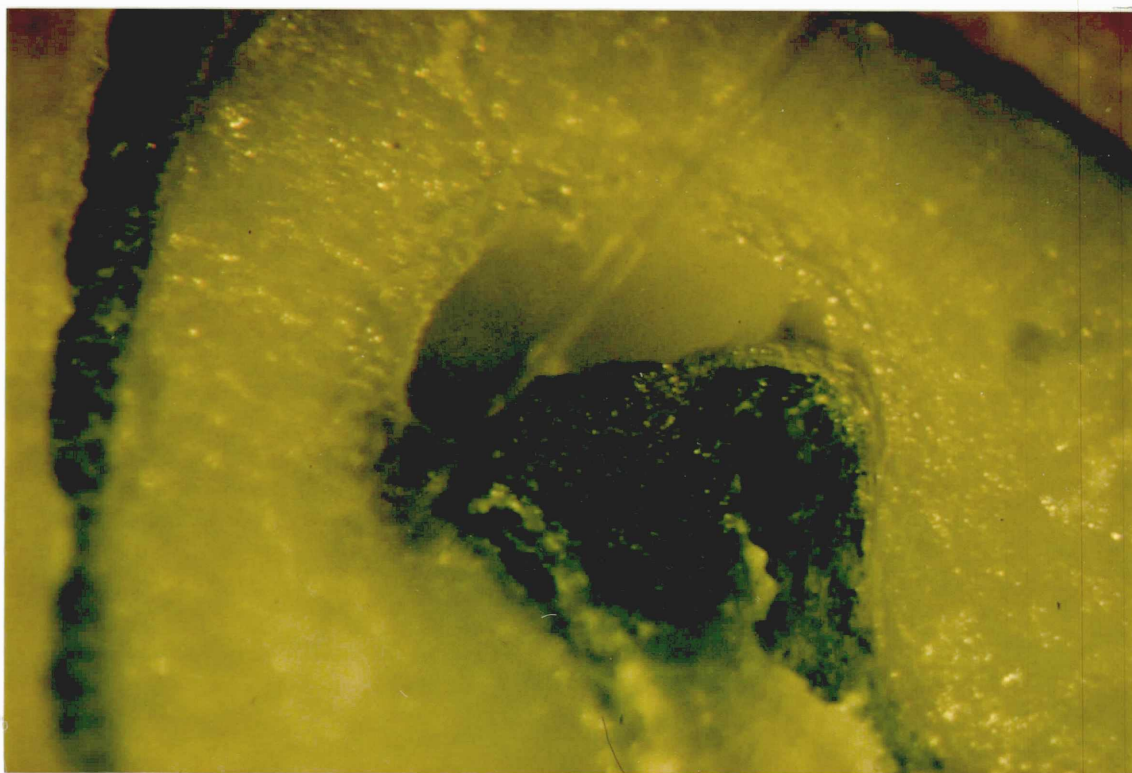
Capillary tube technique

This particular technique proved very difficult and required extensive development. During the period of investigation twenty different populations were grown and tested to refine this technique so that reliable and reproducible results could be obtained (see Appendix V).

The plants of Inia 66 grown in control and saline (4 g l^{-1} NaCl) conditions were used for fluid collection. About 14 days after anthesis, when the endosperm cavities of the grains reached their full size, the grains of the first and second florets from the lower half of the spikelets of the ear (except the first two spikelets) were cut for sap collection while still attached to the rachilla and the plant. Before the grains were cut, the glume and palea were removed with forceps, and the grains were cut in half transversely, using a sharp razor blade (Fig. 2.1a). The upper half of the grain was removed immediately and while the ear was held upward, the cut face of the attached grain observed under a dissecting microscope. The sap of the endosperm cavity, which is quite clear, was collected in a glass capillary tube (Fig. 2.1b). Any contamination of the cavity sap by the endosperm fluid could be observed under the microscope as a dark fluid. To reduce possible contamination, the



(a)



(b)

Fig. 2.1 Transverse cut wheat grain from a control plant, 14 days after anthesis, showing the large endosperm cavity containing a clear sap (a) and a glass capillary tube inserted for sap collection from the endosperm cavity (b).

upper layer of the cavity fluid was removed and discarded. The glass capillary tubes were weighed before and after sap collection on a Sartorius 4503 MP6 electronic microbalance. The tubes were then placed into 4 ml plastic vials containing a known volume of de-ionised water. The collected sap was released into the water by crushing the tubes with a glass rod and this diluted sap analysed for K^+ , Na^+ and sugars.

Paper technique

In order to correct for any contribution by ions released from the glass capillaries a filter paper technique was used for sap collection. Whatman filter paper pieces of about 45 mm^2 with a narrow tip were used to collect sap through absorption. The narrow tips of the preweighed papers were inserted into the cavity for 15 seconds. After being reweighed, they were cut into a vial of de-ionised water. The time between collection of sap and cutting the papers into the vials was kept constant for all collections to avoid creating any differences due to evaporation. The vials were then stirred on a rota-mixer for 10 seconds.

Endosperm fluid collection

After the attached grains were cut, an area in the middle of the endosperm tissues was crushed with a capillary tube. This crushed material was taken as endosperm fluid and collected for analysis for K^+ , Na^+ and sucrose by the same method as the cavity sap.

2.9 Analysis of the fluids

Determination of K^+ and Na^+

A proportion of the fluids collected from the cavity and endosperm containing at least 1000 μg sap in 1-1.5 ml water was used to determine the amount of K^+ and Na^+ present in the fluids. A Corning 410 flame photometer was used for ion measurements. Standard solutions were made for both ions using Corning stock solutions in the range 0.5-4.0 ppm.

Determination of sugars

A gas liquid chromatographic technique was employed to determine the sugar content of samples (Pye Unicam PV4500 chromatograph) (Davison and Young 1969, cited in Chaudhry 1983). Two 5 feet long glass columns of 3 mm internal bore filled with 5% OV17 on 100-120 mesh chromosorb were connected to a hydrogen flame ionization detector. The carrier gas was oxygen free nitrogen, running through the column at 50 ml min^{-1} . Hydrogen flow rate of 50 ml min^{-1} and air 500 ml min^{-1} was used. A temperature programme of 180°C-310°C was employed, at 6°C min^{-1} . The diluted cavity and endosperm fluids were dried in an oven at 60°C, dissolved in pyridine and derivatized with hexamethyldisilane (H.M.D.S.) and trimethylchlorosilane (T.M.C.S.) (7.2.1 v/v). The sugars are converted to volatile derivatives by this procedure, which converted all free hydroxyl groups to trimethyl silyl ethers.

Inositol (30 $\mu\text{g ml}^{-1}$ dissolved in pyridine) was used as an internal standard, since it is known to be absent from the plant

samples. Analar sucrose was used to make a range of standard solutions ($5\text{ }\mu\text{g ml}^{-1}$ - $50\text{ }\mu\text{g ml}^{-1}$). The sucrose/inositol weight ratio was plotted against the ratio of the sucrose/inositol peak area to provide a calibration (Appendix III). The column was calibrated for D-glucose, D-fructose and sucrose. One μl of solution was injected. The retention times of the various sugars are given in Appendix IV.

pH measurement

Using BDH buffer tablets, a series of buffer solutions with a range of pH values (5.0, 5.2, 5.4 up to 8.0) were prepared to obtain narrow range pH papers. A drop of the collected fluid was applied to the pH paper and examined under a dissecting microscope for pH estimation. Alternatively, the narrow tip of a pH paper was inserted into the fluids and then examined for colour change under a dissecting microscope.

2.10 Grain weight measurement

The grains from the 1st and 2nd floret of the 3rd and 4th spikelets on the ears of control and salt treated plants were collected 30 days after anthesis. Ten grains were weighed, dried in an oven at 60°C for 24 hours and reweighed.

3. RESULTS AND DISCUSSION

3.1 Salinity : effect on source leaves

3.1.1 Photosynthesis

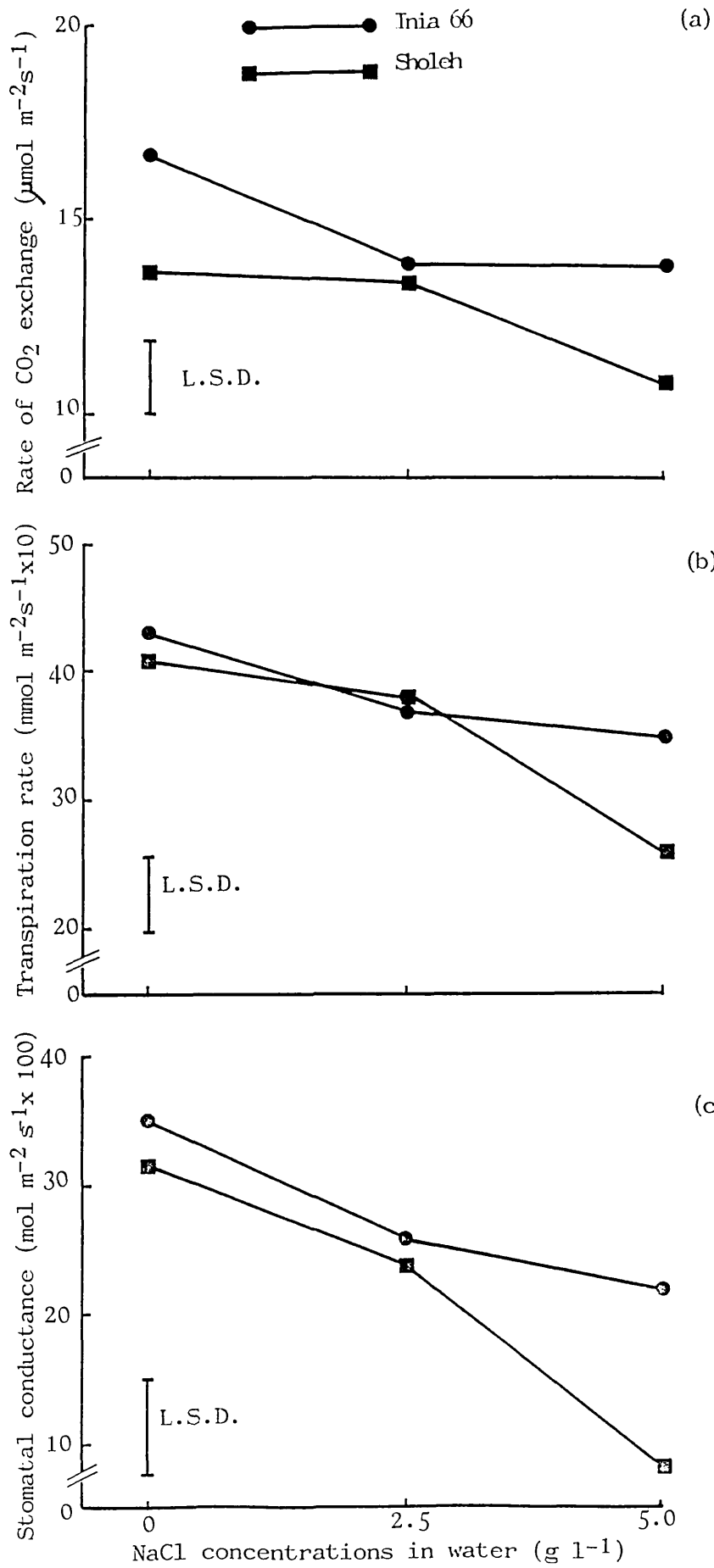
Salt treatments had some effects on the rate of leaf photosynthesis (Fig. 3.1a). At the control level (S1) there was a significant difference between the rates of photosynthesis in the two cultivars employed. Inia 66 took up CO_2 at higher rates than Sholeh, thus showing a higher potential for dry matter production. This supports the view that there are varietal differences in wheat with regard to leaf photosynthesis.

Photosynthesis was reduced under salt stress and Inia 66 responded earlier than Sholeh with increasing salinity. From the agronomic point of view Sholeh, resisting mild salinity (up to 2.5 g l^{-1}) has only a small advantage over Inia 66 in this respect, but with regard to the physiological mechanism there may be a potential for further plant improvement.

The observations here on the effect of salinity on photosynthesis do not agree with those of Robinson *et al.* (1983) who found that in spinach, salt stress did not result in any major decrease in the photosynthetic potential of the leaf. In general, any reduction in photosynthesis may be the result of reduced growth rate as proposed by Greenway and Munns (1980). The observations here are, however, consistent with those of Pitman (1988) who reported that increased salinity is accompanied by a decrease in both net

Fig. 3.1 Effects of salinity on (a) photosynthesis, (b) transpiration and (c) stomatal conductance in two cultivars of wheat.

Each point is the mean of three replicates with standard errors of 0.858 for (a), 3×10^{-4} for (b) and 3.5×10^{-2} for (c).



carbon assimilation and leaf area. The decreased CO_2 uptake observed here may reflect the negative correlation between growth and photosynthetic rates, as observed when the growth responses of the two cultivars are compared, showing higher rates with Sholeh (Fig. 3.5). This is observed not only with plants under stress but more generally, as during the evolution of wheat a parallel increase in leaf size has been coupled with a progressive reduction in the rate of photosynthesis (Evans and Dunstone, 1970).

A decreased leaf photosynthetic rate in salt stressed plants may be attributed to excess Na^+ , or both Na^+ and K^+ , affecting the cell metabolism. Increased vacuolar ion concentrations are accompanied by increased cytoplasmic solute concentrations which can inhibit photosynthesis and other cytoplasmic processes (Pitman, 1988; see also Passera and Albuzio 1979, for the effect of salinity on some enzyme activities in wheat).

3.1.2 Transpiration

The rate of transpiration was reduced by salinity. This reduction was significant with Sholeh at S3 (Fig. 3.1b). This observed reduction in the transpiration rate at higher levels of salt stress is consistent with the 30% reduction in transpiration rate reported for barley under 100 mM salinity (see Munns, 1985). The transpiration of barley seedlings was also reduced at 60 mM external concentration, while it was unaffected by concentrations up to 40 mM (Pitman, 1975). It is known that transpiration can affect the rate of ion uptake by plants and thus the ratios of ions taken up by the plants may be changed. At high external concentrations Na^+ uptake is

increased at the expense of that of K^+ when transpiration is occurring (Pitman, 1988). Therefore the reduction in transpiration rate of plants under saline conditions observed here with Sholeh, may be a mechanism by which excess Na^+ is avoided, thus making these plants relatively tolerant to salinity.

3.1.3 Resistance to CO_2 uptake

Stomatal resistance, which is one of the components restricting CO_2 exchange between the atmosphere and the mesophyll cells is increased with higher levels of salt stress. The stomatal responses of the two cultivars here are very similar to their net photosynthetic responses (Fig. 3.1c) possibly indicating that the reduction in leaf photosynthesis is caused by an increased stomatal resistance to the flux of CO_2 . It has been reported that salinity can have an effect on both stomatal frequency and size in barley (Gill and Dutt, 1982). Also disturbed levels of K^+ may affect stomatal behaviour, as the K^+/Na^+ ratio of the leaf cells may decrease under salt stress, if we can generalize from the results of the study on grain cavity sap presented here (Section 3.4).

The effect of deficiencies of certain elements such as K^+ on stomatal closure is well documented (Beadle *et al.*, 1985). Reduced stomatal opening can lead to a lower rate of CO_2 exchange, and in turn to reduced leaf photosynthetic rates. A parallel reduction in transpiration rate may reflect the relationship between reduced photosynthesis and stomatal resistance observed here. With regard to salinity, other investigators have reported similar findings (Gale, 1975; Kemp and Cunningham, 1981; Yeo, 1983; Beadle *et al.*, 1985).

There is a similarity between salt stress and water stress with regard to photosynthetic and stomatal responses, as the initial reduction in photosynthesis due to moisture stress arises from changes in the conductance of CO_2 through the stomata (Begg and Turner, 1976).

The effect of salinity on leaf structure is illustrated in Fig. 3.2. The shape and arrangements of the mesophyll cells are modified under salt stress when compared with those from the control plants. In salinized plants, these cells are deformed and more compact giving a reduced intercellular air space and decreased leaf thickness. The epidermal cells are also deformed and thicker under salt stress, giving a greater number per unit leaf area. Salinity thus may cause a reduction in leaf expansion compared with normal conditions. In the vascular bundles there is a marked increase in the number of phloem cells affected by salinity. The cells of the bundle sheath are also very small in treated plants when compared with those of control ones.

SEM photographs (Figs. 3.3 and 3.4) illustrate that salt stress has caused considerable thickening on the walls of leaf mesophyll cells. Less expanded cells and in turn less succulence of the leaves are also observed in the salinised plants when compared with those grown under normal conditions.

The observed reductions in photosynthetic rate may be partially related to these anatomical changes, depending on their limiting effects on the rate of CO_2 influx. It may be suggested that



(a)



(b)

Fig. 3.2 Transverse sections from the leaves of wheat plants (cv. Hobbit) under (a) control and (b) salinized conditions. (x 270)

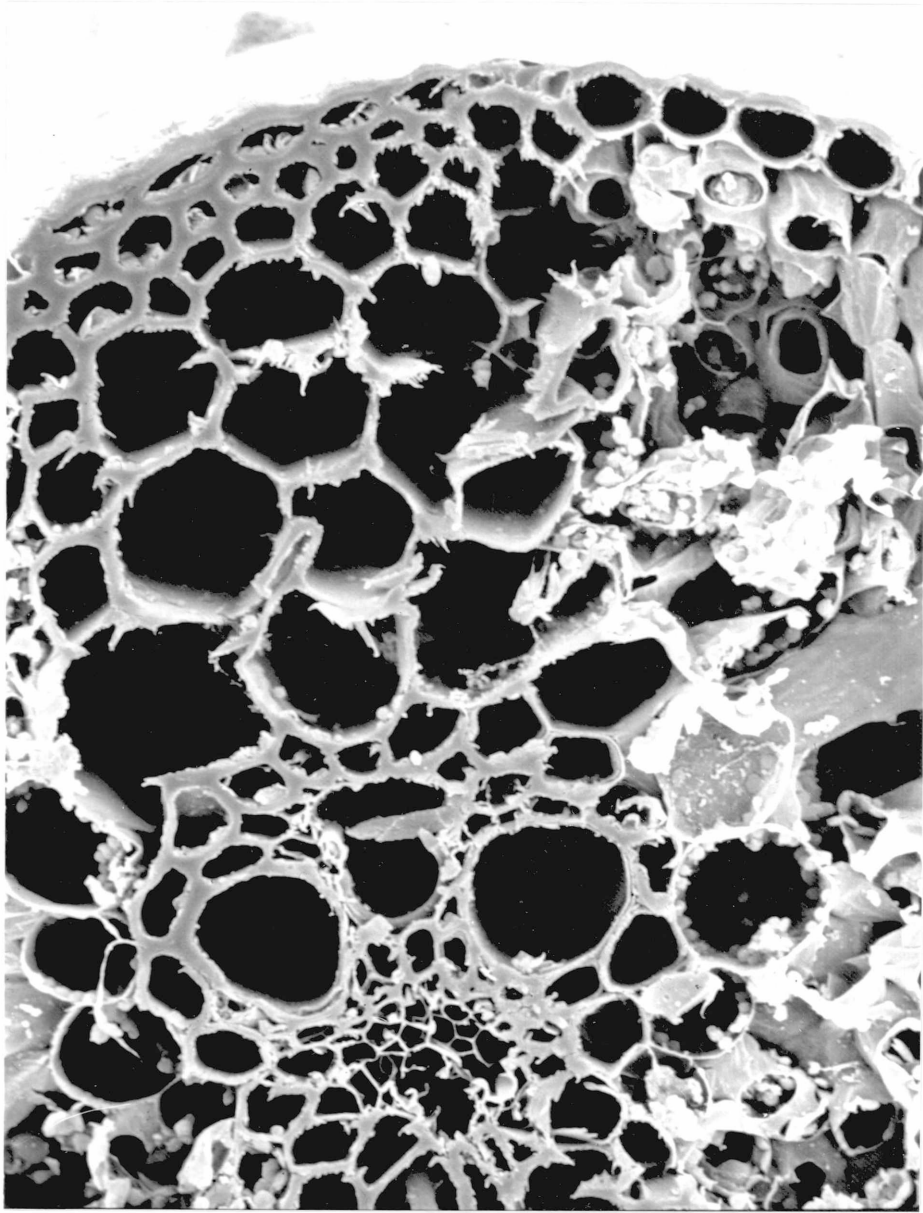


Fig. 3.3 SEM of wheat leaf (cv. Inia 66) in normal conditions (x 575)

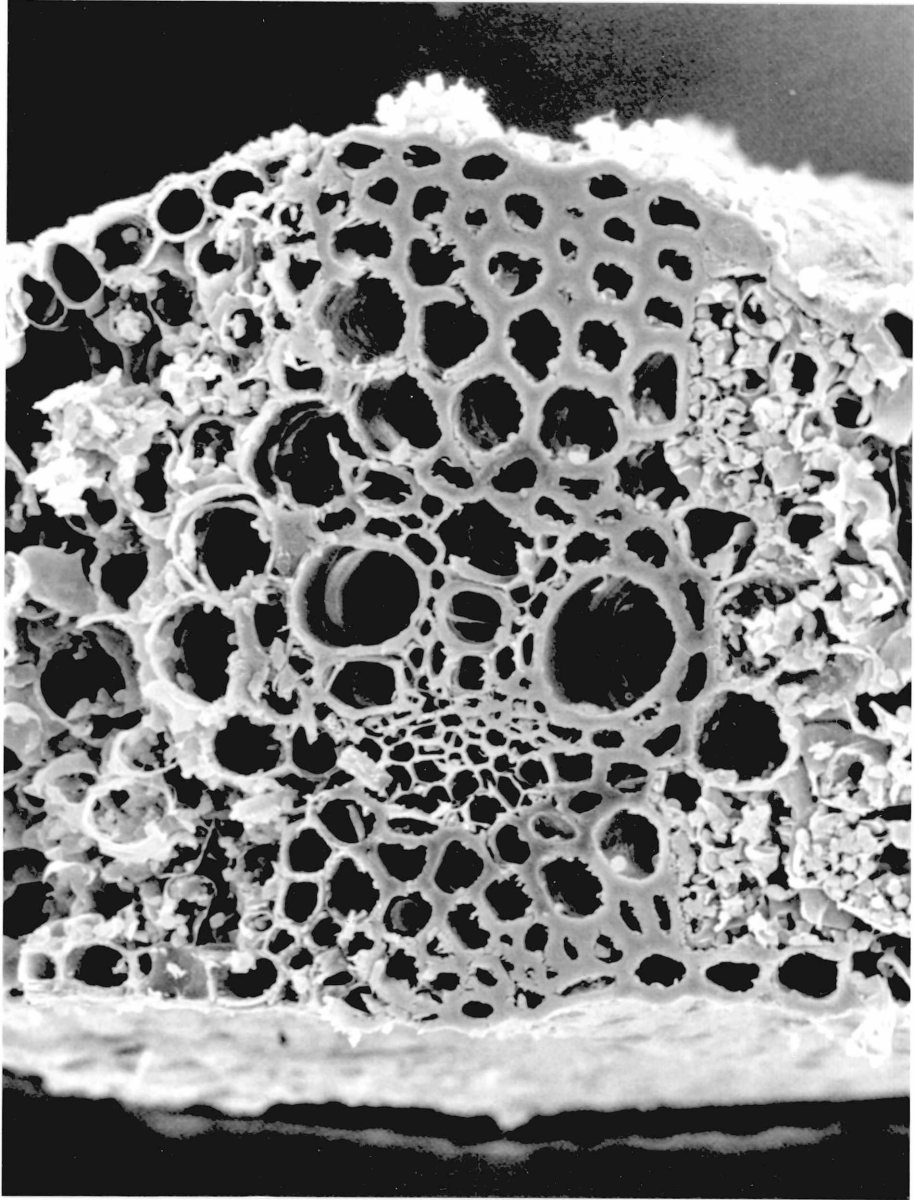


Fig. 3.4 SEM of wheat leaf (cv. Inia 66) under salt stress conditions. (x 575)

the cell wall thickening observed here can increase the mesophyll resistance to CO_2 exchange. This is consistent with the report that the liquid phase of diffusive conductance could be reduced with increased cell wall lignification induced by salt (see Bongi and Loreto, 1989).

The decreased intercellular air spaces caused by salt stress may lead to a reduction in the area of cell surface that can receive the CO_2 gas, although it can be compensated for by decreased cell size and an increased surface/volume ratio (Evans and Dunstone, 1970; Beadle *et al.*, 1985). A reduced cell surface exposed to gas exchange and a further reduction due to the development of an intercellular liquid film, are thought to cause an increased resistance to gas diffusion in olive leaves under salt stress (Bongi and Loreto, 1989).

The reduction in cell size and succulence reported here are consistent with the observations that leaf succulence in wheat was decreased by salinity (Passera and Albuzio, 1979), and that succulence was not observed in wheat leaves with salinity (Chonan, 1972).

The results from preliminary experiments here showed significant reductions in two morphological aspects of the salinised plants, i.e. tiller number and leaf length. These reductions, especially in tiller number, had two distinct responses to increased salt concentrations (over five steps from zero to 5 g l^{-1} salt) with a transient horizontal state of the curve between $2\text{--}3 \text{ g l}^{-1}$. In Fig. 3.1 and Fig. 3.5 changes can be observed in the responses at a salt

concentration of 2.5 g l^{-1} . Pitman (1975) also showed that salt concentrations up to 40 mM had no effect on the transpiration rate of barley seedlings. It may therefore be speculated that the problems which plants face when subjected to low levels of salinity are different from those experienced at higher concentrations. Similar speculations have been made by Woolhouse (1983) with regard to the response of mesophyll and stomatal resistances of plants under water stress. He proposed that these two responses of the plant can appear at different degrees of stress. It may be that water stress and ion toxicity, perhaps the two major components of salt stress, are responsible for the distinctly different plant performances at different salt concentrations.

Assuming the above suggestion is correct, the question as to whether water deficit or ion toxicity is the major problem of either low or higher salinity is yet to be answered. The concept that each of these two parameters of salt stress exerts its individual effect at certain salt concentrations appears to be sustainable, but which one is the most effective at low salinity is open to debate. On the one hand it is believed that, in most instances, growth inhibition in salt-sensitive species even at low salinity levels is caused by ion toxicity (Marschner, 1986), while on the other NaCl up to 87 mM had no effect on the photosynthesis of cowpea, provided that the osmotic potential of the media was equal to that of the leaves (Plaut, Grieve and Federman, 1989). If the assumption of Shannon (1984) that under saline conditions root growth stimulation is due to the osmotic effect of salinity is correct, then probably the increased root/shoot ratio observed here with salinity in the cultivar Sholeh (Fig. 3.5c,

see also section 3.2) can be taken to indicate that water deficit is the primary problem of plants subjected to increasing salinity. Cultivar Inia 66 could also be affected by the same problem as it does not show any decrease in the root/shoot ratio at low salt concentrations.

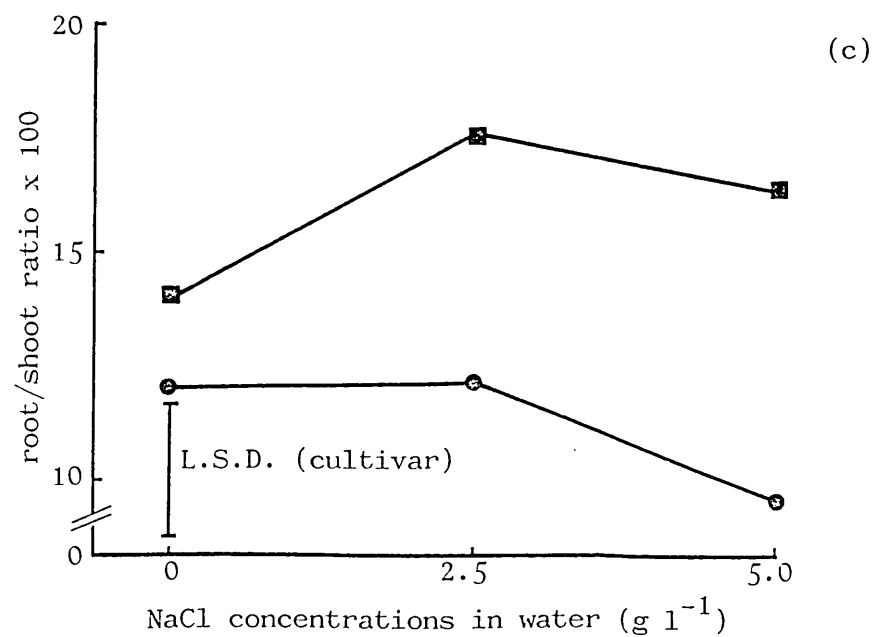
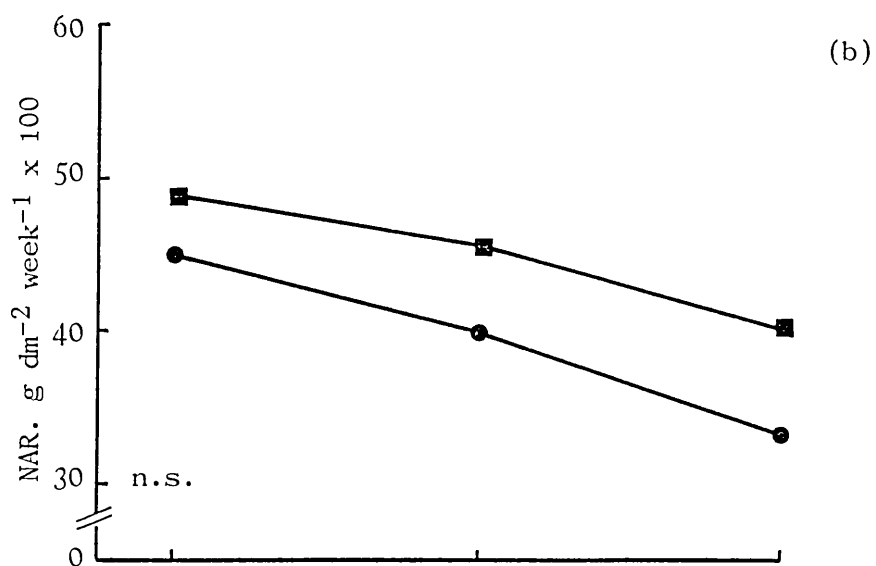
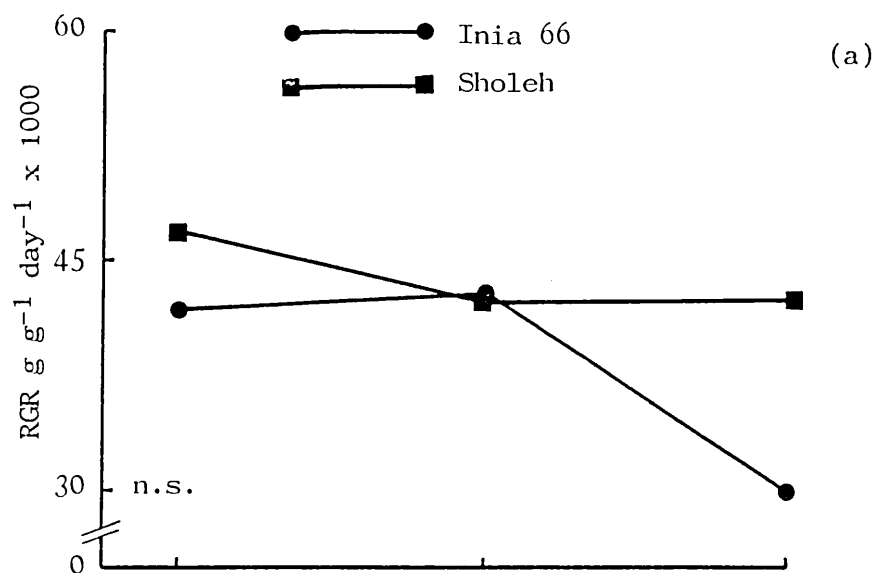
3.1.4 Growth parameters

The effects of salinity on some growth parameters are **with raw data in Appendix VI** illustrated in Fig. 3.5. With regard to the relative growth rate (RGR) and net assimilation rate (NAR) of the plants, there are no significant differences between the two cultivars employed, while a marked difference was observed between them in their photosynthetic rates (see Fig. 3.1a). Moreover, under normal conditions, Inia 66 has a higher photosynthetic rate than Sholeh, whereas the RGR of Sholeh is higher. Therefore, it appears that the growth rate of plants in normal conditions is not directly controlled by photosynthesis but that other factors play a major role.

On the basis of these results that there is no significant difference between the responses of RGR and NAR of these two cultivars to salinity levels, it may be suggested that there is not any link between RGR (or NAR) and photosynthesis under these experimental conditions, i.e. reduction in photosynthesis may not influence or be influenced by growth rates. However, as illustrated by the data of Fig. 3.5 (a and b) RGRs of both cultivars show a tendency to decrease with salinity. This is consistent with the report that at the early tillering stages of barley growth, the RGRs of NaCl (150 m-equiv/l) treated plants fell more quickly with time

Fig. 3.5 Effect of salinity on (a) RGR, (b) NAR and (c) root/shoot ratio of two cultivars of wheat

Each point is the mean of three replicates with standard errors of 4.9×10^{-3} ($\times 1000$) for (a), 4.8×10^{-2} ($\times 100$) for (b) and 1.75×100 for (c).



than those of the control (Greenway, 1962). Although this reduction in RGR is not significant, it reflects an inverse pattern to that of photosynthesis (see Fig. 3.1a) and may be considered as an alternative response to salt stress. For example, with Inia 66 when photosynthesis was reduced at (S2) the RGR remained the same, and at (S3) the situation was reversed. One possible suggestion here is that the reduction in photosynthesis is not directly responsible for the decreased rates of growth observed. To suggest any alternative requires further investigation, since it would imply that there is not any causal relationship between these two and that the nature of the changes is unrelated.

There is a consistency between the observed pattern of changes here and those changes which have been observed in some halophytes at high salt concentrations. For instance, in *Tamarix ramosissima* a decreased growth rate was reported in 200 mM NaCl, while net photosynthetic rates were increased (Winter, 1979).

Non-significant changes in NAR with increased salt concentration may reflect that the dark respiration is almost the same with increasing salinity. If this is the case, then the NAR response of the plants may be expected to follow the photosynthetic changes with salinity. The dissimilarity between these two responses may indicate that the expected reduction in NAR under salt stress has been compensated for by the reduced rates of dark respiration due to lowered growth rates. This, if it happens, involves decreased rates of the growth component of respiration, without marked increases in the maintenance component (McCree, 1986). More investigations are

needed to resolve this issue.

If this suggestion, that photosynthesis is not responsible for the reduced growth rate holds true, it may be proposed that any decline in the growth of the plants caused by salinity can be attributed to a diversion of assimilates from growth, or a non-utilization of the carbohydrates produced. This may include higher rates of respiratory costs of maintenance under salt stress (Schwarz and Gale, 1983; McCree, 1986). Alternatively, accumulation of sugars within the leaves might result in reduced growth rates. This latter is in agreement with the observations of Munns and Termaat (1986) that, in barley, salinity did not reduce the total concentration of reserve carbohydrates. Munns *et al.* (1982) also reached the conclusion that photosynthesis was not limiting growth of barley. This idea was also supported by Munns and Weir (1981), cited in Yeo (1983) who proposed that the reduction of growth with salinity is due to competition with the vacuolar adjustment for carbohydrates, as in *Triticum* sugars were 70-100% of the osmotic adjustment at high irradiance. If this conclusion is correct, it may be suggested that the reduction in photosynthesis may be a feedback effect of reduced growth and carbohydrate accumulation. This is consistent with a number of reports that increases in carbon utilization may increase the rate of fixation (Cheeseman, 1988).

Root/shoot ratios of the two cultivars were not markedly different in normal conditions, although Sholeh had a higher ratio (Fig. 3.5c). With increasing salinity the root/shoot ratio showed a tendency to increase in Sholeh and decrease in Inia 66. The

responses of roots and shoots of these plants are given separately in Table 3.1, where it can be seen that the increased root/shoot ratio of Sholeh with salinity is due to the higher growth rate of its roots under salt stress, compared with that of Inia 66.

It is known that poor root growth can reduce the ability of plants to extract water from the soil (Woolhouse, 1983). It is also proposed that salinity increases the root to shoot ratio as a response to the osmotic effect (Shannon, 1984). Assuming that the problem of salt stressed plants at low salt concentrations is one of reduced water supply, the increased root/shoot ratio (through reduced shoot growth) with Sholeh could benefit the plant, and may account for its tolerance to salinity. Conversely, in Inia 66 the root growth rate under salt stress may not be sufficient to meet the water demands of the shoot with its relatively higher growth rates in this cultivar. This type of change in growth pattern is also reported for barley seedlings where the shoot dry weight decreased as the NaCl concentration in the external solution was increased from 0 to 180 mM (Delane *et al.*, 1982). A similar response was observed in wheat by Cook and Evans (1976) where in water stressed plants the shoot growth was restricted and the root growth enhanced (see also Wyn Jones *et al.*, 1984 for similar responses in some varieties of wheat and Seemann and Crithley, 1985 for the effects on *Phaseolus vulgaris*).

Although the above interpretations are based on water deficits, i.e. physiological drought, the differential growth responses of roots and shoots may be attributed to the different

Table 3.1: Effect of salinity on the root/shoot ratio in two cultivars of wheat after 16 days of salinization.

		Increased dry weight in different levels of NaCl (g l ⁻¹ water)				
		Control	2.5		5.0	
		Weight (g)	Weight (g)	Control %	Weight (g)	Control %
Inia 66	Roots	0.58	0.51	88	0.26	45
	Shoots	4.73	4.15	88	2.71	57
Sholch	Roots	1.05	0.91	87	0.75	71
	Shoots	7.27	4.88	67	4.54	62

ionic contents of these organs, in that changes in the K^+/Na^+ ratio within the shoots may have been causal in their differential growth responses. It is reported that with increasing external ($K^+ + Na^+$) concentrations, the ratio of K^+/Na^+ in the roots was much less affected than that in the shoots. In a comparison between wheat and barley grown under coastal conditions, it was also observed that much more Na^+ was transported to aerial parts in wheat than in barley, thereby disturbing the K^+/Na^+ ratio more in wheat, resulting in a greater injury to this plant than to barley (Gill and Dutt, 1987).

3.2 Salinity : effect on assimilate partitioning

3.2.1 Partitioning in normal conditions

The amount of ^{14}C allocated to the ears, and the fraction of ^{14}C present in flag (fed) leaves is illustrated in Fig. 3.6 and Fig. 3.7, respectively. Only a small amount of ^{14}C has travelled from the flag leaves to the ears during the first hour after $^{14}\text{CO}_2$ application. This portion increased sharply during the next 6 and 24 hours, particularly in control plants. However, this pattern is quite different to that of carbon fixation and location in fed leaves, which mostly appears to have taken place rapidly after feeding, while the fixed carbon travels to other parts of the plants rather slowly. There is an indication here that the plants have no problem in terms of the fixed carbon present within the leaves, even under salt stress. This feature and its interaction with salinity on carbon transport and distribution will be discussed later.

The appearance of tracer in the ears shows that it has travelled a distance (within leaf sheath and stem) of about 50 cm in less than one hour, particularly in control plants (Fig. 3.6). Thus the velocity of assimilate translocation in wheat cultivars employed here could be considered somewhat similar to that cited in Didehvar (1970) as 39 cm/h in the sheath and 87 cm/h in the stem.

The fraction transported to the ear of the total ^{14}C present within the plants 24 hours after $^{14}\text{CO}_2$ application is shown in Table 3.2. The ^{14}C distribution to the roots, other leaves and tillers,

Fig. 3.6. The allocation of ^{14}C into the ears of wheat cvs. (a) Inia 66 and (b) Sholeh in different salt concentrations over three harvests: one hour (T1); 6 hours (T2) and 24 hours (T3) after feeding $^{14}\text{CO}_2$

Each point is the mean of three replicates, with standard errors ($\times 10^{-3}$) of 0.2 at T1, 13 at T2 and 21 at T3.

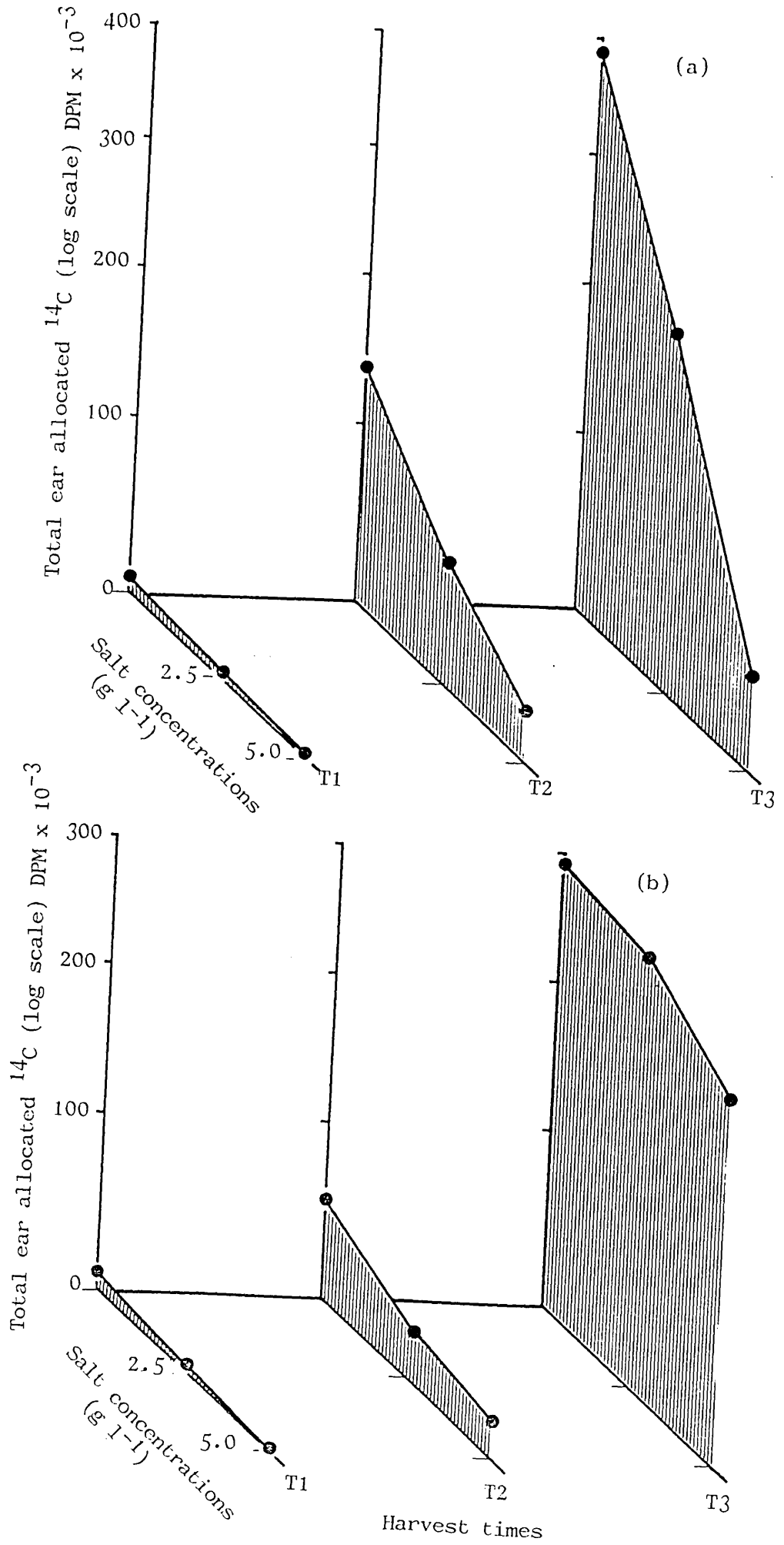


Fig. 3.7 Total ^{14}C present in the flag (fed) leaves of wheat cvs. (a) Inia 66 and (b) Sholeh in different salt concentrations, at three harvests, one hour (T1), 6 hours (T2) and 24 hours (T3) after feeding $^{14}\text{CO}_2$

Each point is the mean of three replicates, with standard errors ($\times 10^{-3}$) of 19.5 at T1, 22 at T2 and 15 at T3.

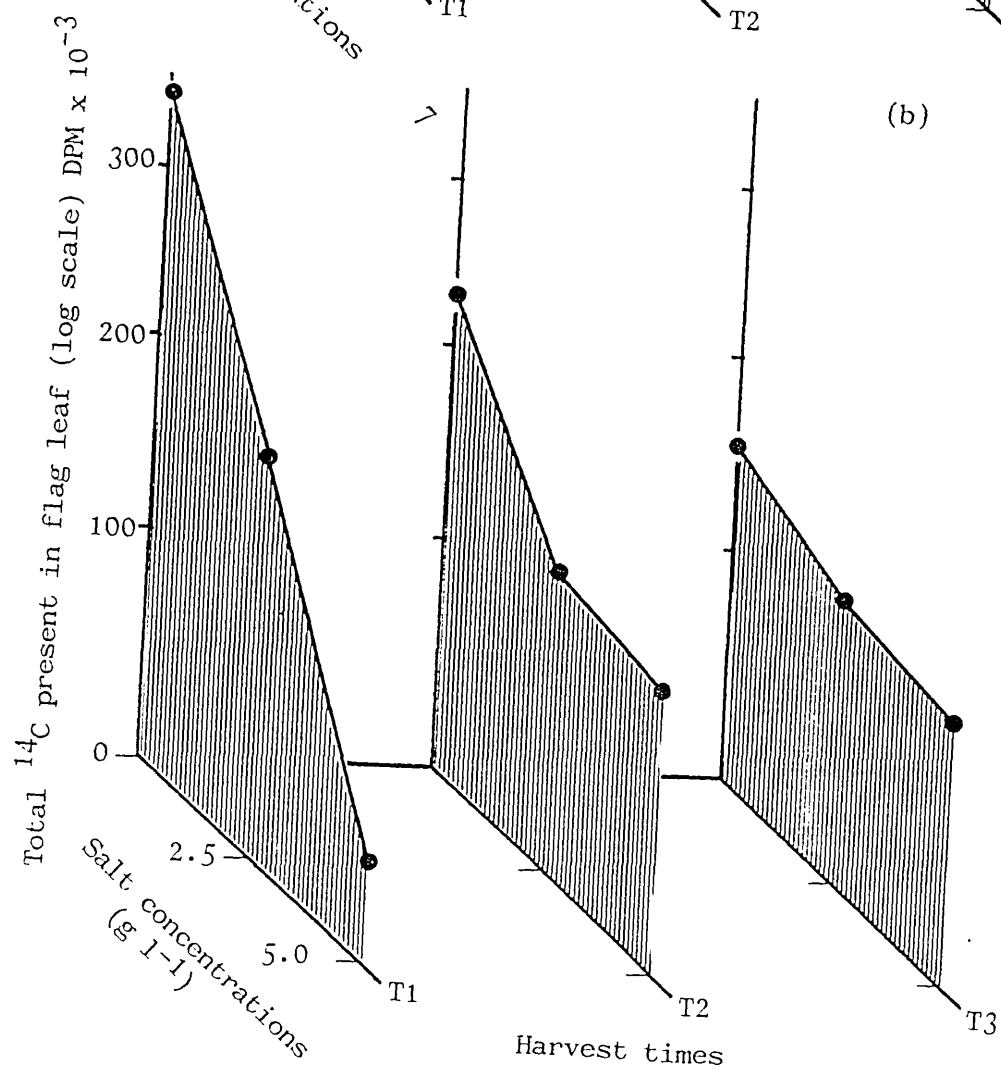
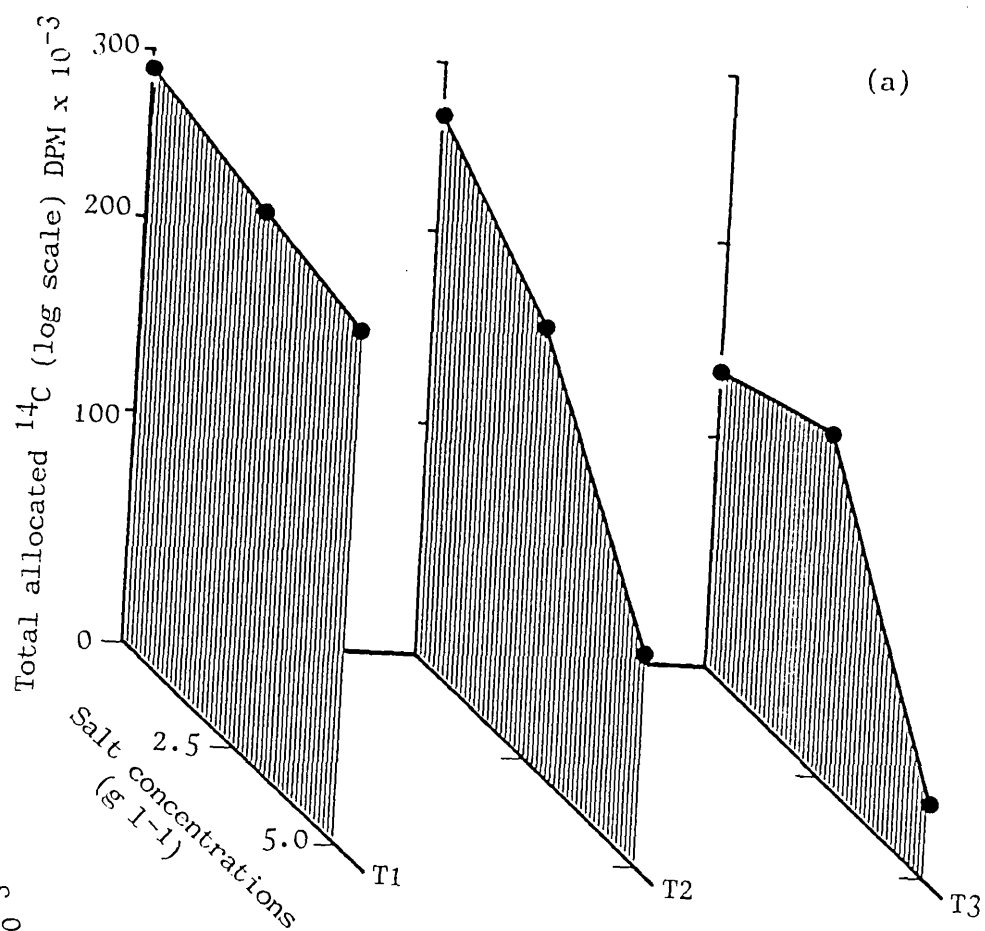


Table 3.2: ^{14}C activity (DPM) in the ears, flag leaves and other parts of the plants as a proportion of the total activities present in the plants 24 h (T3) after $^{14}\text{CO}_2$ application to the flag leaves of two wheat cultivars at different salinities.

Cultivar	Plant parts	Salinity (NaCl g l^{-1})					
		S1 (control)		S2 (2.5)		S3 (5.0)	
		Activity	%	Activity	%	Activity	%
Inia 66	Flag leaf	135896	26.0	121631	39.8	35695	40.2
	Ear	382771	73.4	182523	59.8	52214	58.8
	Other parts	2312	0.44	841	0.27	767	0.86
	<u>Total</u>	520981		304995		88676	
Sholeh	Flag leaf	155993	34.4	108382	27.6	121840	33.9
	Ear	293527	64.8	283376	72.2	236361	65.9
	Other parts	3018	0.66	675	0.17	494	0.13
	<u>Total</u>	452538		392433		359195	

and their proportion of the total ^{14}C present in these three organs are summarised in Table 3.3.

The values of 73% and 64% as the ear ^{14}C fractions of the two cultivars employed agree with the observations of King *et al.* (1967), if the stem content of ^{14}C is also taken into account. They reported that 13 days after anthesis about 45% of the flag leaf assimilates had been translocated to the ear. Assimilate donation by leaf 6 (flag leaf) particularly to the ear was reported to be (37%) at ear emergence, but it was observed that from this stage on the amount of ^{14}C translocated to the ear increased (Rawson and Hofstra, 1969). Carr and Wardlaw (1965) also reported that the proportion of ^{14}C located in the ears of two varieties of wheat 24 hours after assimilation of $^{14}\text{CO}_2$ was 43% and 50%.

Cultivar Inia 66 had a higher efficiency than Sholeh in carbon transport to the ear under normal conditions. This cultivar also showed higher rates of photosynthesis, although its growth rates were lower than Sholeh (section 3.1). This may indicate that cultivar Inia 66 can be more efficient in dry matter production than Sholeh, explaining the field popularity of the Inia 66 cultivar as being more productive in normal conditions compared with the latter.

The pattern of ^{14}C content of the flag (fed) leaves of both cultivars is almost the opposite of that of the ears in non-salinised conditions, decreasing with time (Fig. 3.7). This reduction can be due to a greater ^{14}C translocation with time to the other parts of the plants and/or respiratory losses of carbon and/or lowered CO_2

Table 3.3 ^{14}C activity (DPM) present in roots, other leaves and tillers as a proportion of the total activities present in these three parts of the plants 24 h (T3) after $^{14}\text{CO}_2$ application to the flag leaves of two wheat cultivars at different salinities.

Cultivar	Plant parts	Salinity (NaCl g l^{-1})					
		S1 (control)		S2 (2.5)		S3 (5.0)	
		Activity	%	Activity	%	Activity	%
Inia 66	Roots	650	28.0	178	29.0	119	19.0
	Other leaves	63.3	2.7	61	7.2	26	3.3
	Tillers	1599	69.1	602	71.0	622	81
	<u>Total</u>	2312		841		767	
Sholeh	Roots	495	16.5	172	25.4	146	29
	Other leaves	28	0.9	31	4.5	35	7
	Tillers	2465	82.4	472	69.9	313	63
	<u>Total</u>	2988		675		494	

concentrations available to the leaves. The proportion of ^{14}C in the flag leaf of the total activities retained in the plants 24 hours after $^{14}\text{CO}_2$ application is lower with Inia 66 than Sholeh. Cultivar Sholeh would be expected to have lower amounts of fixed carbon owing to its lower rate of leaf photosynthesis (see section 3.1). These results suggest that assimilate transport into the ear is less efficient in this cultivar. If this inference is correct, it may support the hypothesis that the source is not the limiting factor in assimilate transport and grain filling, as sugar is still available in the leaves but remains unutilised.

3.2.2 Salinity and carbon partitioning

Salinity had a generally depressing effect on photoassimilate distribution, particularly in Inia 66. This can be observed in

Table 3.2, where it can be seen that under salt stress a lower proportion of ^{14}C was transported from the flag leaves and the ^{14}C content of the ears was more or less reduced in both cultivars. This indicates that under salt stress conditions carbon availability in the leaves may not be restricted, and thus may lend support to the view proposed earlier (see section 3.1) that photosynthesis may not play an important role in reducing growth under salt stress conditions. The presence of sucrose in the source leaves, which seems to be available for utilization or transport to other parts of the plant, is observed not only with salinity, but also with normal plants as mentioned earlier in this section. Thus the possibility may not be unlikely that the mechanisms by which carbon transport is restricted under salt stress, are the same as those regulating export under normal conditions. Some of these mechanisms will be discussed

in detail later (see Section 4).

Inia 66 was more affected in terms of ^{14}C allocation to the ear than Sholeh, so that the responses to the two levels of salinity in terms of flag leaf ^{14}C content are significant. With Sholeh there was only a significant difference with the control at the highest level of salinity. The higher proportion of ^{14}C in the flag leaves of Inia 66 under saline conditions than in the control is associated with a lower proportion of ^{14}C in the ears of salt-treated plants compared with the control. This shows a pattern which is the opposite of normal conditions. Sholeh showed a higher efficiency in carbon transport to the ear when subjected to saline conditions (Table 3.2) while its photosynthetic rates were not higher than the other cultivar, being reduced with increasing salinity in parallel with Inia 66 (Fig. 3.1). This indicates that, in contrast to the normal conditions, under salt stress cultivar Sholeh may be more reproductive than Inia 66. This explains the popularity of Sholeh as a salt tolerant cultivar. The non-limiting role of photosynthesis in yield increase can be deduced again from the reduction in CO_2 uptake rates, associated with higher efficiency in carbon transport in Sholeh compared with Inia 66.

Based on these observations it appears that there is a mechanism within the cultivar Sholeh, which makes it more efficient in carbon utilization or partitioning. Wheat plants may be sensitive to disturbed ratios of internal K^+/Na^+ and if this is the case, the lower alterations in the K^+/Na^+ ratio **proposed** in Sholeh may be the reason for its higher efficiency of carbon transport. This is based

on the suggestion that the higher growth rate in this cultivar may have resulted in lower changes in the K^+/Na^+ ratio compared with Inia 66. An increase in leaf $(Na^+ + K^+)$ concentration observed with increasing external NaCl was attributed to decreases in the leaf elongation rate and not to any increase in net ion uptake (Delane *et al.*, 1982). Alternatively, the reduced plant water potential may be the cause of the low carbon transport efficiency, as drought stress in wheat can potentially influence yield by reducing translocation (Clarke, Karamanos and Simpson, 1981). With regard to the water stress component of salinity, the reduced efficiency of translocation in plants might be due to the sensitivity of the transport processes in the phloem to changes in the water potential gradient (Moorby, 1981). With higher root growth rates the plants can obtain more water from the soil (Woolhouse, 1983) and thus the carbon transport in Sholeh should be more efficient than Inia 66.

The pattern of reduced ^{14}C content resulting from salt stress can also be observed in other parts of the plants. The absolute values for other parts of the plants are much smaller than those of the ears, most likely because of the growth stage of the plant at which the observations were made, i.e. 14-16 days after anthesis. This supports the view that when the grains begin to grow the flag leaf and the leaves below it play a greater role in the supply of assimilates for grain growth so that the grains rapidly become the dominant sink for assimilates (Stoy, 1963; Evans and Wardlaw, 1976). However, the overall response to salinity of the three parts of the plants examined here shows that, although the whole carbon balance of the plants is reduced, the proportions in various organs are

different. In Inia 66 a higher proportion of ^{14}C was allocated to the tillers and a lower proportion to the roots, when salinity was imposed on the plants (Table 3.3). Conversely, in Sholeh a higher proportion (than control) of the ^{14}C was located in the roots and lower amounts in the tillers when subjected to salinity. This indicates that in saline conditions the pattern of photoassimilate partitioning can be changed, and that in Inia 66 the carbon was diverted from roots to tillers. The priority given to the roots in Sholeh plants is also reflected in the results from growth analysis (section 3.1.4) which show a higher root/shoot ratio for this cultivar under saline conditions.

Although alterations in the distribution pattern at this stage of growth are small, there is an indication here that the demands for assimilates by the sink organs of plants may differ between normal and saline conditions. This may reflect the fact that under normal conditions any increased demand for assimilates by some sinks can readily be met by a change in distribution (Ho, 1979). This means that the non utilization of assimilates or any other problem in the carbon transport is not the only determinant factor in partitioning patterns, but that there is a potential for the demands of the plant organs to play a role in the distribution pattern under salt stress. Thus the possibility exists for a diversion of carbon to root growth or to any other suitable organ by plant improvement programmes.

3.2.3 Limitations on carbon allocation

As discussed earlier, the current study revealed that under salt stress at about two weeks after anthesis, the translocation of

carbon was more restricting to grain yield than to the carbon distribution pattern in general. This is the situation not only with salt or water stressed plants, but also with those under normal conditions in that not all of the fixed carbon, which is apparently mobile and available in the source leaves, is utilized and allocated to other sink organs. Apart from other environmental factors which reduce the efficiency of carbon transport, the question is which internal factors limit carbon transport processes in the plants, particularly under saline conditions? Terms such as 'translocation' have been widely used here, but this might be misleading as it has not yet been established that it is the efficiency of the transport system which is reduced. The reduction factors could be located anywhere in the source or sink tissues or somewhere in between.

One possible cause for the observed reduction may be the accumulation of sugars within the leaf for osmotic adjustments. Considering this possibility, terms such as 'non-utilized carbohydrates' would become undermined, since such carbon would not be available for transport to other parts. This view is supported by the report that increased carbohydrates, accumulated under saline conditions may have been removed from the pool available for immediate metabolism or growth (Cheesman, 1988).

Other factors which may limit carbon transport are the processes involved in phloem loading, as the uptake of sugars from the free space in *Ricinus* petioles has been reported to be promoted by the presence of K^+ and reduced by Na^+ (Malek and Baker, 1977). Some reductions have also been observed in assimilate transport to

the conducting tissues of wheat plants under water stress, which was proposed to reflect a reduced ability of the leaves to utilize available energy (Wardlaw, 1967). Jeschke (1984a) also proposed that phloem loading of sugars was less efficient with Na^+ than with K^+ present at the loading site.

The structure of the conducting tissues of the phloem may also be considered as a limiting factor in assimilate transport. Wareing and Patrick (1975) suggested that at least in some circumstances, the transport system may restrict transfer of assimilates from source to sink. The increased number of phloem cells in salt-stressed plants observed here (Figs. 3.2, 3.3 and 3.4) may be considered as a response of the plants to the reduced transport processes in the phloem. The accumulation of photosynthates in the leaves of plants under salt stress is also attributed to impaired phloem transport (Maas and Nieman, 1978). On the other hand, it has been reported that the supply of assimilates in wheat does not appear to be limited by the vascular system (Patrick, 1988).

Sink demands for assimilates may also restrict the long distance transport of assimilates under saline conditions. This aspect is discussed in detail in Section 4. It is highly likely that all the factors mentioned here may have the potential and actually do play a role in limiting carbon transport in some circumstances. However, it might be expected that one or other of these factors is more sensitive to salt stress conditions and thus a potential target process for any programme of genetic manipulation aimed at increasing the salt tolerance of crop plants.

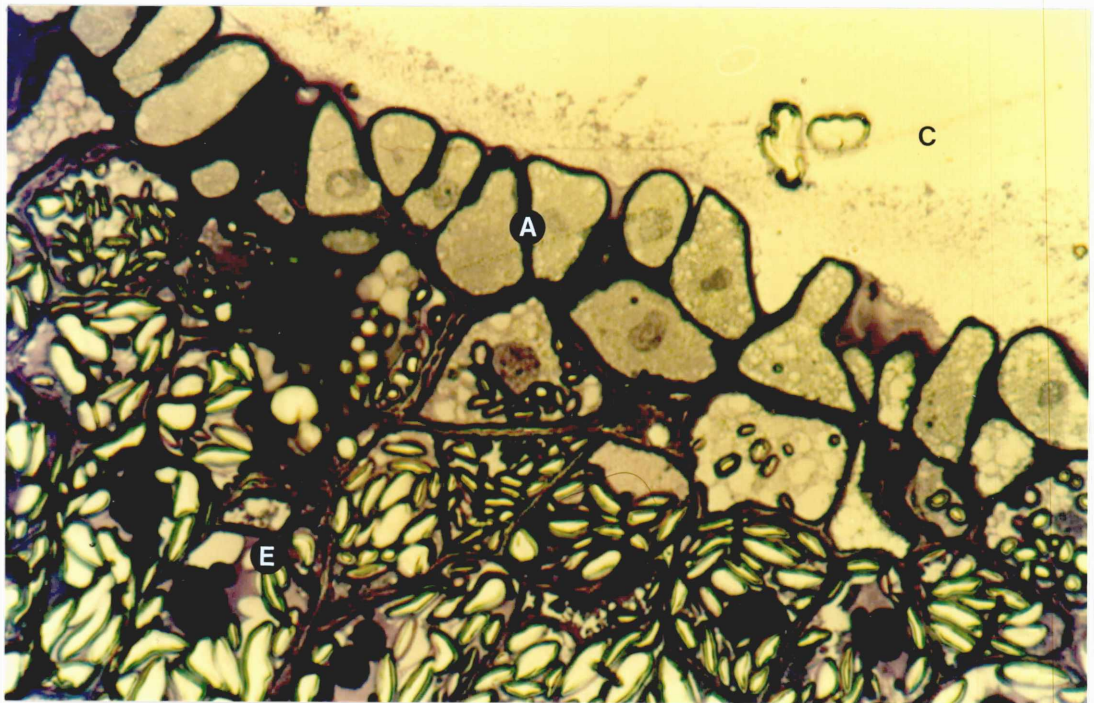
3.3 Salinity and grain ultrastructure

A general view of the structure of the grain from a control plant and its aleurone layer is illustrated in Fig. 3.8. A transverse section of the grain is illustrated in Fig. 3.8a. The maternal tissues, comprising the vascular tissue, pigment strand and nucellar projection are present on one side of the cavity, and the aleurone layer and endosperm tissues on the other. The aleurone layer, which is the first layer of one or two cells facing the cavity, and the endosperm cells are shown in greater detail in Fig. 3.8b.

Electron micrographs of the aleurone layer of grains from salinized and control plants are presented in Figs. 3.9-3.14. These micrographs show the first row of the cells of the aleurone layer and part of the endosperm cavity. In Fig. 3.9 two aleurone cells of a non-salinized plant are illustrated. In these cells the vacuoles are small and the cell walls do not have additional thickenings. A considerable number of mitochondria are present. Fig. 3.10 shows similar cells from a salt-treated plant. These are more vacuolated with thickened cell walls. In addition, a lamellar structure of adcrusting materials is deposited between the cell protoplasts and their walls. At higher magnification (Fig. 3.13) it can be seen that, despite the wall thickenings and adcrustations, the plasmodesmatal connections are still present. The cell walls of control plants having normal thickness are illustrated in Fig. 3.11, and from salinized plants in Fig. 3.12. A comparison of these two figures indicates that considerable differences exist between the



(a)



(b)

Fig. 3.8 Transverse sections from the grain of a control plant of wheat (cv. Inia 66) 15 days after anthesis showing general view (a x 67) and aleurone cells (b x 268). A, aleurone layer; E, endosperm; C, endosperm cavity; M, maternal tissue

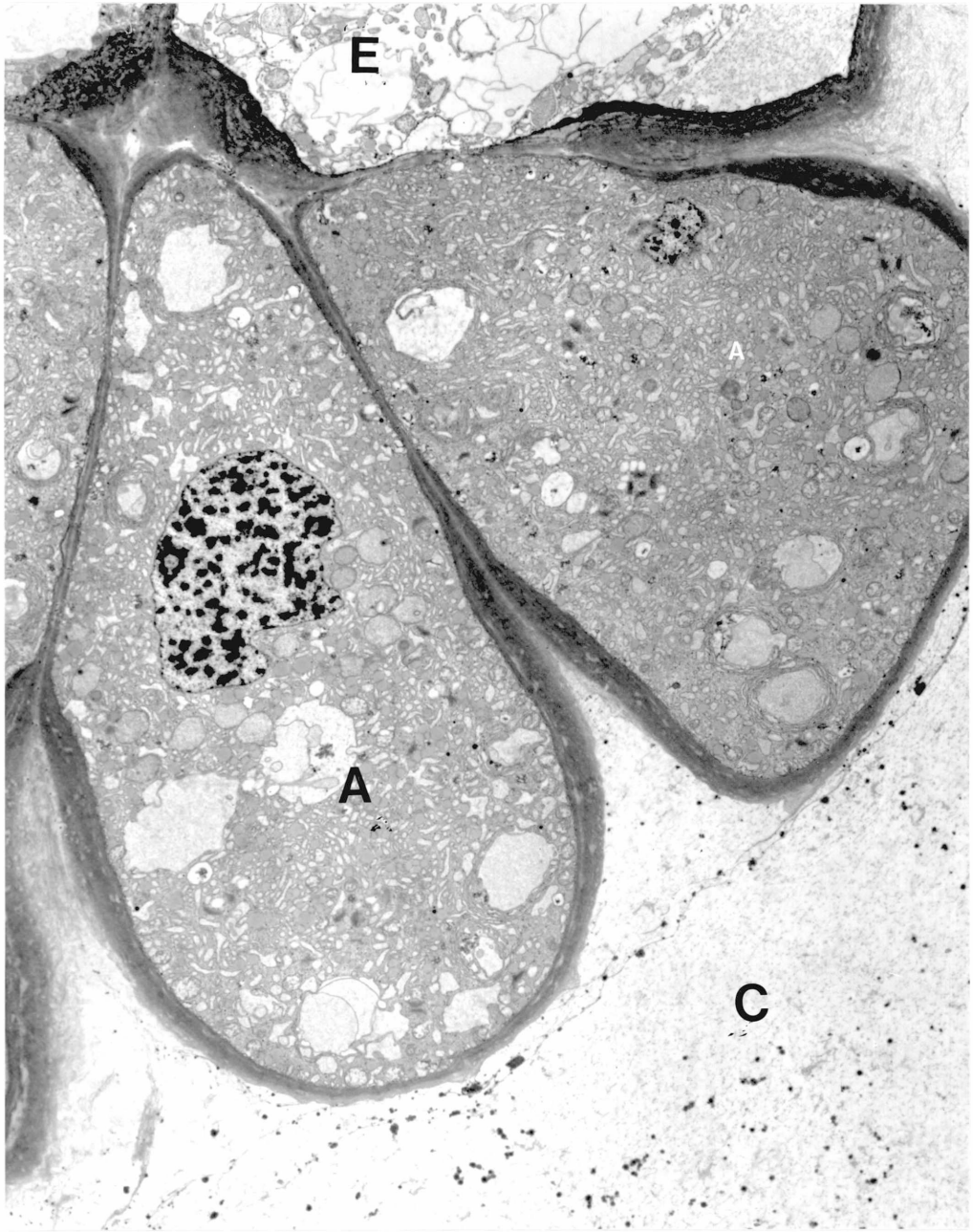


Fig. 3.9 Aleurone cells of wheat (cv. Inia 66) from plants grown under normal conditions, 15 days after anthesis. A, aleurone cell; E, endosperm cell; C, endosperm cavity. (transverse section x 3000)

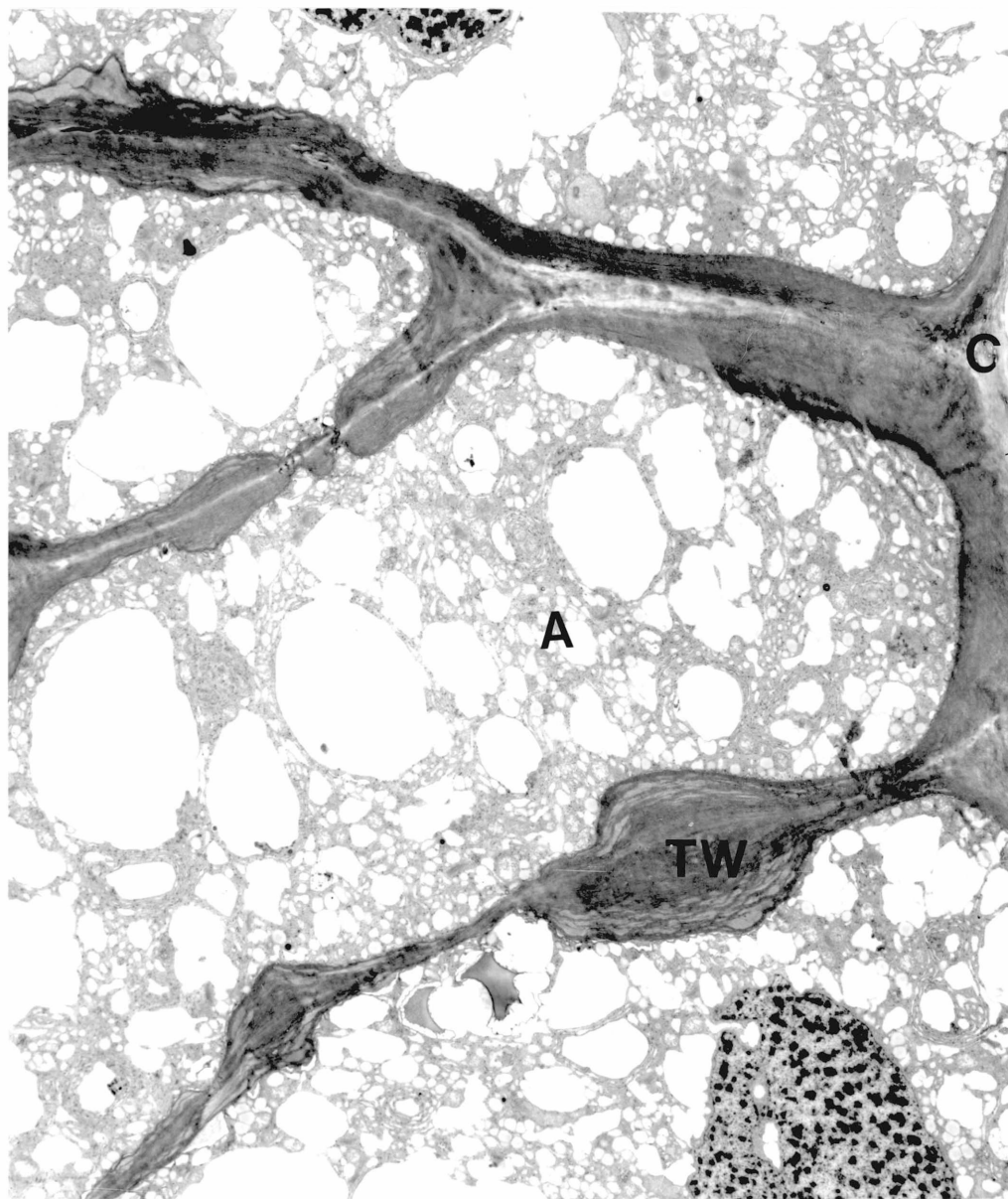


Fig. 3.10 Aleurone cells of wheat (cv. Inia 66) from plants grown under saline conditions, 15 days after anthesis. A, aleurone cell; C, endosperm cavity; TW, thickened cell wall. (transverse section x 3000)

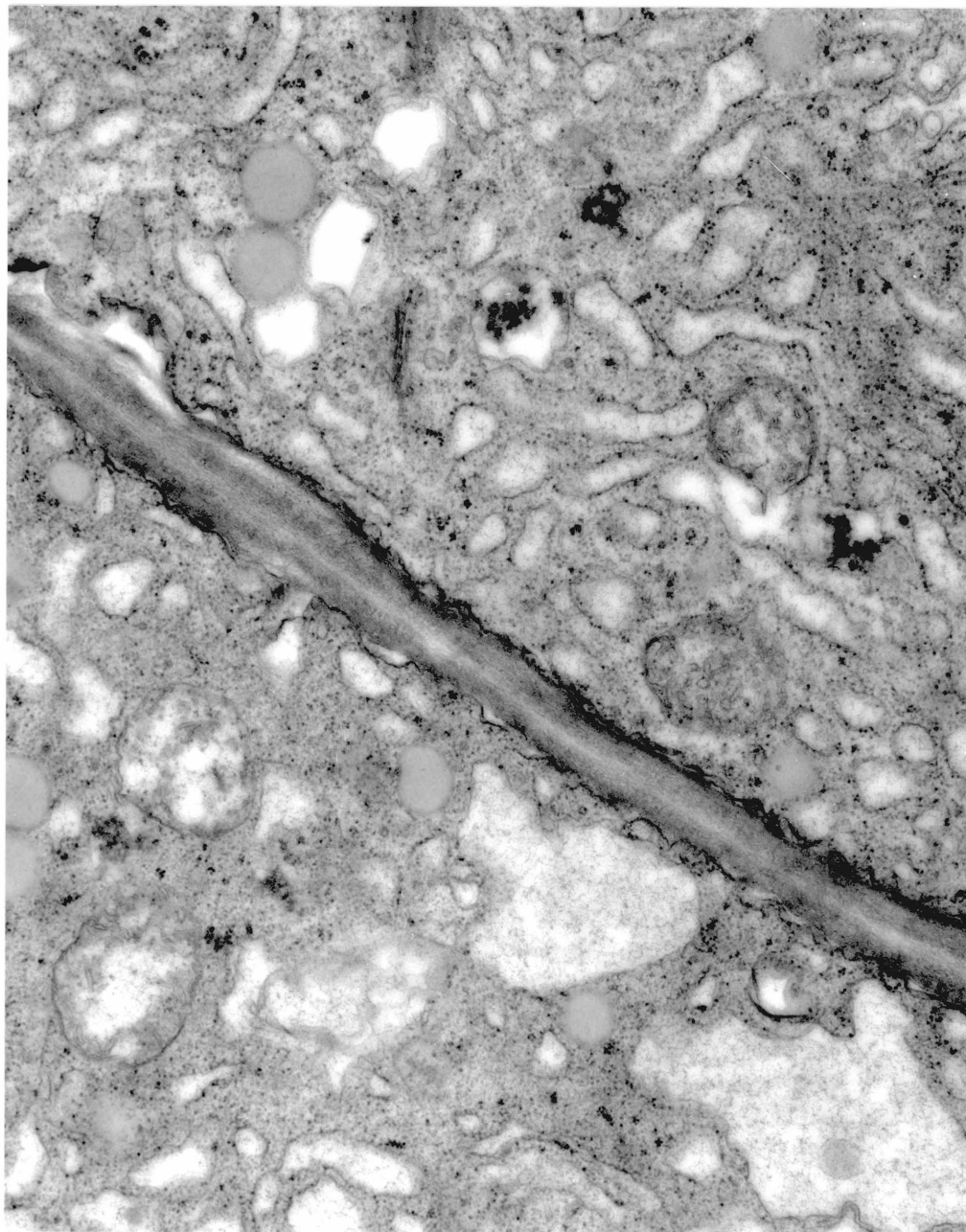


Fig. 3.11 Aleurone cells of wheat (cv. Inia 66) from plants grown under normal conditions, 15 days after anthesis, showing normal thickness of cell walls. (x 24000)

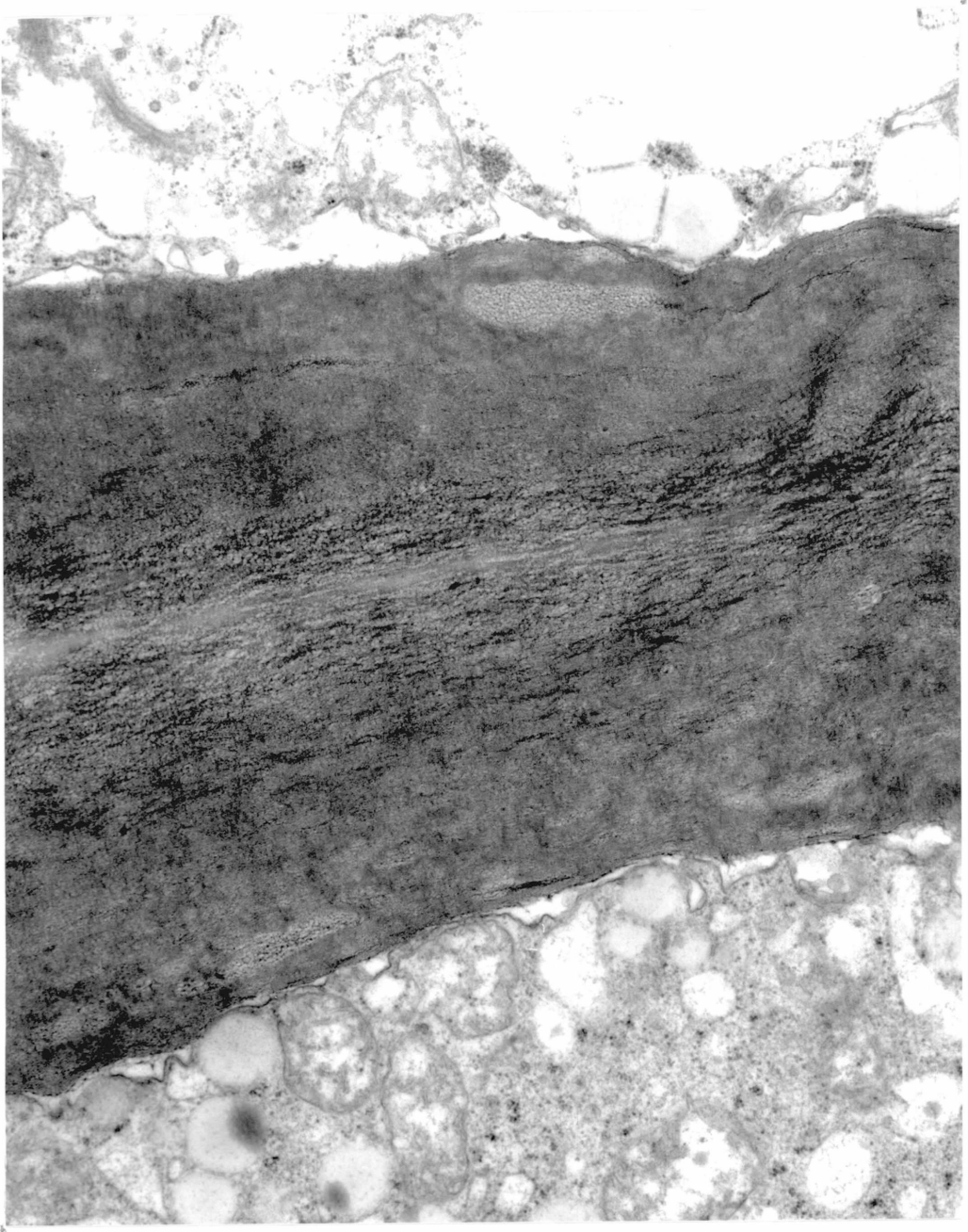


Fig. 3.12 Aleurone cells of wheat (cv. Inia 66) from plants grown under saline conditions, 15 days after anthesis, showing thickened cell walls. (x 24000)

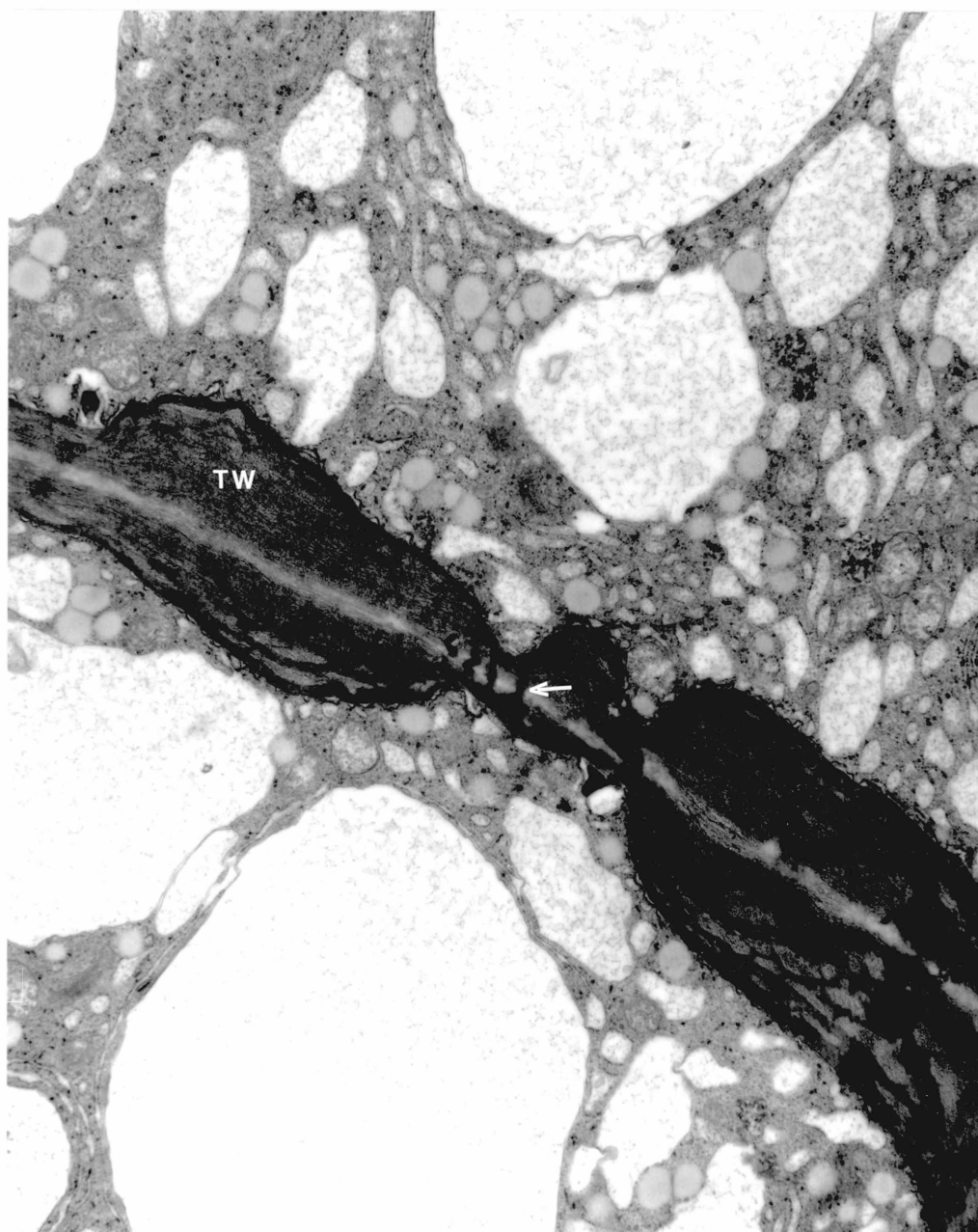


Fig. 3.13 Aleurone cells of wheat (cv. Inia 66) from plants grown under salt stress, showing plasmodesmatal connections (arrows), which traverse the thickened cell walls. TW, thickened cell walls (x 10000)

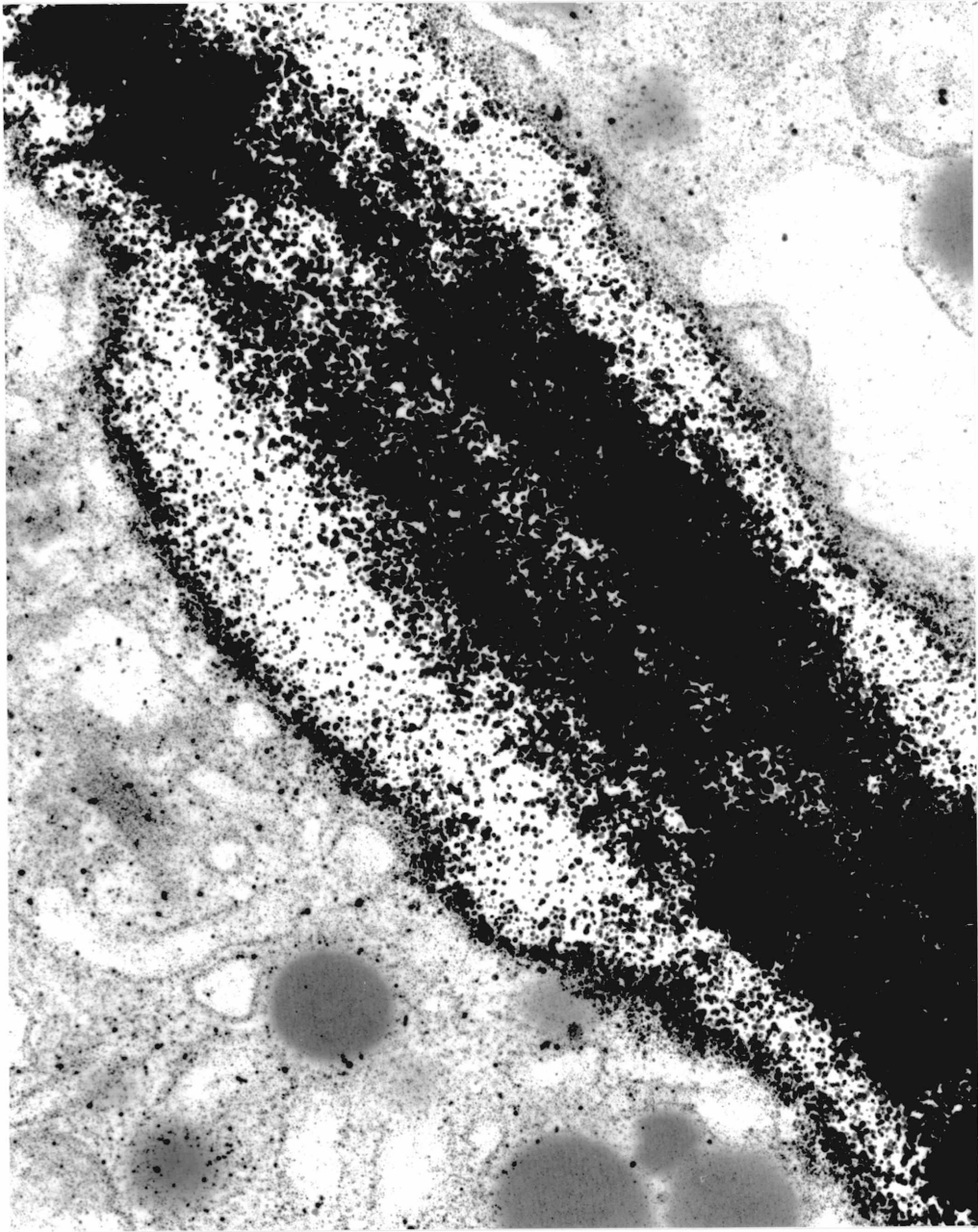


Fig. 3.14 Aleurone cell walls of wheat (cv. Inia 66) stained with silver hexamine from plants grown under salt stress, showing the electron-opaque granular structure in the cell walls (x 40,000)

cell wall structures of the aleurone layer of salt treated and non-treated plants.

The adcrusting depositions reacted strongly with silver hexamine (Fig. 3.14). The thickened cell walls which showed an even structure with uranyl acetate, now evidence an electron-opaque granular material when stained with silver hexamine. This reaction can reflect the presence of free aldehyde groups of suberin (lipid) material, which suggests the existence of a permeability barrier in the cell walls of the aleurone layer in salinized plants.

3.3.1 Cell wall adcrustation

The electron micrographs show that the changes which have occurred in the aleurone cells of treated plants have not, at least at this stage, taken place in control plants. The deposition of adcrusting materials between the protoplast and the cell walls by which cell walls become thickened under salt stress conditions could play an important role in solute transport. If these adcrustations provide an impermeable barrier, then the apoplastic pathway for the import of solutes can be blocked. The reactivity of the deposited materials with silver hexamine observed in the present study indicates that they could be suberized. This is in agreement with the observations of Zee and O'Brien (1970) that the staining reactions of the adcrusting substance in the pigment strand tissue of wheat grains suggest that it is suberized. It is also reported that the suberized walls of the Casparian strip of the root endodermis generally contain lipids of high molecular weight, which react with silver hexamine (O'Brien and Carr, 1970). The characteristics of

lipid materials with respect to water and solute exchange is significant. The suberin is generally believed to be somewhat similar to cutin which seems to be responsible for the resistance of the epidermal cells to water loss (O'Brien and Carr, 1970), or at least the main barrier to passive movement of some solutes, as suggested for suberized endodermis regarding Na^+ and Cl^- flow (Greenway and Munns, 1980). Here, therefore, the suberized depositions observed may be suggested as causing restrictions on solute movement, such that these materials could probably cause an intercellular barrier to solute movement. This supports the observations that the suberin-like material in the pigment strand cells of the rice grain may effectively block the apoplastic pathway for assimilate transfer (Oparka and Gates, 1981a). The possibility of passive transport being restricted by suberized lamellae was also discussed by O'Brien and Carr (1970). The massive wall thickening of aleurone and some cells of nucellar projection of barley caryopsis between 10 and 35 days after anthesis are proposed to prevent the entry of certain molecules from the parent plant (Cochrane and Duffus, 1980).

Similar wall thickenings can also be seen in some endosperm cells of control plants as well as salinized ones (Fig. 3.9). It may be that at the later stages of grain filling in non-treated plants, similar structural modifications occur within the aleurone cells. If this holds true, then the salinity effect may partly be due to a shortening of the grain filling period. As shown in Fig. 3.10 the aleurone cells of salt-treated plants are highly vacuolated, which shows a resemblance to other endosperm cells. Similar changes were

observed in *Suaeda maritima* by Hajibagheri, Hall and Flowers (1984) who found that the volume fraction of the vacuole was greater in plants grown under saline conditions, when compared with those grown under non-saline conditions. These observations support the idea that salinity hastens grain maturity. This is further supported by Carols and Bingham (1973) who found sodium chloride hastened the maturity of wheat grains by about 2 weeks.

3.3.2 Symplast and mitochondria

With the blockage of the apoplastic pathway through the aleurone layer the symplast may remain as the main route for solutes to pass from the cavity into the endosperm. In Fig. 3.13 it can be observed that even when the wall thickenings are present, the plasmodesmatal connections are still intact. This supports the observation of Oparka and Gates (1981a) on pigment strand tissues of rice, that the plasmodesmatal connections traversed both the cellulosic wall and the modified inner layers. It is, however, in conflict with the views of Zee and O'Brien (1970) who believe that the accumulation of adcrusting substances makes symplastic transport impossible. On the other hand, Cochrane (1983) has shown that assimilates from the chalazal tissue continued to reach the endosperm through the symplasm. Also, the perforated lamellae made by plasmodesmata in the mestome sheath of wheat and oat support the idea of plasmodesmatal existence and operation within the suberized tissues (O'Brien and Carr, 1970).

Finally, the presence of a large number of mitochondria observed in the aleurone cells of control plants (Fig. 3.9) could

indicate that these cells may be more involved in energized processes than ordinary cells. This feature of the cells seems to be affected by salinity, as the number of mitochondria was greatly reduced in the aleurone cells of salt stressed plants (Fig. 3.10). The effect of salinity on some energized processes, i.e. facilitating transport of solutes through the aleurone cells will be discussed in Section 4.

In conclusion, from the ultrastructural studies of aleurone cells of wheat grains, it appears that under salt stress, because of cell wall thickening and suberin adcrustations, solute transport from the cavity to the endosperm through the apoplast is restricted or inhibited. Plasmodesmatal connections could be the main pathway for the movement of solutes, if they can enter into the aleurone through the cell membrane.

3.4 Salinity: effect on grain filling

Chemical analysis of cavity sap and endosperm fluid was carried out to determine the concentrations of sucrose, glucose, fructose, potassium and sodium. The data from these analyses and the pH of both fluids are given in Table 3.4. No glucose or fructose were detected in the fluids. Considerable changes occurred in the solute concentrations with salt treatment in that the concentration of sucrose in the cavity sap increased, while in the endosperm it decreased and the concentration of K^+ and Na^+ in the cavity fluid also increased, while in the endosperm fluid K^+ decreased and Na^+ slightly increased. Thus, the K^+/Na^+ ratio in both the cavity sap and the endosperm fluid decreased under saline conditions. No changes were observed in the pH values of the control and salinized samples.

The grain weights presented in Table 3.5 show a decrease in dry weight and water content under salt stress. Among the grains of control plants, a few shrunken ones were found which, instead of having a normal internal structure, were full of a clear sap with a few small pieces of endosperm tissue floating in it. The sap from these grains was collected and analysed. The concentrations of sucrose, glucose and fructose of this sap were 39.4, 8.7 and 3.8 mM respectively, and its K^+ concentration was 11.8 mM. The concentration of Na^+ was not determined in these samples.

Table 3.4: Effects of salinity on the solute composition of the cavity sap and endosperm fluid from wheat (cv. Inia 66) caryopsis.

	Cavity sap		Endosperm fluid	
	Control	Salinized	Control	Salinized
Sucrose	30.7 ± 2.5 mM	46.3 ± 3.7 mM	8.8 ± 0.6 mM	5.1 ± 0.9 mM
K ⁺	6.7 ± 0.3 mM	13.1 ± 2.5 mM	82.9 ± 5.6 mM	64.5 ± 7.3 mM
Na ⁺	9.1 ± 1.6 mM	22.9 ± 2.6 mM	1.12 ± 0.1 mM	1.6 ± 0.2 mM
K ⁺ /Na ⁺	0.74	0.57	74	40
pH	5.4-6.3	5.7-6.3	7.5-7.9	7.4-7.7

Sap collection

The technique used here for sap collection can be an accurate one, although it may not be achieved with all cultivars and/or in all environmental conditions. Sap collected in this way, as it is flowing within the grain attached to the plants (*in vivo*) can reflect the characteristics of the photosynthate coming from the assimilate stream to the sink tissues. The time between grain cut and sap collection was not more than 20 seconds, and thus any changes such as those caused by evaporation were minimized. The collected sap was also free of contamination by endosperm fluid and the tissues of the grain.

However, with regard to the cavity size there might be some limitations to this technique from varietal and environmental differences. In the case of small cavities where the aleurone layer is almost touching the nucellar projection tissues, the size of the cavity and the volume of the sap would not be large enough for the glass tube to be inserted and to collect adequate amounts of uncontaminated sap. This is the case brought about by either varietal differences or by some environmental changes. The cavity sap volume of one variety was reported to be six to eight times more than that of another, and the bigger cavity in the larger grains accounted for a higher proportion of the fresh grain weight (Ho and Gifford, 1984).

Light, in terms of intensity and quality, and temperature, especially during the 3-4 days after anthesis, seem to have a dramatic effect on the endosperm capacity (the number of endosperm

cells) (Brocklehurst, 1979) and in turn probably on cavity size. Fertilizer is also another factor affecting the cavity size, which appears to be larger when higher levels of K^+ in the fertilizer are used. Salt concentrations higher than $EC=10 \text{ mmho cm}^{-1}$ reduced the cavity size of the variety used in this study. In general, a normal grain of a vigorous wheat plant contained 500 to 1000 μg sap in the cavity. From the lower half of the grain, one quarter of that amount would be collected without contamination, provided that all other conditions were suitable.

The fluid collected from the endosperm should reflect the collective properties of all parts of the endosperm. The contents of the endosperm cells can be released by freezing and thawing.

3.4.1 Solute transport in normal conditions

3.4.1.1 Inorganic solutes

Potassium and Na^+ are two inorganic solutes (monovalent cations) whose ratio is of importance in physiological processes. The K^+/Na^+ ratio of 0.74 observed in the cavity sap of control plants here would not be a suitable ratio for sustaining metabolic activities in the cytosol of plant tissues. This ratio needs to be above unity for optimal efficiency (Wyn Jones *et al.*, 1979, cited in Greenway and Munns, 1980; Pall *et al.*, 1984; Gill and Dutt, 1987). Higher ratios are reported for the shoot of barley and a K^+/Na^+ ratio of 8 was observed for grains of the same crop (Greenway and Munns, 1980) although the latter was an average of all parts of the grain. The endosperm cavity can be considered as a passage for the assimilates and appears to be the part of the grain having sap with

the lowest K^+/Na^+ ratio, reflecting that the aleurone layer may be involved in ion regulation within the grain. It is known that in addition to mechanisms whereby sodium can be partially excluded during uptake as for example by barley roots (Nassery and Baker, 1972, 1974), K^+ is preferentially selected over Na^+ in both initial ion uptake (Pitman, 1984), and ion transport to the vegetative and reproductive organs (Jeschke, 1984a). Consequently, the ratio of K^+/Na^+ in the shoot is maintained higher than that in the roots and higher again than that in the external solution (Jeschke, 1979; Pitman, 1988). The concentration of K^+ and Na^+ in phloem sap is reported as 20-85 mM and 0.06-0.3 mM respectively (MacRobbie, 1971; see also Malek and Baker, 1977 for K^+ concentration). These analyses indicate that only a small amount of Na^+ is transported towards the grain. Despite this a high concentration of Na^+ was observed in the cavity in the present study. Metabolism in the grain is stated to be more sensitive to salinity than the other parts of the shoot (Greenway, 1962) and therefore it may be postulated that there is another selective system sited within the caryopsis, which acts as a barrier for certain ions, e.g. Na^+ . This could be the aleurone layer which lies between the cavity and endosperm. On this basis the endosperm cavity can be considered as a pool in which unnecessary or harmful ions such as Na^+ are retained while other ions like K^+ are accumulated by the endosperm.

Ion concentrations in the endosperm fluid are quite different to those of the cavity sap (Table 3.4). Comparison between these values and those of the cavity sap, particularly for K^+ , show marked differences in the concentrations of both ions. The endosperm K^+

concentration in controls was twelve times higher than in the cavity and the Na^+ was seven times less. These steep gradients are over a short distance with only a single layer of cells, the aleurone, in between. The resultant K^+/Na^+ ratio in the endosperm fluid is 74 which is suitably high for the metabolic activities associated with starch synthesis (Wyn Jones, Brady and Speirs, 1979).

The aleurone cells of the wheat caryopsis are reported to serve as transfer cells through which solutes are transported to the endosperm (Ayre and Angold, 1979). Inevitably, the steep gradient of inorganic solutes described here could be established by these aleurone cells as a result of selective ion uptake. This selective system may be attributed to the higher affinity of the membrane carrier to K^+ which has a smaller ionic radius than Na^+ . A reported lower uptake ratio of 42% for Na^+ compared with K^+ has been related to the larger radius of Na^+ (calculated from data given by Marschner, 1986; based on Jacobson *et al.*, 1960). Some aspects of K^+ transport through the aleurone membrane will be discussed in Section 4.

3.4.1.2 Sucrose

The sucrose concentration of the cavity sap from control plants presented here (Table 3.4) does not agree with the value of 30 mg ml^{-1} (85 mM) reported by Jenner (1974b) and 65 mM reported by Fisher and Gifford (1986). It does, however, agree with the concentration found by Ho and Gifford (1984) which is between 10-50 mM. The inconsistency of the observations in this respect could be due to different varieties and/or environmental conditions. It may also be due to the grain age at which the sap was collected, as

sucrose uptake by the endosperm tends to be more rapid in the first 20 days after anthesis. Alternatively, the position of spikelets in the ear and the grains in the spikelet, and the location of a sample in the length of the cavity may have different effects on the sucrose concentration observed. However, another factor that may have changed the apparent sucrose concentration of the cavity is contamination by the endosperm fluid resulting from squeezing or centrifugation methods employed by other investigators.

A comparison between the amount of sucrose in the cavity with that in the endosperm fluid in control plants (Table 3.4) shows that there is a downhill gradient for sucrose from the cavity to the endosperm. This is in agreement with the observations of Jenner (1974b) that there is a downhill gradient in sucrose concentration from the phloem to the cavity and then to the endosperm. A ratio of 8.3 has been reported for the sucrose concentration of the sieve tube relative to the cavity in wheat grain (Fisher and Gifford, 1986).

The relatively high sucrose concentration gradient between these two compartments can create a driving force for the movement from the unloading site into the cavity. Thus the sucrose transport here may well be passive, with no need to invoke a metabolic step. Sucrose could cross the membrane through either a carrier or a non-carrier mediated system. The former is suggested to be quantitatively important at low sucrose concentrations (Ho and Gifford, 1984). Sucrose uptake and re-accumulation into the endosperm symplast could also be passive in response to the observed gradients and there is no requirement for active transport on these

grounds.

The solute gradient described here occurs over a short distance within the grain, i.e. from the crease tissues up to the endosperm, indicating that there are some resistant sites in this region, while restrictions to assimilate movement in the sieve tube are thought to be unimportant under most conditions (Fisher and Gifford, 1986). Some resistance may occur before solute entry into the cavity within the maternal tissues in either the symplastic or apoplastic compartments. Between the cavity and the endosperm, with the aleurone layer in between, a further major gradient of sucrose concentration has been identified in the present study which may point to a second site of resistance to assimilate transport. This supports the view that the aleurone layer is one of the five locations in the grain resistant to sucrose transport from the phloem to the endosperm cells (Jenner, 1974b).

3.4.2 Solute transport under salt stress

3.4.2.1 Inorganic solutes

Salt treated plants received tap water with 4 g salt (NaCl) per litre, a concentration equivalent to $EC = 10 \text{ mmho cm}^{-1}$. Salinity had a marked effect on the balance of the monovalent cations K^+ and Na^+ in the grain. The decreased K^+/Na^+ ratio of the cavity sap from 0.74 to 0.57 by salinity indicates that with extra salt in the root medium, not only the vegetative organs but the grain, which is the most sensitive part of the plant, is also affected. This reduction is in reality greater since the normal transport of K^+ to the endosperm has been reduced, resulting in a lowering of the K^+

concentration in the endosperm fluid by 22%.

These changes in ion concentrations probably reflect alterations in the systems involved in ion distribution within the plant. This may include the extrusion of sodium from the root, as reported for K^+/Na^+ selectivity in barley plants as the result of an active Na^+ - extrusion pump as well as a selective uptake of K^+ over Na^+ (Nassery and Baker, 1972). The root K^+/Na^+ selectivity may also be affected reducing the K^+/Na^+ ratio in the plant. The K^+/Na^+ ratio was reported to be 3.2 when the K^+/Na^+ selectivity in the inflorescence (without grain) was 68, while in the grain a higher K^+/Na^+ selectivity of 130 resulted in a higher K^+/Na^+ ratio of 6.4 (Jeschke, 1979). High external ion concentrations are observed to be a factor affecting the selectivity of ions in barley roots (Pitman, 1988).

The plasmalemma of xylem parenchyma cells may also be a site of K^+ preference in the selective release of ions (Jeschke, 1984a), which may be affected by salinity (see Jeschke, 1984b). It appears that all the systems in the plant involved in the regulation of ion transport to the shoots can be affected to some extent by salinity, and thus the grain, as the most sensitive and uppermost part of the plant, loses this protection.

The K^+ concentration in the cavity sap of salinised plants increased by 95% relative to the control, which may be attributed to a reduced K^+ transport to the endosperm. This was reflected by a reduced K^+ concentration in the endosperm fluid. Reductions of K^+

flow across the membrane under saline conditions may be attributed to the lowered potential differences of the aleurone and/or endosperm cell membranes, as it is known that when metabolism is inhibited the membrane potential is reduced (Pitman and Luttge, 1983). As a result more K^+ has remained in the cavity. This may explain the observation that there was no increase in K^+ in the grain of barley with salt treatment, while Na^+ concentration increased considerably (Greenway *et al.*, 1965).

Whether there is another reason for the increase in K^+ concentration in the cavity sap is not clear. Redistribution of ions from the other parts of the plant could contribute to this increase. With increasing external K^+ and Na^+ concentrations, a redistribution of excess K^+ and Na^+ from the leaves of *Ricinus* via the phloem is reported by Baker and Chaudhry (1986) and from older to younger leaves by Pitman (1988).

Another effect of salinity on the grains is the changed concentration of monovalent cations ($Na^+ + K^+$) collectively. Using the average of the values for K^+ and Na^+ (Table 3.4), the concentration of these ions was 15.7 and 36 mM in the cavity sap and 84 and 66 mM in the endosperm fluid of control and salinized plants respectively. The concentration of these monovalents in the cavity sap of salinized plants was twice that in control plants. This increase is the result of two components, first reduced transport of ions to the endosperm (K^+) and the second, increased ion concentration (mainly Na^+ based on present data) within the plant.

The increased ion content of the plants through uptake from the root medium can be considered as the mechanism by which the plant achieves an internal water potential low enough to adjust osmotically with the root medium under salt stress conditions. The increased concentration of ($K^+ + Na^+$) confirms the previously held view that at higher external ($K^+ + Na^+$) concentrations, there is a tendency in many species for the K^+ (or $K^+ + Na^+$) concentration to increase (Greenway and Munns, 1980). In contrast to this view, Delane *et al.* (1982) attributed the increase in ($K^+ + Na^+$) concentration with increasing external NaCl to decreases in the elongation rate and not to an increase in net ion uptake.

3.4.2.2 Sucrose

The effect of salinity on the sugar balance of the grains can be shown by comparing sucrose concentrations from control and salinized plants (Table 3.4). Under saline conditions the concentration of sucrose in the endosperm has been decreased by more than 40%, while the cavity sap sucrose concentration increased by 50%. The concentration of sucrose in the cavity sap is about ten times greater than that in the endosperm fluid under salt stress conditions, whereas in control plants it is only three times higher.

The data from the cavity sap show that the sucrose availability is not limited even though transport into the grain or unloading may be reduced. This type of change, i.e. reduced sucrose transport, as well as decreased CO_2 uptake with salinity has been observed in the present study (Sections 3.1 and 3.2). However, this does not show a direct causal relationship *per se* between these

processes and sugar uptake in the grain. Sucrose accumulation in the cavity of the grain could probably explain the situation in that the inhibition of sucrose transport from the cavity may partially be responsible for the lower sucrose levels in the endosperm. This is consistent with the observations from the ultrastructural studies presented here that the apoplastic pathway through the aleurone layer appeared to be blocked by cell wall thickenings under salt stress (see Section 3.3). The possible mechanisms of this inhibition of sucrose transport through the aleurone plasma membrane will be discussed in Section 4.

The effect of the increased concentration of sucrose in the cavity can be transmitted back through the crease tissues up to the unloading site. The negative feedback effect of higher levels of sucrose could affect the unloading processes, and in turn, the loading of sucrose into the phloem, via the reduced gradient of turgor pressure between the two ends of the sieve tubes. Changes in phloem turgor can alter the vascular plasma membrane permeability via an effect on carriers (Thorne, 1985). Turgor and phytohormones are proposed to be signals for transmission of sink demand to the phloem loading process (Giaquinta, 1983; Baker, 1985, cited in Patrick, 1988). The possibility that some other salt induced resistances occur throughout the route to the source tissues must also be considered. Some of these possibilities were considered in Section 3.2.

It is speculated here that there is a negative feedback effect on sugar transport resulting from accumulation in the grain cavity.

This is consistent with the observations in the present study that salinity reduced sucrose export from the source leaves (Section 3.2).

Finally, if the interpretations made here are correct, the reduced rate of sucrose transport to the endosperm under salt stress is partially responsible for the reduction in dry matter accumulation in the grain recorded in Table 3.5. This pattern of reduction in

Table 3.5: Effect of salinity on grain weights, 35 days after anthesis

	Ten grain weight (mg)	
	Control	Salinized
Fresh weight	606 ± 19.8	530 ± 1.7
Dry weight	244 ± 9.3	220 ± 2.4
Water content	362 ± 16.9	310 ± 4.1

grain yield under saline conditions has been frequently reported in the literature. Here the values given in Table 3.5 show that a reduction in grain weight due to salinity occurred which reflects the reduced rate of grain filling as one of the components of the effect of salinity. Another component of decreased grain yield could be the duration of grain filling which may become critical in decreasing grain weight in the late period of grain growth.

4. GENERAL DISCUSSION

As discussed earlier (Section 3.4), there is a downhill gradient in sucrose concentration from the phloem to the endosperm which should be maintained by processes which reduce the sugar concentration in the endosperm. These processes could be either sucrose hydrolysis or its relatively direct conversion into other compounds, mainly starch.

Sucrose is known to be hydrolysed during movement into the endosperm in C_4 plants (Glasziou and Gayler, 1972, cited in Thorne, 1985). The cavity sap of wheat grain was therefore analysed for glucose and fructose to determine whether any sucrose was hydrolysed during transfer through the cavity. Glucose and fructose were not detected in the cavity sap indicating that sucrose is transported to the endosperm without hydrolysis, supporting the previously held view that in wheat grain extracellular sucrose hydrolysis is not an obligatory step in the passage of sucrose through the ear into the endosperm (Jenner, 1973; Baker, 1978a; Sarah and Chevalier, 1984). Similarly, sucrose cleavage was not observed in barley grains, during movement from the phloem to the endosperm (Chevalier and Lingle, 1983). Salinity had no qualitative effect on this aspect of sucrose transport in the experiments reported here.

Analysis of the endosperm fluid of wheat also showed only small traces of glucose indicating that sucrose is not cleaved before conversion to starch. This confirms reports that the wheat endosperm is better equipped to convert apoplastic sucrose, rather than its

hydrolysed products, to starch (Jenner, 1973; Rijven and Gifford, 1983a). Salinity had no qualitative effect on this sucrose metabolism pathway.

As no hydrolysis of sucrose occurs in the grain endosperm, a relatively direct conversion of sucrose to starch should be the alternative way for starch synthesis. It appears that starch is synthesised through a rapid sucrose conversion reaction, the nature of which can be important in terms of its effect on the sucrose concentration gradient and, in turn, transport. It is known that enzymes are involved in these reactions (Duffus and Cochrane, 1982), and sucrose transport from the sieve tube to the endosperm may thus respond to environmental changes which can influence these enzyme activities.

Starch synthesis in the endosperm is thought by some investigators to be the rate limiting factor for sucrose transport to the grain (Kumar and Singh, 1981; Lingle and Chevalier, 1984; Fisher and Gifford, 1987). Other workers suggest that there is a higher potential for starch synthesis than that usually observed (Jenner, 1970; Gifford and Bremner, 1981a; Ho and Gifford, 1984). These two conflicting possibilities will have quite different effects on the sugar balance, especially under stress conditions. With the central role of starch synthesis, the level of sucrose observed in the cavity may to some extent reflect the rate and efficiency of starch formation. However, with the dominant role for sucrose in starch synthesis, the level of sucrose in the cavity may point to factors reducing movement towards the endosperm. Circumstantial evidence

indicates that under normal conditions, the former situation appears to prove critical in the late grain filling period, and the latter in the early stages of grain fill. The interaction of these processes with salinity will be discussed later.

A further process which may influence sugar transport from the sieve tube to the endosperm is facilitated sucrose transport. If this process happens within the developing wheat grain, the rate-limiting factor for grain filling may not be solely passive movement. Although assimilate transport within the grain is basically down its concentration gradient, the observed chemical properties of the cavity and endosperm fluids indicate the possibility of a facilitated active transport.

To reach the endosperm cells sucrose must cross a number of membranes, including those of the aleurone and/or endosperm cells, and of the sieve element and cells of the maternal tissues. Active transport could thus occur on either side of the cavity. Sugar transport across these membranes may be carrier mediated as it is reported that in one cultivar of wheat they account for 66% of the sucrose uptake by the endosperm (Ho and Gifford, 1984). The carrier transporting the sugar molecule binds a proton, forming a ternary complex as the mechanism for secondary active transport of organic solutes (Baker, 1978). Sucrose at the aleurone membrane is postulated to be co-transported in response to the gradient of electrochemical potential for protons ($\Delta\mu_{H^+}$). After dissociation of the complex at the internal membrane surface, the carrier returns to the external surface. The carrier which is

thought to be involved in active transport may not be the same as that for passive transport.

For the operation of this system, metabolic energy is not directly coupled with the carrier but instead a proton gradient can create an electrochemical potential difference ($\Delta\mu_{H^+}$) across the membrane which can provide the driving force for sugar H^+ -cotransport. The formation and maintenance of such a gradient requires an electrogenic pump which transports H^+ out of the cell (in the case of aleurone cells) without an accompanying ion of negative charge, and without transport of an ion of positive charge in the opposite direction. The occurrence of such proton pumping in higher plants is now widely accepted (Baker, 1978b; Ho and Baker, 1982; Pitman and Lüttge, 1983).

Having such a proton pump within a system will result in a relatively low pH in the apoplast. A pH gradient of 1.5 units was considered more than sufficient together with an average potential membrane of -150 mV, to meet the energy required for the 100-fold accumulation of sucrose measured in *Ricinus* cotyledons (Komor, 1977).

In the current study a low pH was observed in the cavity sap compared with that in the endosperm fluid, with 1.9 pH unit difference (Table 3.4). The pH requirement for a proton/sucrose cotransport therefore appears to be met. The cavity protons could originate from the aleurone and endosperm cells and from the cells of the crease tissues, i.e. phloem and/or other tissues. This is consistent with the suggestion of Ho and Gifford (1984) of an

extrusion mechanism for protons, based on the low pH of the cavity sap which they observed.

The proposed H^+ pump requires the expenditure of metabolic energy which may be provided by ATP hydrolysis or by electron flow associated with certain redox reactions (Baker, 1978b). The coupling of the proton transport to the potential energy of ATP is proposed to be mediated by a membrane-bound ATPase (Baker, 1978a). The localisation of ATPase on the plasma membrane of the sieve element in *Ricinus* and in a number of other species has been demonstrated by some authors (Browning, Hall and Baker, 1980; Cronshaw, 1980; cited in Ho and Baker, 1982). In wheat grains, using enzyme localization techniques and electron microscopy, ATPase activity has been observed on the plasma membrane of aleurone cells, particularly those parts facing the cavity (Didehvar, personal communication). Localisation of ATPase is also reported in the endoplasmic reticulum and Golgi apparatus of barley aleurone cells. These two organelles were viewed as components of the intracellular transport system that facilitates movement of ATPase to autophagic vacuoles and to the cell exterior (Jones, 1987). In the present study a large number of mitochondria were observed in aleurone cells of control plants of wheat (Section 3.3). This may lend support to the proposal that these cells play an essential role in energising the uptake processes. Thus it is reasonable to propose here the existence of an electrogenic proton pump on the membrane of the aleurone cells. This can support the hypothesis that in wheat grain sugar-proton co-transport may be involved in sucrose uptake by the endosperm cells (Ho and Gifford, 1984). This is also consistent with the view that

passive diffusion in the crease region is unlikely to be adequate to meet the sink demand during peak periods of sucrose transfer (Patrick, 1988). This hypothesis proposes the same sucrose transport process as that reported for a number of other plant systems (Malek and Baker, 1977; Baker, 1978a; Ho and Baker, 1982; Rijven and Gifford, 1983a; Baker, 1985; Baker and Chaudhry, 1986).

The sensitivity of various enzymes to the ionic status of the cells would affect the processes involved. A high performance of these processes depends on the concentration of certain ions and therefore the sugar transport may respond to ionic conditions via this sensitivity of the enzymes. It is suggested that the presence of K^+ has a positive effect on the ATPase activity and thus in turn on the sucrose/proton co-transport. With regard to phloem loading, the possibility of ATPase stimulation by high K^+ levels in the phloem sap has been considered (Ho and Baker, 1982). It has also been observed that the presence of K^+ as well as low pH promoted uptake of sucrose from the free space in *Ricinus* (Malek and Baker, 1977; see also Marschner, 1986; Patrick, 1988). The K^+ concentration of the endosperm fluid observed here (Table 3.4) is high enough to provide suitable conditions for ATPase activities. The concentration of K^+ within the phloem sap is also reported to be high (MacRobbie, 1971; Malek and Baker, 1977) and this can serve to stimulate the ATPase for a high performance regarding the probable loading and unloading of sugar into and out of the sieve tubes.

The K^+ concentration in the endosperm cells is maintained high relative to its level in the cavity sap where it is much lower.

Although K^+ is being transported against its chemical gradient, an active transport need not be postulated. For uncharged solutes like sucrose, the driving force is the gradient of chemical potential only, while for ions the driving force has an additional component arising from the electrical potential difference (Baker, 1978b). Therefore the tendency of ions such as K^+ to move down their concentration gradient, i.e. from the endosperm towards the cavity can be balanced by the tendency to move in the opposite direction down an electrical potential gradient. According to the Nernst equation at a negative electropotential of -59 mV, K^+ would be in electrochemical equilibrium across a membrane even if its concentration in the aleurone or endosperm cells were 10 times higher than that in the cavity sap. In the present study it is possible that a proportion of the K^+ concentration gradient observed between these two compartments (Table 3.4) can be maintained by passive K^+ transport, depending on the magnitude of the membrane potential, which is not known. There may also be some secondary active transport of K^+ energised by an electrogenic H^+ pump. Potassium could be transported from the sieve tube to the cavity through either a passive or facilitated transport process.

If it is accepted that some of the requirements for a facilitated transport of sucrose within the grain have been met by the present observations, the model of proton sucrose co-transport given by Malek and Baker (1977) may be modified to fit the situation in the cavity of the wheat grain (Fig. 4.1). Sucrose transport through the aleurone membrane is proposed to be coupled with the inward movement of protons down the gradient of increasing pH. The

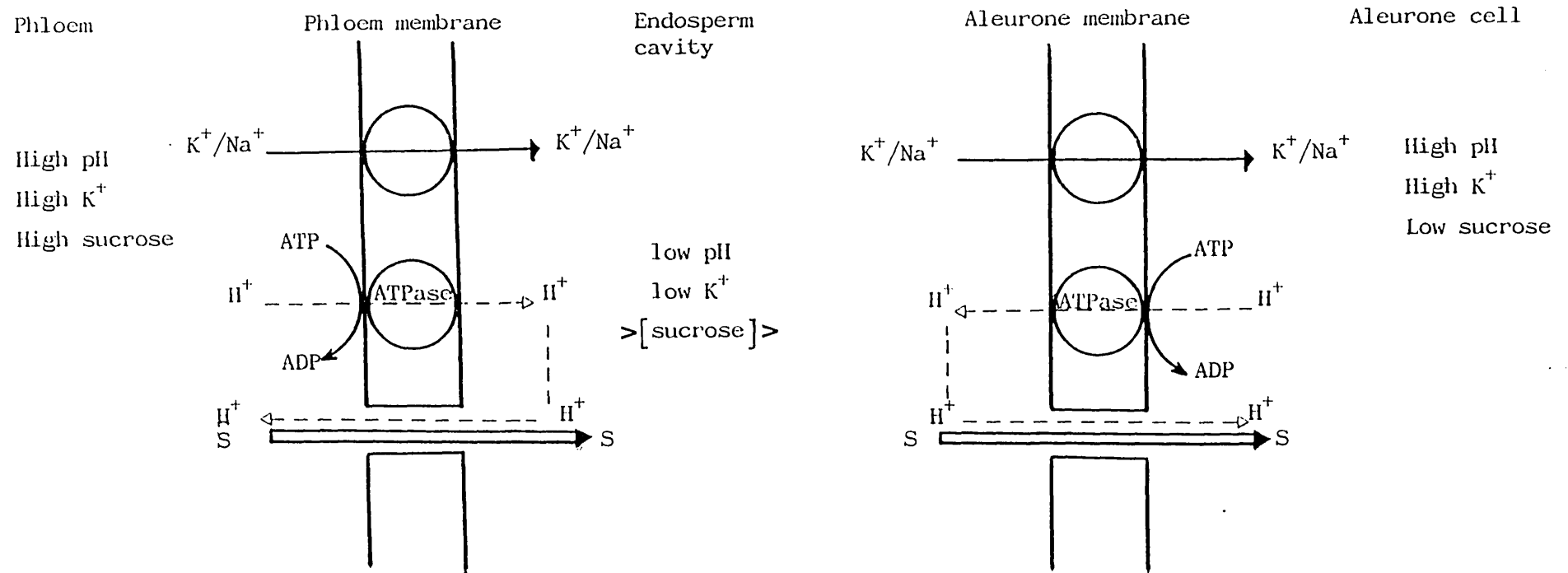


Fig. 4.1 A scheme for a proton-sucrose antiport at the plasma membrane of the phloem and a proton sucrose symport at the aleurone plasma membrane, driven by a proton pumping ATPase which extrudes protons into the endosperm cavity thus maintaining the necessary proton gradient.

gradient is maintained by a proton efflux pump from the endosperm to the cavity. This pump is envisaged to be a membrane bound ATPase which pumps protons when ATP is hydrolysed.

At the maternal side of the cavity it is known by analogy with other species that the pH of the phloem sap will be somewhere between 7.4-8.7 and its K^+ concentration about 100 mM (Malek and Baker, 1977). The sucrose concentration of the phloem sap has been reported as being about 500 mM (Fisher and Gifford, 1986; Patrick, 1988). Potassium could move down the gradient by diffusion or facilitated transport across the phloem membrane towards the cavity. Here there may be competition with Na^+ , resulting in higher levels of Na^+ in the cavity particularly under saline conditions. Sucrose may also be transported down its gradient from the phloem to the cavity, either by diffusion or by proton antiport. In order to maintain the proton gradient between the phloem and the cavity sap there may be an energized pump transporting protons from the phloem or other maternal tissues into the apoplast or cavity.

Salinity has a depressive effect on the transport of sucrose to the endosperm. This is reflected in the reduction in grain dry weight of plants under salt stress (Table 3.5). In Section 3.4 it was reasoned that the reduced sucrose level in the endosperm may not originate from any decrease in CO_2 uptake or sugar mobilization since sucrose is found to be accumulated in the cavity under saline conditions. The question therefore is what does decrease the amount of starch in the grain under salt stress? The possibility that the rate-limiting factors for grain filling under normal conditions have

been reinforced under salt stress may be the major effect. The interaction between some of the processes outlined above and salinity will now be considered.

One explanation for the reduced amount of starch in the grain (grain dry matter) under saline conditions could be a reduced capacity for starch synthesis due to the effect of salt on the enzymes involved in this process. A lower starch content and higher soluble carbohydrate (particularly sucrose) level have been observed in leaves in which a high proportion of K^+ is replaced by Na^+ (Hawker Marschner and Downton, 1974). However, this effect does not seem to be the dominant factor here for two reasons. 1) If the depressive effect of salinity starts with starch formation, then sucrose should be accumulated within the endosperm. This did not occur and in fact sucrose levels decreased compared with the controls (Table 3.4). 2) The increase in the amount of Na^+ in the endosperm is physiologically trivial, and this small amount of Na^+ is unlikely to affect starch metabolism and may even have a positive effect on starch synthesis, as it is reported that Na^+ stimulated sucrose conversion into insoluble matter (Rijven and Gifford, 1983b). The reduced amount of K^+ in the endosperm under saline conditions should not have drastically affected the rate of starch synthesis as the K^+ level of the endosperm fluid under these conditions (Table 3.4) is still within the K^+ requirement range for ADP-glucose starch synthase (Wyn Jones, Bradey and Speirs, 1979). In barley K^+ appears to increase grain yield more by its effect on assimilate supply (sucrose) than by its influence on the rate of synthesis of storage products (Beringer and Haeder, 1981).

The accumulation of sucrose in the cavity indicates that there may not be limitations on sucrose delivery to this compartment, but some restriction may be imposed on sucrose transport from the cavity to the endosperm. Sucrose uptake by the endosperm cells is partially dependent on assimilate entry into the endosperm intercellular space from the cavity. This entry of sucrose into the endosperm apoplast may be one restriction point, i.e. through the intercellular pathways of the aleurone and endosperm tissues which, with the development of cell wall thickenings demonstrated here, would appear to provide severe limitations to solute transport through this pathway (Section 3.4). Consequently, the endosperm cells would receive sucrose only through the symplastic continuity from the aleurone cells. Plasmodesmatal connections between these cells have been observed in the present study (Section 3.4).

Uptake of sucrose through the aleurone cells would occur through the outer face of these cells as a consequence of the partial blockage of the cell walls. These may also be vulnerable to the adverse effect of salinity as far as active transport is concerned due to the effect of alterations in the ionic content of endosperm cells on certain enzyme activities. ATPase is postulated to be stimulated by high K^+ levels in the phloem sap so that the influx of K^+ into the sieve elements during phloem loading may exert a positive feedback (Ho and Baker, 1982; see also Briskin and Pool, 1983, cited in Marschner, 1986 for the stimulatory effect of K^+ on Mg-ATPase). With the decreased level of K^+ in the endosperm resulting from salinity (Table 3.4), the ATPase activity would be

less, resulting in a reduced proton pumping at the aleurone and endosperm cell membranes.

In addition, some reduction in ATPase activity might result from an adverse effect of the higher concentration of Na^+ present in the endosperm under saline conditions. The higher Na^+ concentration in the cavity may also have affected the aleurone membrane-bound ATPase. Sodium stimulation of ATPase is reported to be suppressed when the plants were exposed to salinity (see Kuiper, 1984). It is also reported that the ATP/ADP ratio is reduced as salinity is increased (see Rains, 1972; O'Neill and Spanswick, 1984, cited in Marschner, 1986). Also of relevance is the report that the distribution of Na^+ -stimulated ATPase amongst higher plants is restricted (Jennings, 1976). Also of significance is the reduction in the number of mitochondria in aleurone cells of plants under salt stress, suggesting that less respiratory energy is available for uptake processes (Section 3.3).

A further explanation which may be proposed for the reduced sugar transport is the effect of some phytohormones on the electrochemical potential difference for protons ($\Delta\mu_{\text{H}^+}$). Abscissic acid (ABA) for example, is reported to have an inhibiting effect on the proton efflux (Malek and Baker, 1978). Zeevaart (1977) observed that the ABA level in phloem exudate of *Ricinus* plants increased from 1.0 to 1.7 $\mu\text{g ml}^{-1}$ under water stress conditions. Hoad (1978) also reported an increase in ABA level from 200 to 700 $\mu\text{g ml}^{-1}$ in seeds of white lupin plants subjected to water stress. The level of this hormone has been found to increase in the xylem exudate

of maize roots exposed to salinity (Lachno and Baker, 1986). A rise in ABA level has also been observed in leaves of wheat and some other species when wilting under water stress (Aspinall, 1980; Milborrow, 1981).

Since the pH of the cavity sap remained constant with salinity, any reduction in proton extrusion from the endosperm should be paralleled by a decrease in the proton-sucrose co-transport from the cavity. Sucrose accumulation in the cavity may be an indication of this reduction. It would appear that because of the low sucrose level in the endosperm resulting from a reduced sugar transport, the rate of starch synthesis and subsequently grain yield is decreased. A disturbed starch-sugar balance was reported as responsible for the reduced yield under salt stress in developing grains of barley (Gill, 1979).

The drop in the level of K^+ present in the endosperm cells observed in this study may be attributed to salinity-induced structural modifications. Additionally the reduced proton pumping may contribute to this reduction as a direct result of the Na^+ effect on ATPase activities. Moreover, K^+ uptake may also be affected by any extrusion of Na^+ from the endosperm into the cavity.

Whether the sucrose transport from the sieve tube to the cavity *per se* is directly reduced by salinity is not evident. It may be speculated that the unloading of sucrose would be reduced under salt stress, although the nature of this reduction may not be the same as that for the aleurone layer. Such a reduction may be due

to the increased Na^+ level rather than the decreased K^+ concentration. The disturbed ratio of K^+/Na^+ could have an effect on the enzymes involved in energy-producing processes. The resultant reduction, however, is not reflected in a reduced sucrose level in the cavity but could contribute to a decreased sucrose mobilization from the source leaves.

It may be concluded that an active system could be involved in sucrose transport from the phloem to the endosperm, particularly between the cavity and the endosperm. This system may be affected by alterations in ionic content induced by salt stress, which together with structural modifications of the aleurone cells, could create severe restrictions on sucrose transport to the endosperm.

5. GENERAL CONCLUSION

Salinity is a deteriorating problem in many regions of the world which restricts the growth and ultimately reduces the yield of crop plants. Improvement of plants, as a biological solution for the utilization of salt-affected lands, requires adaptations of physiological characters and functions to saline environments. This reflects the need for considerable information on the physiological aspects of plants under salt stress.

Some of the physiological responses of wheat plants to salinity, which are related to grain yield, have been examined in the course of the present study. These include photosynthesis, assimilate partitioning, and grain filling. Changes imposed by salinity were observed in almost all aspects studied. A reduction in photosynthetic rates, observed at different salt levels with different cultivars, can be related to a reduction of the stomatal openings, as transpiration rates were decreased, and/or to an increased mesophyll resistance, as leaf structural modifications reduced intercellular air spaces. Whatever the cause of the reduced CO_2 uptake the effect of salinity was also reflected in reductions of the RGR and NAR. These reductions were less with the salt-tolerant cultivar Sholeh, but this cannot simply be attributed to its intrinsic tolerance, since there may be some secondary effects of the interaction between salinity and growth. It may be suggested that reductions in photosynthesis are not directly responsible for reductions in dry matter production, but that non-utilized carbohydrates may affect CO_2 uptake rates and/or are used for osmotic

adjustment.

Assimilate partitioning is affected by salinity, more in allocation and translocation of photosynthates rather than distribution into the plant organs. Lower rates of carbon allocation are observed from the flag leaf to the ear in saline conditions, while considerable amounts of carbon are present in the fed leaf. This may provide grounds for the suggestion that the availability of photoassimilates is not the rate limiting factor in grain yield. The cultivar Sholeh showed significantly higher rates of carbon allocation under salt stress. The small fractions of carbon allocated to other parts of the plant are not comparable to the ear fractions and may be due to the relatively later stage of growth at which it was studied (14-16 days after anthesis). However, these values show different patterns of carbon partitioning under salt stress, i.e. more to the tillers with Inia 66 and more to the roots in Sholeh. The performance of the latter is consistent with its high root/shoot ratio observed in the growth analysis study, indicating that there may be a link between assimilate distribution pattern and salt tolerance, although a causal relationship is not implied.

Collection of sap from the endosperm cavity of the grains, while they are attached to the plants, can provide information on very similar conditions (in vivo). This was achieved using glass capillary tubes and filter paper techniques.

ATPase activities located in the plasma membranes of the aleurone cells, particularly those parts facing the cavity (Didehvar,

personal communication), and the large number of mitochondria observed in aleurone cells in normal conditions indicate that energy metabolism may be involved in assimilate transport into the endosperm. These observations together with the low pH in the cavity sap (5.7-6.3) and its high value in the endosperm fluid (7.5-7.9), may provide supportive evidence for the hypothesis of a proton co-transport movement of sucrose from the cavity to the endosperm similar to that described for sucrose transport in other systems (Ho and Baker, 1982; Rijven and Gifford, 1983a; Baker, 1985).

The 50% increase in the concentration of sucrose in the cavity sap and its 42% decrease in the endosperm fluid under salt stress conditions indicates an inhibitory effect of salinity on the transport of sucrose from the cavity to the endosperm. Alterations in the ionic composition of these fluids as well as ultrastructural modifications of the aleurone cells may be responsible for this inhibitory effect. In the cavity sap $[K]^+$ and $[Na]^+$ were increased by 95% and 152% respectively, whereas in the endosperm fluid $[K]^+$ decreased by 22%, but $[Na]^+$ increased by 42%. Altered ratios of K^+/Na^+ in the fluids may have influenced metabolism, i.e. ATPase activities within the aleurone cells, reducing the energy supplied to the postulated active system of sucrose transport. This will affect any symplastic movement of sucrose in saline conditions. On the other hand, under salt stress the apoplastic route (suggested by Jenner, 1974b) can also be partly blocked through suberized wall thickenings of the aleurone cells.

In general, the effect of salinity can be observed in almost

all structural, physiological and chemical aspects of plants. These features do not seem to be affected individually by salinity, but circumstantial evidence indicates that these events are closely linked. Among these processes photosynthesis and assimilate partitioning do not appear to reflect any primary effects of salinity on the plant. It is proposed that the limitations made by salinity on grain yield reflects events within the caryopsis where the inhibitory effects of a disturbed ratio of K^+/Na^+ will affect sucrose transport from the cavity to the endosperm. This view is consistent with the highly documented hypothesis that it is primarily the sink activities which regulate source-sink relationships.

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APPENDICES

APPENDIX I

Standard compost

- 1. 4 parts medium peat (sphagnum moss) + 1 part 5-6 mm grit
- 2. Bio potting base (constituents below) containing NPK + trace elements and base of neutralising agent to bring pH of compost to 6.5.

Analysis Bio P. base

NPK Fertilizer
6.8:2.5:6.1

Total nitrogen (N)	6.8%
Total phosphorus pentoxide (P_2O_5)	2.5% (P 1.1%)
Phosphorus pentoxide (P_2O_5) soluble in water	2.5% (P 1.1%)
Total potassium oxide (K_2O)	6.1% (K 5.1%)
Magnesium oxide (MgO)	10.5% (Mg 6.3%)
Boron	0.13%
Copper	0.13%
Iron	0.85%
Manganese	0.17%
Zinc	0.27%
Molybdenum	240 mg/kg
Peat conditioner (inner bag)	
Neutralising value (CaO)	51.3%

APPENDIX II

Fertilizer

The fertilizer used was a mixture (50/50) of Libfeed 214 and Libfeed 311, constituents below:

I. Libfeed 214

Total N (as N)	16%
Total phosphorus pentoxide (P_2O_5)	8.0% (P 3.5%)
Phosphorus pentoxide (P_2O_5) soluble in water	8.0% (P 3.5%)
Phosphorus pentoxide (P_2O_5) insoluble in water	0%
Total Potassium oxide (K_2O)	32% (K: 26.5%)
*Magnesium oxide (MgO)	502 mg/kg (Mg 301mg/kg)
*Iron (as Fe)	67 mg/kg
*Manganese (as Mn)	34 mg/kg
*Copper (as Cu)	34 mg/kg
Boron (as B)	17 mg/kg
*Zinc (as Zn)	12 mg/kg
Molybdenum (as Mo)	1 mg/kg

Low in chlorin

*Chelated elements (Boron and Molybdenum present as inorganics)

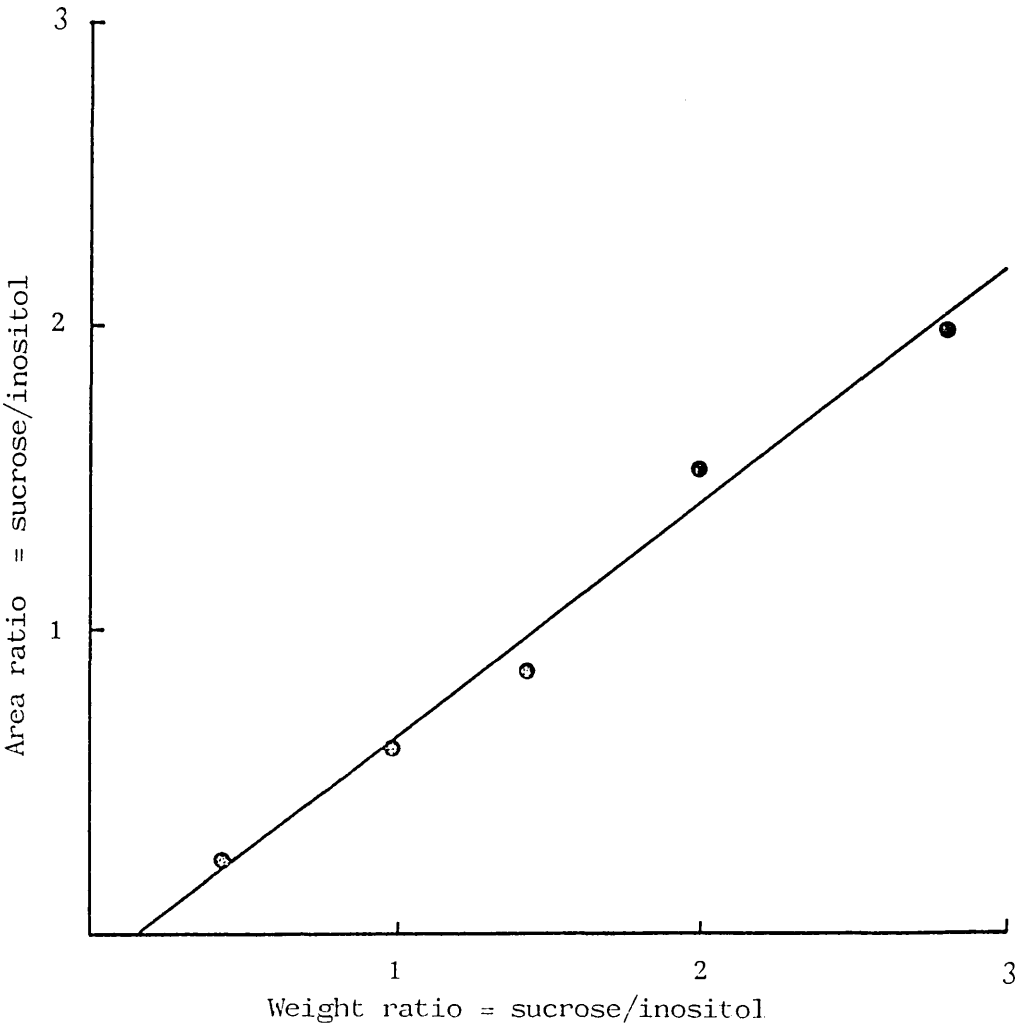
Contd over....

II. Libfeed 311

Nitrogen (as N)	31.0%
Soluble Phosphorus (as P_2O_5)	10.0%
Potassium (as K_2O)	10.0%
*Magnesium (as Mg)	225 ppm
*Iron (as Fe)	67 ppm
*Manganese (as Mn)	34 ppm
*Copper (as Cu)	34 ppm
Boron (as B)	17 ppm
*Zinc (as Zn)	12 ppm
Molybdenum (as Mo)	1 ppm

*Chelated elements (B and Mo do not form chelates)

APPENDIX III



Calibration of the G.L.C. for sucrose

APPENDIX IV

Relative retention time and elution temperature of fructose, glucose and sucrose separated by G.L.C.

	Retention time (min)	Elution temp (°C)	No. of peaks
D-fructose	2.3	194	1
D-glucose	3.5	201	1
Myo inositol	5.1	211	1
Sucrose	12.6	256	1

APPENDIX V

There are some limitations to the technique used for sap collection with regard to the grain cavity size required. Growing plants under saline conditions showed that at the level of salt stress employed for the earlier experiments the plants failed to produce grains with cavities large enough for the microcapillary tubes to be inserted. Therefore, the salt concentration was reduced from 5 g l^{-1} to 4 g l^{-1} , and the duration of salt treatment shortened so that salinization started 30 days after germination, i.e. more than 2 weeks before heading. Cultivar difference was another limitation, as Sholeh proved impossible to use for this technique. Therefore the study was carried out only on Inia 66. Of the environmental factors, when the temperature rises above 21°C , the cavity size becomes too small to sample. To avoid this problem, the experiments were conducted during times when the temperature was below this critical value.

APPENDIX VI

Leaf areas and dry weights for the experiment of growth analysis; each value is the mean of three replicates

Parameter	Treatments					
	S_1V_1	S_2V_1	S_3V_1	S_1V_2	S_2V_2	S_3V_2
<u>First harvest:</u>						
Leaf area (cm ²)	85.5	68.0	79.7	87.3	103.6	82.4
Dry weight (g)	0.48	0.39	0.5	0.42	0.5	0.43
<u>Second harvest:</u>						
Leaf area (cm ²)	208.9	286.7	178.5	535.9	382.9	364.7
Dry weight (g)	2.25	1.95	1.49	3.19	2.42	2.20

Levels of salinity (NaCl):

S_1 = control
 S_2 = 2.5 g l⁻¹
 S_3 = 5 g l⁻¹

Cultivars:

V_1 = Inia 66
 V_2 = Sholeh