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Tillering response to salinity in contrasting wheat cultivars

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Abstract

Background and Aims Tillering is essential for the establishment of yield in wheat plants, but the function of tillering in salt tolerance is rarely investigated. Therefore, this study aimed at: (i) identifying the effects of salinity on the growth of individual tiller by manipulating the tillers of the different orders; (ii) obtaining information on the possible role of tillering in improving salt tolerance of wheat; and (iii) understanding the possible mechanisms of salt tolerance related to salt distribution among tillers of contrasting wheat cultivars.

Materials and Methods Contrasting spring wheat (*Triticum aestivum* L.) cultivars (salt-tolerant; salt-sensitive) were grown in soil with or without salinity in a greenhouse. Five levels of detillering treatments were applied to understand the mechanisms of tillering related to salt tolerance. The plants were harvested at both vegetative growth stage and maturity. The growth parameters, biomass, yield components and mineral element contents (Na^+ , Cl^- , K^+ , Ca^{2+} , Mg^{2+} , and NO_3^-) in the mature leaves of the mainstem tiller and subtillers were determined.

Key Results Salinity affected the dry weight and grain yield of the subtillers more than those of the mainstem tiller in contrasting wheat cultivars. The mineral contents in the leaves revealed that more reduction in the growth of subtillers under saline conditions was due to higher accumulation of Na^+ and Cl^- and /or greater reduction in NO_3^- , K^+ , Mg^{2+} or Ca^{2+} concentrations compared with the growth of mainstem tiller. Under saline conditions, the differences in the ratios of salt to nutrient between the mainstem tiller and subtillers became larger in the salt-sensitive cultivars than in the salt-tolerant ones. We also found that the growth of subtillers in the salt-sensitive cultivars is more sensitive to salinity than that in the salt-tolerant cultivars. The results, therefore, indicates that plant salt tolerance may be depending on the number of subtillers. Regardless of cultivars, the less tiller number per plant could result in the higher Na^+ accumulation and higher ratios of Na^+/K^+ and/or $\text{Na}^+/\text{Ca}^{2+}$ under saline conditions, indicating that there may exist the collective defense to resist the salt toxicity by increasing ion selectivity in tillers. Furthermore, fewer tillers per plant for both contrasting cultivars not only increased the dry weight and grain yield per tiller, but also altered salt tolerance in the different tillers and the whole-plant. Under saline conditions, only one tiller or two tillers per plant increased the reduction

in dry weight and grain yield per plant in the salt-tolerant cultivar due to higher accumulation of toxic ions, indicating the advantage of having more tillers per plant. In the salt-sensitive cultivar, however, the reduction in dry weight and grain yield per plant with fewer number of tillers became smaller, suggesting that for salt-sensitive cultivars, the plant growth under saline conditions can be improved by having fewer tiller number per plant.

Conclusions Results in this study suggest that tillers may have the function of the collective defense. The tillers could control the plant salt tolerance by the subtillers because they accumulate higher Na^+ and Cl^- than the mainstem tiller, which lead to the greater difference in the growth between mainstem tiller and subtillers. Under saline conditions, the accumulation of mineral contents as well as tiller growth in tillers can be regulated by the different tiller number per plant. In the salt-tolerant cultivar, the exclusion of toxic ions in the individual tiller proposes that more tillers enhance the plant salt tolerance, while in the salt-sensitive cultivar, fewer tillers are better to enhance the tiller growth of wheat plants because the tissue tolerance to toxic ions is increased.

Zusammenfassung

Hintergründe und Ziele Die Bestockung ist von herausragender Bedeutung für die Ertragsbildung der Weizenpflanze, allerdings ist die Bedeutung der Triebzahl für die Salztoleranz der Pflanzen bis jetzt nur wenig erforscht worden. Aus diesem Grunde hatte diese Arbeit die Ziele (i) die Einflüsse von Salzstress auf das Wachstum der einzelnen Bestockungstriebe von Weizenpflanzen zu bestimmen, indem die Bestockungstriebe unterschiedlichster Ordnung gezielt beeinflusst wurden, (ii) mehr Informationen über die Bedeutung der Bestockung zur Verbesserung der Salztoleranz bei Weizen zu gewinnen und (iii) ein besseres Verständnis für die Mechanismen der Salztoleranz, die möglicherweise in Zusammenhang mit der Salzverteilung zwischen den Bestockungstrieben stehen, in unterschiedlichen Weizensorten zu entwickeln.

Material und Methoden In ihrer Salztoleranz stark unterschiedliche Sommerweizensorten (*Triticum aestivum* L.) (salztolerant; salzempfindlich) wurden in Gewächshausversuchen in Boden mit bzw. ohne Salzstress angebaut. Es wurden fünf Behandlungen der selektiven Triebentfernung durchgeführt, um die Bedeutung der Bestockung für die Salztoleranz der Gesamtpflanze zu verstehen. Die Pflanzen wurden an zwei Terminen beerntet, während der vegetativen Wachstumsphase und zum Reifestadium. Die Wachstumsparameter, der Biomasseaufwuchs, die Ertragsparameter und der Nährstoffgehalt (Na^+ , Cl^- , K^+ , Ca^{2+} , Mg^{2+} und NO_3^-) der ausgewachsenen Blätter des Haupttriebes und der Nebentriebe wurden erfasst.

Ergebnisse Der Salzgehalt beeinflusste in den unterschiedlichen Weizensorten die Trockenmasse sowie den Kornertrag der Nebentriebe deutlicher als diejenigen der Haupttriebe. Die Nährstoffzusammensetzung der Blätter zeigte, dass die Wachstumsdepression der Nebentriebe unter Salzstress auf eine höhere Anreicherung von Na^+ und Cl^- und/oder größere Reduzierung der NO_3^- , K^+ , Mg^{2+} , oder Ca^{2+} Konzentrationen verglichen zu den Gehalten im Haupttrieb zurückzuführen war. Unter Salzstressbedingungen waren die Unterschiede zwischen Haupt- und Nebentrieben in ihren Verhältnissen der Gehalte an Salzen zu Nährstoffen in den salzempfindlichen größer als in den salztoleranten Sorten. Das Wachstum der Nebentriebe in den salzempfindlichen Sorten reagierte ausgeprägter auf den Salzstress als dies bei den salztoleranten Sorten der Fall war. Die Ergebnisse lassen deshalb vermuten, dass die Salztoleranz der Pflanzen von der Anzahl der Nebentriebe

abhängig sein könnte. Unabhängig von der Sorte könnte eine niedrigere Anzahl von Nebentrieben unter Salzstressbedingungen zu einer höheren Anreicherung von Na⁺ und einem größeren Na⁺/K⁺- und/oder Na⁺/Ca²⁺-Verhältnis führen. Dies könnte das Vorhandensein einer kollektiven Verteidigungsstrategie der Gesamtpflanze gegenüber Salztotoxicität anzeigen, die darauf beruht die Ionenselektivität der Bestockungstriebe zu erhöhen. Außerdem erhöhte eine geringe Anzahl von Bestockungstrieben pro Pflanze bei den beiden unterschiedlichen Weizensorten nicht nur die Trockenmasse und den Kornertrag pro Bestockungstrieb, sondern veränderte auch die Salztoleranz in den unterschiedlichen Bestockungstrieben sowie in der Gesamtpflanze. Unter Salzstressbedingungen führte eine Bestockungsintensität von nur einem bzw. zwei Trieben pro Pflanze in den salztoleranten Sorten zu einer Reduktion der Trockenmasse sowie des Kornertrages. Dies war durch die höhere Akkumulation toxischer Ionen bedingt und zeigt den Vorteil einer größeren Anzahl von Nebentrieben bei diesen Sorten an. In der salzempfindlichen Sorte dagegen, verringerten sich mit abnehmender Anzahl an Bestockungstrieben die Einbußen an Trockenmasse und Kornertrag pro Pflanze, was darauf hindeutet, dass das Pflanzenwachstum bei salzempfindlichen Pflanzen durch eine geringere Anzahl an Nebentrieben verbessert werden kann.

Schlussfolgerungen Die Ergebnisse dieser Arbeit lassen vermuten, dass die Bestockungstriebe die Funktion einer allgemeinen Verteidigungseinrichtung haben könnten. Die Nebentriebe könnten die Salztoleranz der Pflanze steuern, weil sich in ihnen höhere Gehalte an Na⁺ und Cl⁻ als im Haupttrieb ausbilden, was zu größeren Unterschieden bezüglich des Wachstums zwischen Haupt- und Nebentrieben führt. Unter Salzstressbedingungen kann die Konzentrationserhöhung des Mineralgehaltes in den Nebentrieben ebenso wie das Triebwachstum durch die Anzahl an Bestockungstrieben pro Pflanze reguliert werden. In den salztoleranten Sorten lässt der Einschluss toxischer Ionen in einzelnen Bestockungstrieben vermuten, dass mehr Nebentriebe die Salztoleranz der Pflanze erhöhen, während in den salzempfindlichen Sorten weniger Bestockungstriebe zu einer Erhöhung des Triebwachstums der Weizenpflanzen führen, weil die Toleranz des Pflanzengewebes gegenüber toxischen Ionen erhöht ist.

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General Introduction

1.1 Background

Salinity and agriculture

Salinity in agricultural terms is defined as the concentration of dissolved mineral salts in soil or water. The major dissolved mineral salts in saline soil solutions consist of cations, Na^+ , K^+ , Ca^{2+} and Mg^{2+} and anions, Cl^- , NO_3^- , SO_4^{2-} , HCO_3^- and CO_3^{2-} (Hu and Schmidhalter 2004).

Based on the FAO/Unesco Soil Map of the World, over 6% of the land throughout the world (= 800 million hectares) is affected by either salinity or sodicity (FAO 2006). At global level, saline area has a dangerous trend of about 1% per year increase (Massoud 1974; FAO 2006). In arable lands, as much as 20% of irrigated lands are salt-affected soils that tend to be increased with about 10% per year, and there are about 2% of salt-affected soils in drylands (Ponnampereuma 1984; Oldeman *et al.* 1991). As an ever-present threat to agriculture, salinity in arable lands is of major concern because of its challenge for the demands of agricultural product in increasing global populations (Hillel 2000). It is reported that the saline soil has recently caused average about 65, 68 and 62 % yield losses of wheat, rice and sugarcane in Pakistan, respectively (Qureshi *et al.* 2003). From the late 1970s to the late 1980s, salinity had reduced cotton yields from average 2800 to 2300 tonnes/ha in the central Asian republics (Gardner 1997). It is estimated conservatively that annual losses due to salinity in agriculture are about 12 billion US dollars per year and are expected to increase as soils are further affected (Ghassemi *et al.* 1995). Hence, increases in plant salt tolerance are needed to sustain crop productivity in many regions of the world.

Salt tolerance of plants

Plant salt tolerance is defined as the ability of plants in survival and in biomass production over prolonged periods under saline conditions. It could be typically expressed as relative plant biomass or yield on saline versus non-saline soils, or in terms of biomass or yield decrease associated with salinity increase (Maas and Hoffman 1977; Hu and Schmidhalter 2004). The general response of plants to salinity is the reduction in the biomass production, but there are dramatic genetic differences in response to salinity within plant species. According to the salt level of relatively optimal growth, plant species are classified as halophytes (grow better at high salt level) and glycophytes (grow better at low salt level). Most common crops belong to glycophytes, i.e. nonhalophytes. Based on the maximum salt level of tolerance and relative reduction in yield with increasing salinity, common crops are further rated into sensitive, moderately sensitive, moderately tolerant and tolerant ones (Maas 1985). Furthermore, among cultivars (genotypes) of one common crop, genetic variability does also result in difference of their salt tolerance, depending on plant organs and growth stages in combination with environmental factors, and cultivars are rated into salt-sensitive, moderately salt-tolerant and salt-tolerant ones (Zeng *et al.* 2002; El-Hendawy *et al.* 2005a). In any case, the variation of salt tolerance is closely related to leaf growth under salt stress, as leaves are the most sensitive parameter to salinity (Hu *et al.* 1997; El-Hendawy *et al.* 2005a).

Under saline conditions, mechanisms of plant salt tolerance are involved in ion selectivity, ion accumulation, osmotic adjustment, organic solutes and water use efficiency (Shannon 1997). These components may be described more elaborately as: (a) the selective uptake of nutrient ions over toxic ions as a function of maintaining normal nutrient ranges of plant growth; (b) the sequestration of toxic ions in special organs (e.g. vacuoles, glands) as a function of maintaining normal physiology of plant cell; (c) the resistance of decrease of plant osmotic potential as a function of maintaining normal turgor pressure, cell expansion and gas exchange; (d) the efficient uptake for organic solutes (e.g. sugars, proline) as a function of maintaining osmotic balance and preserving enzyme activity; (e) the better water use efficiency (e.g. fewer stomata, higher root-shoot ratio) as a function of decreasing adverse effects of water deficit.

Therefore, plant salt tolerance should depend on the morphological and physiological complexity of plants. The intricate response of plants to salinity could be a result of ionic, osmotic and nutritional interactions under salt stress.

1.2 Response of wheat growth to salinity

It has been reported that wheat (*Triticum aestivum* L.) is moderately tolerant to salinity (Maas and Hoffman 1977). In the field, if the electrical conductivity rise up to about 10 dS m⁻¹, rice will almost die before maturity, while wheat only will have about 28% yield loss (Maas 1985). It is well known that plant growth in wheat is an orderly sequence with time and generally divided into three growth stages, vegetative, reproductive and mature stage. Effects of salinity on a particular stage at whole-plant level have been described in wheat in previous studies and summarized as follows: (1) salinity affects tiller number per plant, size of leaves, leaf number per culm, spikelets per spike and kernels per spike during the vegetative stage (Maas and Grieve 1990; Grieve *et al.* 2001); (2) salinity affects the survival of tiller and florets per spikelet during the reproductive stage (Kirby 1988; Francois *et al.* 1994); (3) salinity affects seed number, seed weight and seed size during the mature stage (Maas and Poss 1989). Therefore, the response of wheat to salinity is the most sensitive during the vegetative stage, less sensitive during the reproductive stage and least sensitive during the mature stage. Furthermore, among cultivars in wheat, they display different and/or identical salt tolerance at the different growth stages due to genetic diversity. For example, El-Hendawy *et al.* (2005a) reported that Kharchia and Sakha 8 are relatively salt-tolerant in all growth stages while Sids 1 and Gemmeza 7 are relatively salt-sensitive during the vegetative stage but become moderately salt-tolerant from the reproductive stage to the mature stage.

Under saline conditions, wheat plants undergo a two-phase growth response to salinity, resulting in the reduction in plant biomass (Munns 1993). The first phase of growth inhibition presents the osmotic or water-deficit effect of salinity outside the plant (soil solution) rather than ion-specific effect (Munns 2002). Greenway and Munns (1980) pointed out that water deficit could decrease cell turgor pressure to limit plant cell expansion under saline conditions. As much, low rates of metabolic activity due to water deficit caused by high salinity cause cells to grow very slowly (McIntyre 2001). The second phase of growth inhibition presents salt-specific effects

(toxicity) of salinity inside the plant (tissue), which is driven by water evaporation in leaves. This phase is characterized by the old leaves having higher salt concentration than in the young leaves at a given time (Munns 1993). In this phase, the genetic variation to salinity in wheat cultivars (sensitive, moderately tolerant, tolerant) presents the plant potential for resisting salt toxicity under saline conditions. Salt-sensitive cultivars show faster transport of salt within the plant and the inability of compartmentalizing salt in vacuoles, resulting in earlier death of leaves (Munns 1993). For salt-tolerant cultivars, they resist salt-specific effects by two ways (Flowers *et al.* 1977; Greenway and Munns 1980; Husain *et al.* 2004): a) salt exclusion, low rates of salt entry into the plant; b) tissue tolerance, low concentration of salt within cell cytoplasm. However, in any case, salt-specific effects on the inhibition of plant growth are due to inducing nutrient deficiency (e.g. K^+ , NO_3^-) and/or imbalance (e.g. Na^+/K^+ , Cl^-/NO_3^-) of plant, but there is a different degree among cultivars, resulting in their differences at photosynthetic capacity, net assimilation rate and enzyme activity (El-Hendawy *et al.* 2005b).

1.3 Roles of tillering in wheat growth and effect of salinity on tiller growth

1.3.1 General pattern of tillering in wheat

The wheat seedling appears as a single blade at first, which starts branching at or near the surface of the ground relatively early in its development. The culms of seedling are developed from seed and leaf axils on its shoots. The first culm to emerge from the seed is termed the mainstem tiller (MS), and any other culm to emerge from shoots is termed the subtiller (Fig. 1.1). According to the positioning of subtiller buds in relation to the mainstem tiller, sub tillers are further identified as primary, secondary, tertiary, and so on (Klepper *et al.* 1982). Primary tillers are those arisen from leaf axils of the mainstem tiller, with a designation of TN , where N refers to the parent leaf number of the mainstem tiller, e.g. T1 emerging from the axil of leaf 1 on the MS. Secondary tillers are those arisen from primary tiller leaves and designated as TNN , where the first N refers to the parent primary tiller number and the second N is the parent leaf number of the parent primary tiller, e.g. T11 emerging from the leaf 1 of the primary tiller T1. Similar naming systems are applied for tertiary and higher order

tillers. Based on tillering capacity, a plant may or not produce subtillers during the growth.

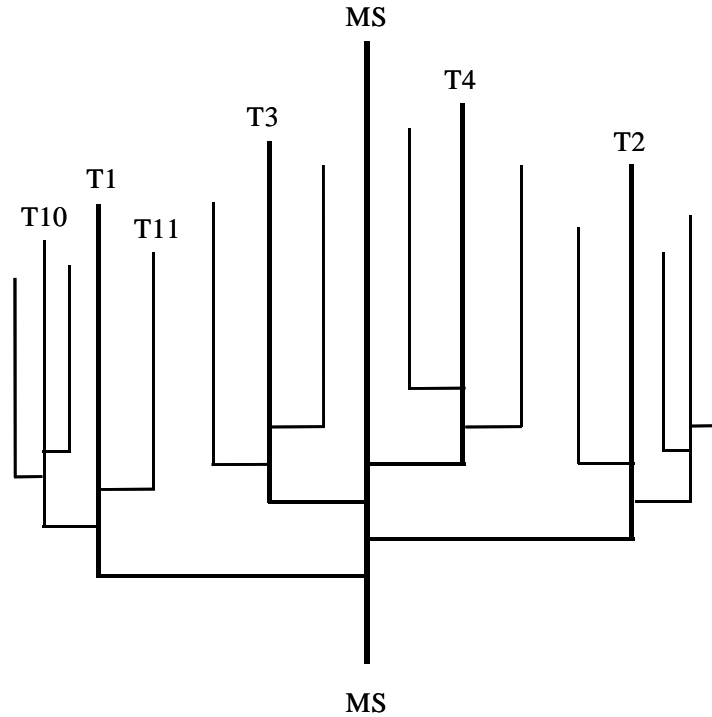


Figure 1.1 Sketch of tiller distribution in the plants of wheat. MS: mainstem tiller; T1, T2, T3 and T4: primary tillers; T10 and T11: secondary tillers.

1.3.2 Relationships between tiller and yield

The yield potential of wheat (*Triticum aestivum* L.) is highly dependent upon the tillers of plant that are markedly affected in the early life cycle. It has been reported that tillering regulated grain yield by its predominant influence on the spike number in wheat (Simons and Hunt 1983). Lupton *et al.* (1974) proposed that both the maximum tiller number and the proportion of tiller death contributed to the differences of both spike number and yield between cultivars. Therefore, the tillering pattern, a particular pattern of cultivars to adapt to particular environments, is of importance in establishing grain yield.

The tillering pattern is involved in tiller emergence, tiller number, tiller mortality and tiller survival (Darwinkel 1980; Hucl and Baker 1989; García del Moral and García del Moral 1995). Darwinkel (1980) pointed out that the time of tiller emergence largely determined subsequent grain yield of tiller in winter wheat. Early

tiller emergence appears to be relatively less unaffected in grain yield compared with late tiller initiation. It is further elaborated by the report of Hucl and Baker (1989), in which the mainstem, T1, T2 and T3 of spring wheat contributed an average of 26, 22, 19, 12% to plant grain yield under a semiarid environment, respectively. García del Moral and García del Moral (1995) noted that tiller survival (i.e. spike-bearing) depends on tiller emergence and the subsequent growth of tillers to form spikes. However, the competition between tillers for a limited supply of resources may suppress the growth and formation of spike-bearing tillers, resulting in the reduction in grain yield (Lauer and Simmons 1988). In order to improve grain yield, two breeding strategies for tillers have been proposed: 1) to develop low-tillering cultivars (Common and Klinck 1981); or 2) to select high-tillering cultivars with low tiller mortality (Benbelkacem et al. 1984). Low-tillering cultivar was found to present lower competition of nutrient within tillers, longer tiller growth and better seed-set per tiller (Dofing and Karlsson 1993). For high-tillering cultivars, low tiller mortality could increase the formation of spike-bearing tillers on a single plant.

However, in any strategy, tiller growth plays a key determinant of grain yield by affecting the spike formation on tillers. The better spike development could raise yield capacity of spikes, which is highly dependent on the number of fertile spikelets, the number of kernels per spike and weight per kernel. Hucl and Baker (1989) found that the early seeding date could produce significantly more spikes per unit area than the late seeding date under field conditions due to longer periods of tiller growth. It is proposed that the delayed seeding may not affect tiller emergence (Stern and Kirby 1979), but causes the severe tiller death and sterility in higher order tillers to drastically reduce grain yields in plants (Black and Siddoway 1977). As much, the optimum effective plant population may also be beneficial to tiller growth. Darwinkel (1978) observed that wheat could show the maximum grain yield at a moderate plant population by the maximum contribution rate of tillers. High plant population could cause higher tiller mortality and fewer spikelets and kernels on spike-bearing tiller (Darwinkel 1978; Sharma 1995).

The contribution of non-survival tillers to the growth of survival tillers is still in the argument. The spikeless tillers may accumulate assimilate and nutrients to support the growth of spike-bearing tillers (Palfi and Dezsi 1960; Lupton and Pinthus 1969). However, non-survival tillers could also be the competitors for assimilate and

nutrients (Langer and Dougherty 1976). The important aspect is that, regardless of the interaction between non-survival and survival tillers, tiller mortality appeared to cause direct yield reduction in wheat (Sharma 1995).

1.3.3 Dynamic development of tiller formation

Tiller development is defined as an interaction of physiological and environmental events, controlled by internal and external factors. In general, tillering starts when the fourth leaf on the mainstem emerges and tillers do not emerge until their parent leaf is fully expanded. There is no way to interpret tiller formation without sufficient knowledge for factors that affect tiller growth.

Temperature

Tiller formation and development are heat driven, controlled by temperature. It is proposed that temperature affects wheat tillering by its effects on morphogenetic characters of tiller, i.e. leaf emergence. Bos and Neuteboom (1998) pointed out that leaf appearance could be considered to be a function of tiller appearance at early growth stages of plants. An optimum temperature of tillering is consistent with that of leaf emergence (Friend 1965). Increasing temperature could improve the rates of tiller appearance and leaf emergence until reaching an optimum temperature (22-25°C) although the raised ratio of tillers is lower than that of leaves (Friend 1965; Cao and Moss 1989).

Effects of temperature on tillering have been postulated to be related with the supply of assimilates, in which high temperatures inhibit the translocation of assimilates from stem to other meristematic tissues (Friend *et al.* 1962; Langer 1963; Aspinall and Paleg 1964). Duru and Ducrocq (2000a) further revealed that high temperatures accelerate lamina expansion rate and decrease lamina growth duration to reduce the assimilate supply required for tiller emergence and development. However, temperatures lower than optimum for tiller emergence from the late vegetative stage could decrease floral sterility on the tiller to reach the maximum grain-bearing tiller (Porter and Gawith 1999).

Light

Light intensity is an important factor in regulating the tiller formation. High light intensity can promote the rate of tillering by reducing leaf number interval between tiller emergences, which is considered as an expression of the increased plant growth due to the enhanced photosynthesis (Friend 1965). In contrast, low light density reduces tillering due to light competition among plants, bringing about low availability of local assimilate for tiller appearance (Bos and Neuteboom 1998). As much, light competition could further affect tillering by mediating responses of plant to light (photomorphogenesis) due to the variation of light quality. It has been reported that lowered red: far-red ratio plays a role in suppressing tiller formation on its filling-site in wheat (Kasperbauer and Karlen 1986; Casal 1988) and barley (Davis and Simmons 1994). As one of the critical determinants of plant growth, photoperiod affects tillering capacity of wheat by altering the duration of vegetative stage and the rate of leaf emergence. With the increase of photoperiod, the duration from sowing to terminal spikelet initiation and rate of leaf emergence were decreased in wheat, resulting in delayed tillering time and low rate of tiller emergence (Lafarge 2000; Miralles and Richards 2000). If plants are grown in a continuous light, leaves could be burned (Jiang *et al.* 1998). Duggan *et al.* (2002) further demonstrated that long photoperiod at low temperature could induce the expression of a tiller inhibition (*tin*) gene in wheat.

Soil water content

Water deficit has a marked effect on the patterns of tiller development. Plants growing under water deficit often failed to produce tiller in the axil of the first foliar leaf or delayed tiller emergence (Richman *et al.* 1983). Aspinall *et al.* (1964) reported that a short period of water stress during early tiller development initially reduced tillering, and a large, temporary increase in tillering subsequently occurred with rewatering to optimum level, which resulted in larger maximum tiller number compared to water control. Actually, a continuous water deficit beginning at almost any growth stage is likely to inhibit tiller formation and growth (e.g. small tiller size and low tiller number) but more pronounced during early vegetative growth (Oosterhuis and Cartwright 1983; Lawlor *et al.* 1981). If the periods of water stress extend into the late vegetative growth, there usually is the maximum reduction in tiller populations (Stark and

Longley 1986; Blum *et al.* 1990). Therefore, the adequate early-season water availability is important in developing uniform tillering pattern and high yield potential.

Nutrition

It is generally accepted that the availability of nutrients plays an important role at plant growth. Theoretically, the deficiency of any nutrient cannot increase the tillering of plant until one limited nutrient has been eliminated. Among the major nutrients, effects of nitrogen on tillering are most interesting, because nitrogen is not only a crucial component for protein synthesis, but is also frequently limited available under natural conditions. There are evidences that the addition of nitrogen in plants increases tiller number at the vegetative stage and spike-bearing tiller number at maturity when applied at seeding (Needham and Boyd 1976; García del Moral *et al.* 1984). The increased tillering could result from: a) the enhanced development of higher order tillers by improving leaf length and leaf appearance rate in lower order tillers (García del Moral *et al.* 1984; Duru and Ducrocq 2000b); b) the increased growth of lateral roots related to nitrogen uptake and translocation (Pan *et al.* 1985; Wang and Below 1992); c) the improved productivity of cytokinin promoting the growth and development of tiller buds (Sharif and Dale 1980). Furthermore, the supply of phosphorous is also important in the increase of tillering. Phosphorus deficiency in wheat is found to delay and suppress the tiller formation by slowing leaf emergence (i.e. increasing the phyllochron) and reducing the maximum rate of tiller emergence on each tiller without the reduction in the final number of leaves (Rodríguez *et al.* 1998; 1999).

Plant hormone

Plant growth is regulated by endogenous chemical signals, involving in single or integrated activity of several independent groups of plant hormones. Hormones regulate tillering by plant metabolism and cell growth within the plants. Among plant hormones, auxin, gibberellic acid and abscisic acid are the inhibitors of tiller formation. Auxin produced by the apical meristem could inhibit the outgrowth of tiller buds at the early growth stages through the regulation of the supply of assimilates (Jewiss 1972; Langer *et al.* 1973). Gibberellic acid reduces tillering by increasing

stem extension and leaf expansion to have assimilates unavailable for tiller bud growth (Jewiss 1972; Laidlaw and Berrie 1974). Abscisic acid may reduce tillering in relation to its inhibition on plant growth, in which abscisic acid increases the permeability of cell membrane to both water and solutes to cause the reduction in cell turgor (McIntyre 1997). As the promoter of tillering, cytokinin obviously increases tiller formation by enhancing the growth of tiller buds (Sharif and Dale 1980). A possible reason is that cytokinin could increase the availability of assimilates for tiller bud growth due to its suppression on root growth (Johnston and Jeffcoat 1977). No growth hormone can substitute for the function of cytokinin in the control of tiller growth. However, under adequate conditions for mineral nutrients or cytokinin, the additions of some hormones that inhibit tillering (e.g. gibberellic acid) could stimulate the growth of actively growing tiller buds (Sharif and Dale 1980).

1.3.4 Response of tiller formation and senescence to salinity

Plants display diverse adaptations and responses to salinity by tillering with two categories: the reduction in tiller formation and the enlargement in tiller senescence. Developmental responses of tillers under saline condition can be placed into either category during the plant growth.

Tiller formation could be considered to involve in tiller number and tiller biomass. Under saline conditions, salinity reduces tiller number by delaying and reducing tiller emergence at the vegetative stage (Maas and Poss 1989; Grieve *et al.* 2001). As an illustration, in most instances, seedlings appear later and reach the growth stages earlier under saline conditions (Maas and Poss 1989). Due to the closely linked relationship between leaves and tillers, later leaf appearance results in the delayed tiller emergence, and the decreased rate of leaf emergence due to shorter growth stages results in the reduction in the rate of tiller emergence (Kirby *et al.* 1985; Grieve *et al.* 2001). After tiller emergence, the growth of tillers through all stages is inhibited by salinity due to its damage on the essential metabolic reaction in plants, resulting in low tiller biomass and small tiller size (Maas and Poss 1989; Grattan and Grieve 1999)

According to the observation of Rawson (1971), tillering reaches the maximum at terminal spikelet differentiation with subsequent beginning of tiller senescence. Here, it is noted that tiller senescence is properly composed of tiller mortality (without

spike formation) and floret abortion on spike-bearing tillers. Tiller mortality usually ends at about anthesis on the mainstem, and is followed by floret abortion on the spike until grain-filling (Gallagher and Biscoe 1978; Francois *et al.* 1994; McMaster *et al.* 2005). Under saline conditions, the continuous salinity induces the increases of both tiller mortality and floret abortion on spike-bearing tiller by causing nutrient unavailability for plants. Tiller mortality and floret abortion on the tillers may diminish the competition between tillers or florets for the limited resources under saline conditions.

In any case, salt concentration is the most important parameter affecting tiller formation and tiller senescence. Mass *et al.* (1994) reported that higher than 7.5 dSm⁻¹ of salt levels in soil water could eliminate most of the secondary tillers and greatly reduce the formation of T3 and T4 tillers during tiller development. However, compared with high salinity, moderate salinity only greatly inhibits tiller formation but do not increase tiller mortality in wheat (Mass *et al.* 1994).

1.4 Mechanisms of the inhibition of tillering by salinity

The mechanisms of tiller reduction under salinity have been seldom studied in wheat up to now. According to the tillering pattern, however, tiller reduction under saline conditions should consider to be closely associated with the effects of salinity on leaf and shoot growth. Any factor inhibiting their growth could also limit tiller development under salt stress. Tiller reduction is considered as including the reduction in total tillers and spike-bearing tillers.

Inhibition of plant growth

The tiller emergence in wheat is an orderly sequence at a specific time corresponding to the appearance of particular leaves on higher order shoot (Klepper *et al.* 1982). Limitation of salinity to tillers is considered as due to the reduction in leaf growth and delay of leaf emergence because leaves are the most sensitive plant parts to salinity. Williams *et al.* (1975) found that the initial appearance rates of tiller buds were closely related to those of subtending leaf primordia. Grieve *et al.* (1993) proposed that the decreased rate of leaf primordium initiation under salinity reduced the rate of tiller bud initiation. The report by Hu *et al.* (1997) showed that salinity causes water deficit in the plant tissue, resulting in the decrease of cell turgor pressure. Greenway

and Munns (1980) pointed out that the reduction in cell turgor pressure is a major reason for the inhibition of cell expansion under saline conditions. It was further found that the turgor pressure was significantly decreased in the apical meristem with increasing salinity (Fricke and Peters 2002), but not in the fully developed tissue (Hu *et al.* 1997). Therefore, the initiation of tiller bud primordium as well as leaf primordium could greatly be decreased by low turgor pressure with the increase of salt level.

The inhibition of plant growth due to salinity could be the results of the osmotic effect, ionic imbalance and photosynthetic depression (Grattan and Grieve 1999; Muranaka *et al.* 2002). It was reported that salinity reduced relative growth rate (RGR), net assimilation rate (NAR) and photosynthetic rate in plants through all growth stages (El-Hendawy *et al.* 2005b). However, wheat cultivars with low tiller reduction under salinity show much lower decreases at relative growth rate, net assimilation rate and photosynthetic rate than those with high tiller reduction (El-Hendawy *et al.* 2005a; 2005b), indicating that tiller reduction could be related to the production, transport and distribution of photosynthates during the plant growth (Iyengar and Reddy 1994). In addition, high tiller reduction under salinity shows a decline of plant capacity in controlling ion distribution away from cytoplasm and chloroplasts during the growth (Noble and Rogers 1994).

Timing of salinity

The occurring time of salinity during the plant growth is a more important factor affecting tillering in wheat. In general, if salt stress is imposed from seed imbibition to terminal spikelet differentiation, larger reduction in total tillers and spike-bearing tillers could occur, while if salt stress is imposed from terminal spikelet differentiation to maturity, the effect of salinity is little in tiller reduction (Maas and Poss 1988; Francois *et al.* 1994). Recently, Grieve *et al.* (2001) has further elaborated that effects of salinity on the tillers of different order during the different early growth stages. For example, primary tiller emergence could be suppressed when a short time of salinity was applied at late leaf primordium initiation or double ridge stage but not at terminal spikelet differentiation. However, the effects of timing of salt stress on the tiller reduction depend on salt concentration and the duration of salt stress. Francois *et al.* (1994) found that the application of high salinity after terminal spikelet differentiation

could result in greater tiller mortality compared with low and moderate salinity, and a continuous salinity caused a larger tiller reduction than a short salinity during early growth stages.

Nutrient availability

Due to the competition between ions, soils contain the extreme ratios of $\text{Na}^+/\text{Ca}^{2+}$, Na^+/K^+ , $\text{Na}^+/\text{Mg}^{2+}$, $\text{Cl}^-/\text{NO}_3^-$, and so on, under saline conditions. Low nutrient availability in soil solution due to salinity largely reduces nutrient uptake of roots, consequently causing nutrient deficiency and imbalance in plant tissues (Hu and Schmidhalter 2004). Therefore, salinity greatly inhibits the growth of shoots and roots. Hu and Schmidhalter (1997) and Hu *et al.* (1997) observed that nutrient deficiency and imbalance in the solution largely reduced the number of total tiller and spike-bearing tiller in wheat, and the rate of tiller reduction could further be increased with decreasing nutrient concentration in the growth medium at more than 30 mM NaCl level. Anderson-Taylor and Marshall (1983) proposed that the vigor of tillers is closely related to root biomass of plant. As the nutrient supplier, the lower productivity of roots under saline conditions could be associated with less tiller initiation and development. As a result, nutrient deficiency and imbalance could intensify the competition between tillers for a limited nutrient, resulting in tiller reduction. Lauer and Simmons (1985; 1988) attested that, due to the requirement for their own survival, there is a competition for available nutrients among tillers through the life cycle, especially at the time after the appearance of flag leaf on the mainstem. Therefore, the severe nutrient deficiency and imbalance under high salinity could totally eliminate or inhibit the appearance of high-order tillers, i.e. secondary tillers (Maas *et al.* 1994).

Salt tolerance

Due to genetic differences for resisting salt toxicity, wheat cultivars show the different responses for tiller development to salinity, which are classified as salt-tolerant and salt-sensitive (El-Hendawy *et al.* 2005a). Under saline conditions, low salt level may not reduce the number of total tiller in salt-tolerant cultivars even though the leaf area and shoot biomass are reduced, while for salt-sensitive cultivar, there could be a great reduction (El-Hendawy *et al.* 2005a). Effects of salt tolerance on tiller reduction

should relate to those of toxic ions on shoots during plant growth. At present, the relationship between Na^+ or Cl^- accumulation and salt tolerance has been well established in wheat. It was observed that wheat plants use Na^+ or Cl^- exclusion and inclusion mechanisms in response to saline substrates (Husain *et al.* 2003; El-Hendawy *et al.* 2005b). For Na^+ or Cl^- exclusion, low build-up of Na^+ or Cl^- could decrease their specific damage in cells, e.g. disrupt protein synthesis (Tester and Davenport 2003). For Na^+ or Cl^- inclusion, Na^+ and Cl^- are compartmentalized into vacuoles to avoid the inhibition of various enzyme activities in the cytosol and/or organelles (Greenway and Munns 1980; Munns and James 2003). In fact, tillers may not decrease until a given salt level (threshold) is reached whatever for salt-tolerant or salt-sensitive cultivars. Due to the different capacity of cultivars in resisting salt toxicity, salt-tolerant cultivars show higher threshold salinity than salt-sensitive ones. Regardless of salinity, salt-tolerant cultivars always show relatively lower rate of reduction in total tillers and spike-bearing tillers than salt-sensitive ones, resulting in their higher grain yield (El-Hendawy *et al.* 2005a).

Capability of tillering

According to tiller number, wheat cultivars could be distinguished as four types of population: unicum, low-tillering, moderate-tillering and high-tillering. The potential of tillering is observed to positively correlate with tiller number and yield (Hucl and Baker 1991). Up to now, there has been little information available about effects of salinity on tiller development in the cultivars with different tillering. Based on the observation of El-Hendawy *et al.* (2005a) in the different wheat cultivars, it could be considered that if cultivars only produce mainstem tiller (unicum) or few tillers (low-tillering) per plant, their tiller reduction could be lower than cultivars with moderate-tillering or high-tillering under the same saline conditions. Benbelkacem *et al.* (1984) has reported that more tillers could result in higher tiller mortality due to higher competition for resources among tillers under non-saline conditions. However, the relationship between the mechanisms of tillering and salt tolerance is unclear. Therefore, we hypothesize that tillering may regulate the plant salt tolerance by adjusting the physiology and morphology of plants to resist salt toxicity.

1.5 Aims of the thesis

Objectives of this study were: (1) to obtain fundamental information on utilizing tillering as a means of increasing salt tolerance of wheat cultivars; (2) to investigate the growth of mainstem tiller and subtillers and distribution of toxic ions (Na^+ and Cl^-) and inorganic nutrients among mainstem tiller and subtillers in contrasting wheat cultivars under saline conditions; (3) to understand how salinity affects the growth and ionic content in the individual tillers of contrasting cultivars through removing either mainstem tiller or subtillers; and (4) to enhance the understanding of the mechanisms of salt tolerance in wheat by comparing the salt distribution between the mainstem tiller and subtillers of contrasting wheat cultivars.

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2

Differential Effect of Salinity on Growth and Ion Contents in Mainstem and Subtillers in Contrasting Wheat Cultivars

Abstract Tillering in wheat is known to be closely associated with grain yield under saline conditions. However, the mechanisms behind the inhibition of tillering remain uncertain. Two spring wheat (*Triticum aestivum* L.) cultivars with differing salt tolerance (tolerant: Sakha 8; sensitive: Thasos) were grown in a greenhouse in soils with or without salinity. Both above-ground dry weight and leaf area at day 55 after sowing, as well as grain yield, grain number, spikelet number, straw dry weight and above-ground dry weight at plant maturity were determined. Inorganic ion content in the young leaves was also analyzed at day 55 after sowing. The results showed that above-ground dry weight and leaf area in the subtillers (T1 and T2) at day 55 after sowing were greatly reduced by salinity, and more than that in mainstem for both cultivars. Grain yield in the mainstems of both cultivars was also less affected by salinity compared to subtillers for possible reason, namely the less effect of salinity on the growth in the mainstems of both cultivars at the vegetative stage. In contrast to Thasos, the mainstem and subtillers for Sakha 8 were able to largely exclude Na^+ and Cl^- and to accumulate more K^+ under salinity, suggesting that both mainstem and subtillers of the salt-tolerant cultivar have a better ion selectivity under salinity. Compared with the mainstem, the deficiency and/or imbalance of NO_3^- , K^+ , Ca^{2+} or Mg^{2+} due to higher Na^+ and Cl^- accumulation were observed in the subtillers in both Sakha 8 and Thasos under saline conditions, which may lead to greater reduction in the growth of their subtillers, especially in the salt-sensitive cultivar.

2.1 Introduction

Salinity has drawn extensive attention throughout the world because over 6% of the earth's land area (= 800 million hectares) is affected by either salinity or the associated condition of sodicity (FAO 2006). Hence, increases in crop salt tolerance are needed to sustain food production in many regions of the world. Wheat represents a major food crop in most countries where saline soils exist or might develop (Ashraf and McNeilly 1988), and is reported by Maas and Hoffman (1977) to be moderately tolerant to salinity.

Yield capacity of wheat is highly dependent on the number of spike-bearing tillers and the number of grains per spike. Salinity as an environmental factor depresses both the growth and development of wheat, most notably causing a reduction in tiller number and biomass, even though secondary effects on wheat also occur during the later growth stages (Maas and Grieve 1990; Nicolas *et al.* 1994; Hu *et al.* 1997; El-Hendawy *et al.* 2005a). It has been reported that the mainstem in salt-stressed wheat does not suffer from as much of a reduction in yield with increasing salinity as do the sub tillers (Maas *et al.* 1996; Hu *et al.* 1997). It is generally assumed that apical dominance in plants could be one of the reasons for the inhibition of sub tillers by the mainstem apex. Because competition among tillers for mineral nutrients, carbon and water resource, and/or hormones could exist, a key to enhance salt tolerance in wheat is to understand what inhibits tillering in these plants.

The inhibitory effects of salinity on plant growth are related to Na^+ and Cl^- accumulation together with the uptake of water and essential nutrients such as K^+ and Ca^{2+} (Hu and Schmidhalter 1997; El-Hendawy *et al.* 2005b). High Na^+ and Cl^- levels in soils can cause a range of ionic and osmotic problems in plants. For example, high Na^+ concentration can decrease K^+ and Ca^{2+} concentrations by interfering with the uptake of these ions by the root plasma membrane (Cramer 1991; Siberbush and Ben-Asher 2001) as well as induce a water deficit to decrease the cell turgor pressure related with cell expansion (Greenway and Munns 1980). The translocation of Na^+ and Cl^- from the roots to the shoots is driven by the rapid transpiration stream in the xylem, through which the roots control the salt levels in the plant by exporting the salt ions to either the soil solution or to the shoot. Hence, the ability of a wheat plant to exclude Na^+ and Cl^- can be used as a physiological trait to screen for salt tolerance (Shah *et al.* 1987; Ashraf and O'Leary 1996; Rashid *et al.* 1999; El-Hendawy *et al.*

2005b). By contrast, Na^+ and Cl^- can also be recirculated via the phloem from the shoots to the roots. Munns *et al.* (1986) reported that the retranslocation of Na^+ and Cl^- in the phloem could play a role in controlling the NaCl level in the leaves of barley. It is known that, under saline conditions, high concentrations of toxic ions (Na^+ and Cl^-) inhibit the tillering of plants by reducing biomass, tiller and leaf number in wheat (Maas and Poss 1989; Maas *et al.* 1994; El-Hendawy *et al.* 2005a, 2005b). However, it is unclear whether the accumulation of toxic ions (Na^+ and Cl^-) in the different tillers is associated with the inhibition of their growth in wheat.

Recently, the two wheat cultivars Sakha 8 and Thasos have been identified according to multiple agronomic parameters at the different growth stages as being salt-tolerant and salt-sensitive cultivars, respectively (El-Hendawy *et al.* 2005a). This finding provides a unique opportunity to understand the influences of salinity on tillering in contrasting wheat cultivars. Therefore, the objectives of this study were to investigate the relationship between plant growth and ion-partitioning in tillers under salinity in these two contrasting wheat cultivars, and to provide a comprehensive understanding of the mechanism reducing tillering under saline conditions.

2.2 Materials and methods

2.2.1 Plant growth

Thirty seeds for each of Sakha 8 and Thasos were sown in plastic pots containing 10 kg of dry loamy soil with or without salt stress. Soil collected from the soil surface (0-15 cm) was air-dried, sieved through a 5-mm screen, and mixed with 30% sand to achieve good leakage. Soils were initially salinized by adding 120 mM NaCl solutions layerwise to four layers within the pot. Due to soil absorption, however, the electrical conductivity in soil solution was finally about 7.0 dS m^{-1} , which was measured before the experiment started. According to our preliminary experiment, the moderate salinity was applied in this study because high salinity could cause the tiller to be skipped. Soil water content was maintained at 25% (dry soil basis). To ensure that no nutrient deficiency existed during plant growth, 0.57 g NH_4NO_3 was applied to each pot at days 20, 40 and 60 after sowing, and 0.2 g KH_2PO_4 and 0.2 g K_2SO_4 were similarly applied at day 20 after sowing. At the two-leaf stage, the seedlings were thinned to twenty per pot.

Differential effect of salinity in mainstem and subtillers

The experiment consisted of a split-plot layout with three replications. The main plot consisted of salt levels, with the two cultivars being allocated to the subplots. Plants were grown in a greenhouse at day/night temperatures of about 18/13°C, and with a 14-h light period of photon flux density 550 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$.

At day 55 after sowing, six randomly selected plants were harvested per pot, and the two youngest fully developed leaves were separated from the mainstem and the two subtillers (i.e. the primary tillers from leaves one and two, T1 and T2, on mainstem). Leaf areas for the mainstem, subtillers (T1 and T2) and remaining tillers were determined using a leaf-area meter. Plant material was dried at 65°C for 48 h for the determination of dry weight.

At plant maturity, five plants per pot were harvested at random, and then separated into the mainstem, T1, T2 and remaining tillers from which the leaves, stem and spikes were removed as appropriate. After drying at 65°C for 48 h, straw dry weight, spike dry weight, grain number per spike, grain yield per spike and spikelet number per spike were measured in each of the mainstem, T1, T2 and remaining tillers. The above-ground dry weight at plant maturity was equal to the spike dry weight plus the straw dry weight.

2.2.2 Analysis of ion contents

The two youngest fully developed leaf samples from the mainstem, T1 and T2 harvested at day 55 after sowing were ground after oven-drying into a fine powder by passing them through a 0.5-mm diameter sieve. To determine Na^+ , K^+ , Mg^{2+} and Ca^{2+} concentrations, 150-mg plant samples were ashed at 560°C in a muffle furnace for 6 h and then digested with 2 ml of 20% HCl for 5 min at 60°C using a heating block, and finally diluted to a volume of 25 ml with distilled-deionized water. The concentrations of Na^+ , K^+ , Mg^{2+} and Ca^{2+} were determined with an Inductively Coupled Plasma Emission Spectrometer (ICP model Liberty 200, Varian Australia Pty. Ltd., Mulgrave Victoria, Australia).

For determination of NO_3^- and Cl^- concentrations, 50 mg of ground sample was shaken with 25 ml distilled water for one hour and then filtered. Chloride and NO_3^- were determined using an ion chromatography analyser (Model LC20-1, Dionex, Sunnyvale, CA 94086, USA).

2.2.3 Statistical Analysis

All variables were analyzed using the General Linear Model (GLM) procedure implemented in SAS (SAS Institute Inc., 2004). All growth parameters were analyzed using individual analyses of variance (ANOVA) with individual model for the mainstem, T1, T2 and the total plant. Individual model for the mainstem, T1 and T2 was also employed for individual ANOVAs for each inorganic ion. All tests employed a nominal alpha level of 0.05.

2.3 Results

2.3.1 Effects of salinity on growth parameters of the whole shoot, mainstem and sub tillers (T1 and T2)

Above-ground dry weights and leaf areas of the whole shoot and individual tiller (mainstem, T1 and T2) at day 55 after sowing decreased significantly with increasing salinity for both cultivars (Fig. 2.1). However, compared with the salt-tolerant Sakha 8, the reductions in above-ground dry weight and leaf area in the mainstem, T1, T2 and the whole shoot were greater for the salt-sensitive Thasos. Importantly, above-ground dry weights and leaf areas of the sub tillers (T1 and T2) at day 55 after sowing were reduced to greater degree in both cultivars under salt stress than in the mainstem. For instance, the above-ground dry weights of the mainstem, T1 and T2 were reduced by 20%, 56% and 38%, respectively, for Sakha 8 and by 46%, 72% and 69% for Thasos.

As compared with the non-saline control, grain yield, grain number and above-ground dry weight per plant at plant maturity in Sakha 8 were reduced by salinity by 22%, 23% and 29%, respectively. For Thasos, the corresponding values were 32%, 32% and 34% (Figs 2.2 and 2.3). By contrast, larger reductions in both straw dry weight and spikelet number per plant for Sakha 8 (41% and 38%, respectively, as compared with the non-saline control) than for Thasos (38% and 28%) were apparent (Fig. 2.3). Furthermore, whereas the reductions in grain yield and straw dry weight per plant in Thasos were significant, only that for straw dry weight was in Sakha 8. Under salinity, significant decreases in grain number and spikelet number per plant in Thasos and in only spikelet number per plant in Sakha 8 were also found (Figs 2.2 and 2.3).

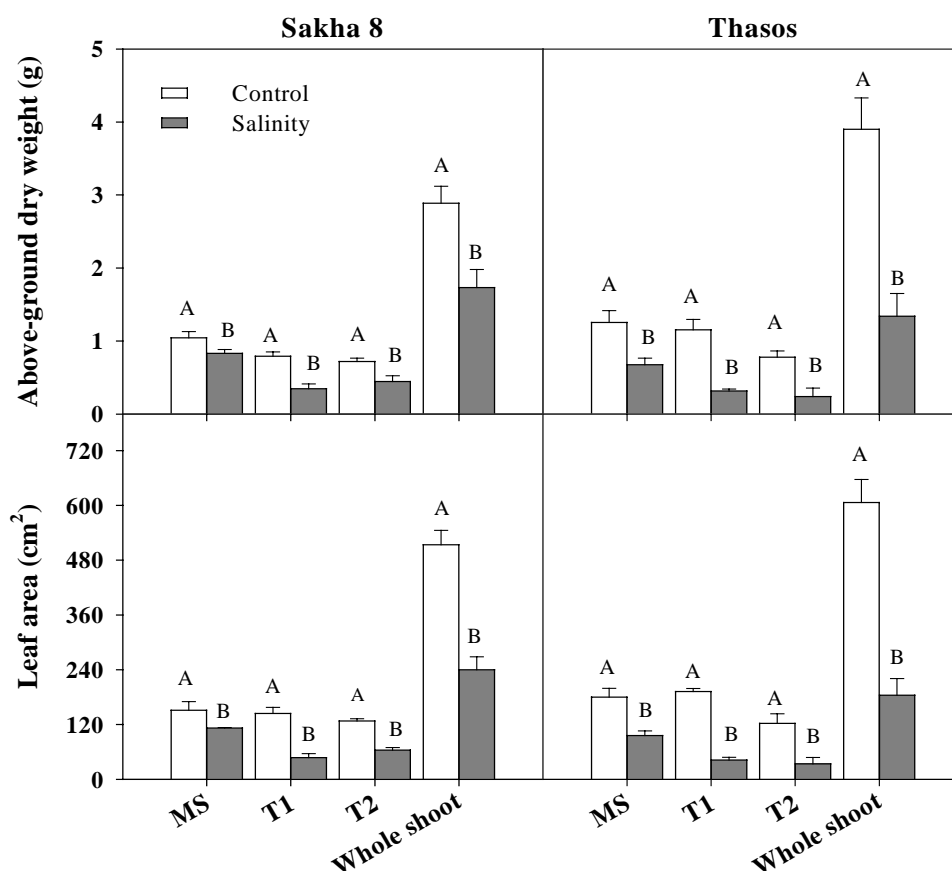


Figure 2.1 Effect of salinity on above-ground dry weight (per tiller or per plant) and leaf area (per tiller or per plant) of the mainstem (MS), T1, T2, and the whole shoot for Sakha 8 and Thasos at day 55 after sowing. Bars with the same letter are not statistically different ($P \leq 0.05$) between the control and salinity.

Similar to the responses of the whole shoot to salinity, the mainstem, T1 and T2 of Sakha 8 also showed better salt tolerance than those of Thasos with respect to grain yield, grain number and above-ground dry weight at plant maturity. However, straw dry weights and spikelet numbers of the mainstem, T1 and T2 underwent smaller reductions in Sakha 8 than in Thasos as compared to over the whole shoot (Figs 2.2 and 2.3). Grain yields, grain numbers and above-ground dry weights of the mainstem, T1 and T2 for Sakha 8 were only slightly decreased or even increased at the mature stage by salinity, such that significant reductions were observed only for straw dry weights of the mainstem and T1, and spikelet numbers of T1 and T2 in Sakha 8. The slightly increased grain yields and grain numbers of the mainstem and T2 for Sakha 8

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under salinity suggest that the remaining tillers were mainly responsible for the reduction in these parameters per plant (Figs 2.2 and 2.3). For Thasos, salinity caused significant reductions in all of grain yield, grain number, spikelet number, straw dry weight and above-ground dry weight at maturity in all of the mainstem, T1 and T2.

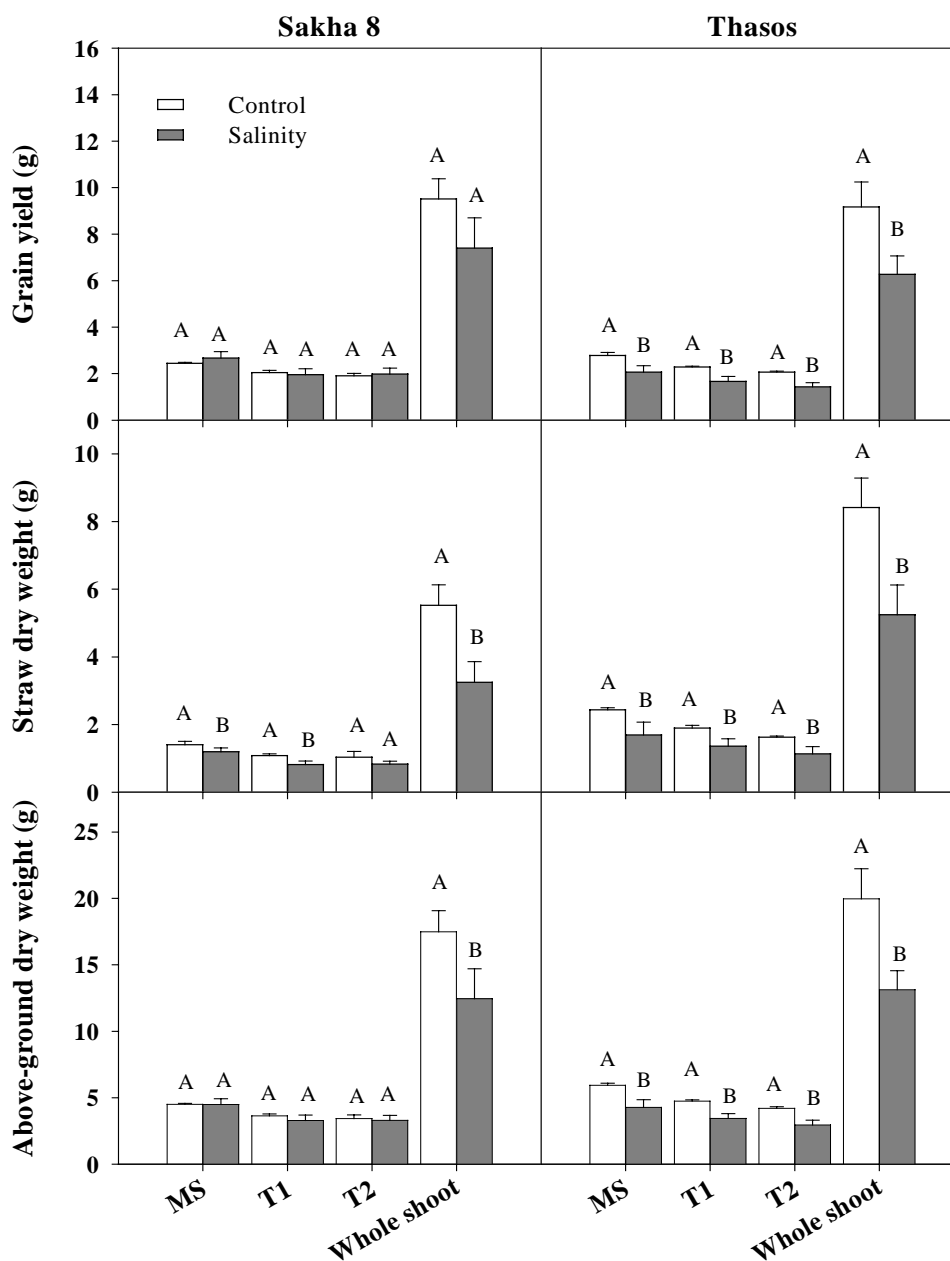


Figure 2.2 Effect of salinity on grain yield, straw dry weight and above-ground dry weight (all per tiller or per plant) of the mainstem (MS), T1, T2, and the whole shoot for Sakha 8 and Thasos at plant maturity. Bars with the same letter are not statistically different ($P \leq 0.05$) between the control and salinity.

Differential effect of salinity in mainstem and sub tillers

In each of the two cultivars, the mainstem always showed a higher salt tolerance than did T1 and T2 in grain yield (Fig. 2.2). For example, the difference between the two treatments for Sakha 8 was -9 %, 4% and -4% in the mainstem, T1 and T2, respectively, and 26%, 27% and 30% for Thasos. Results further show that, compared to the mainstem, both the above-ground dry weights and straw dry weights of T1 and T2 for Sakha 8, and spikelet numbers of T1 and T2 for Thasos showed larger decreases under salinity.

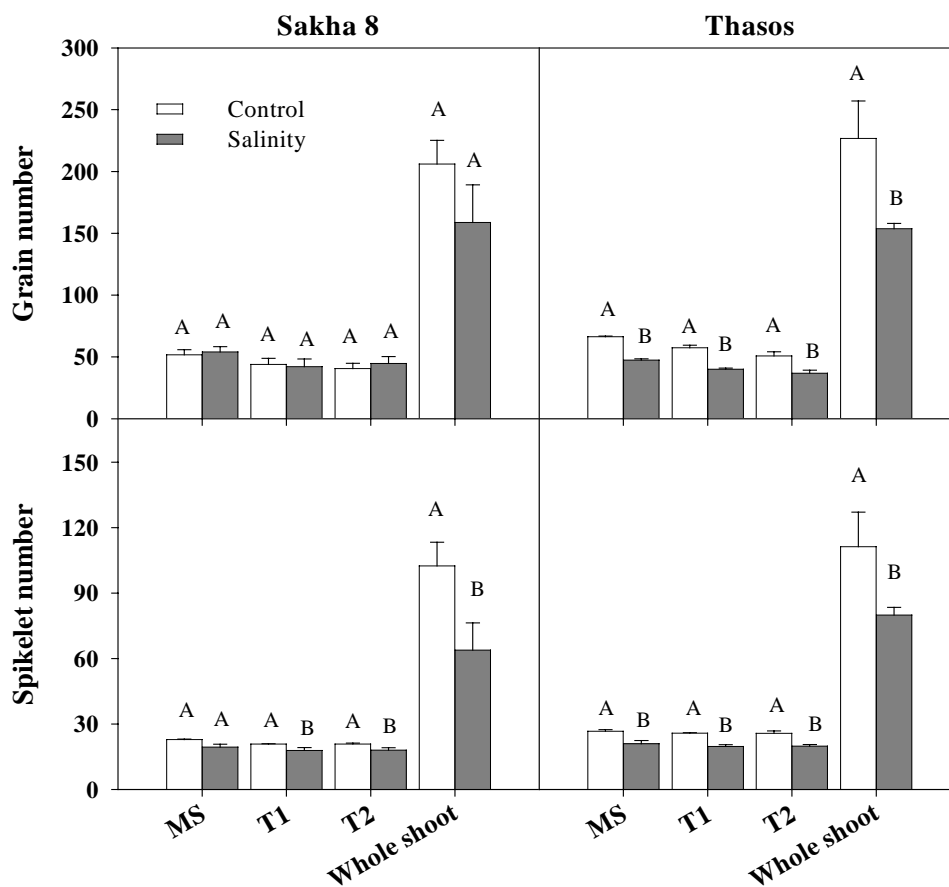


Figure 2.3 Effect of salinity on grain number and spikelet number (both per tiller or per plant) of the mainstem (MS), T1, T2, and the whole shoot for Sakha 8 and Thasos at plant maturity. Bars with the same letter are not statistically different ($P \leq 0.05$) between the control and salinity.

2.3.2 Distribution of inorganic ions in the mainstem, T1 and T2 at day 55 after sowing

In both cultivars, salinity significantly increased the Na^+ concentration in the mainstem, T1 and T2 (Fig. 2.4). Compared to the non-saline control, the Na^+ concentration in Sakha 8 was increased by about two times in the mainstem and T1, and by about three times in T2. The accumulation of Na^+ in Thasos under saline conditions was even higher about eight times in the mainstem, six times in T1, and 12 times in T2. The salt-sensitive Thasos was also observed to accumulate significantly higher Na^+ levels in the mainstem, T1 and T2 than the salt-tolerant Sakha 8. The sub tillers (T1 and T2) of Sakha 8 showed a significant higher build-up of Na^+ than the mainstem under salinity, and the similar result was also observed in T2 of Thasos (Fig. 2.4).

The effect of salinity on the K^+ concentration in the leaf tissues differed depending on the combination of the tillers and cultivars. For example, K^+ concentrations in the mainstem and T2 of both Thasos and Sakha 8 and in T1 of Thasos increased slightly under saline conditions, whereas that in T1 of Sakha 8 decreased slightly (Fig. 2.4). Compared to the mainstem, the K^+ concentration significantly increased in T1 and T2 of Sakha 8 under salinity, whereas there was no significant difference between the mainstem and sub tillers in Thasos (Fig. 2.4). Regardless of the treatment, all tillers of Sakha 8 accumulated higher K^+ concentrations in the young leaves than those of Thasos. However, no differences between the two treatments were significant for any individual tiller of either Sakha 8 or Thasos. Large differences in the Na^+/K^+ ratios between the two cultivars under saline conditions were observed, ranging from 0.05 to 0.06 for the tillers of Sakha 8 and from 0.33 to 0.38 for those of Thasos. An increase in the Na^+/K^+ ratio for T1 or T2 compared to the mainstem was observed in both cultivars.

The influence of salinity on Ca^{2+} concentrations in the young leaves also varied according to the tiller and cultivar. Under salt stress, Ca^{2+} concentrations in Sakha 8 declined slightly in all tillers, whereas they increased slightly in Thasos. In Sakha 8, the degree of the reduction in Ca^{2+} concentration induced by salinity increased from the mainstem to T1 and T2, although these differences were not significant. By contrast, Mg^{2+} concentrations in each of the mainstem, T1 and T2 decreased with salinity in both cultivars (Fig. 2.5). In general, Mg^{2+} concentrations in tillers of Sakha

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8 were higher than the corresponding ones of Thasos, although the reduction in Mg^{2+} concentrations under salinity was greater in Sakha 8. Finally, there was no significant difference in Mg^{2+} concentration among tillers under saline conditions regardless of the cultivar.

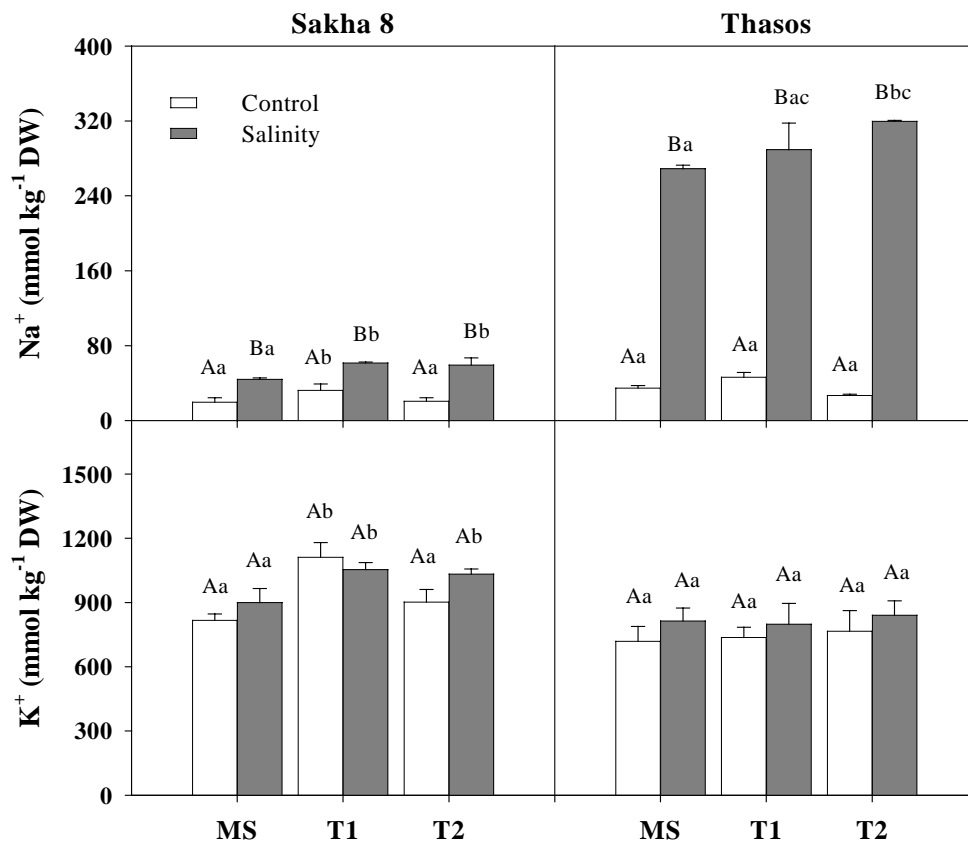


Figure 2.4 Effect of salinity on the Na^+ and K^+ concentrations of the two youngest fully developed leaves of the mainstem (MS), T1 and T2 for Sakha 8 and Thasos. The same upper case letters in the different colour bars or lower case letters in the same colour bars are not statistically different ($P \leq 0.05$).

Differential effect of salinity in mainstem and subtillers

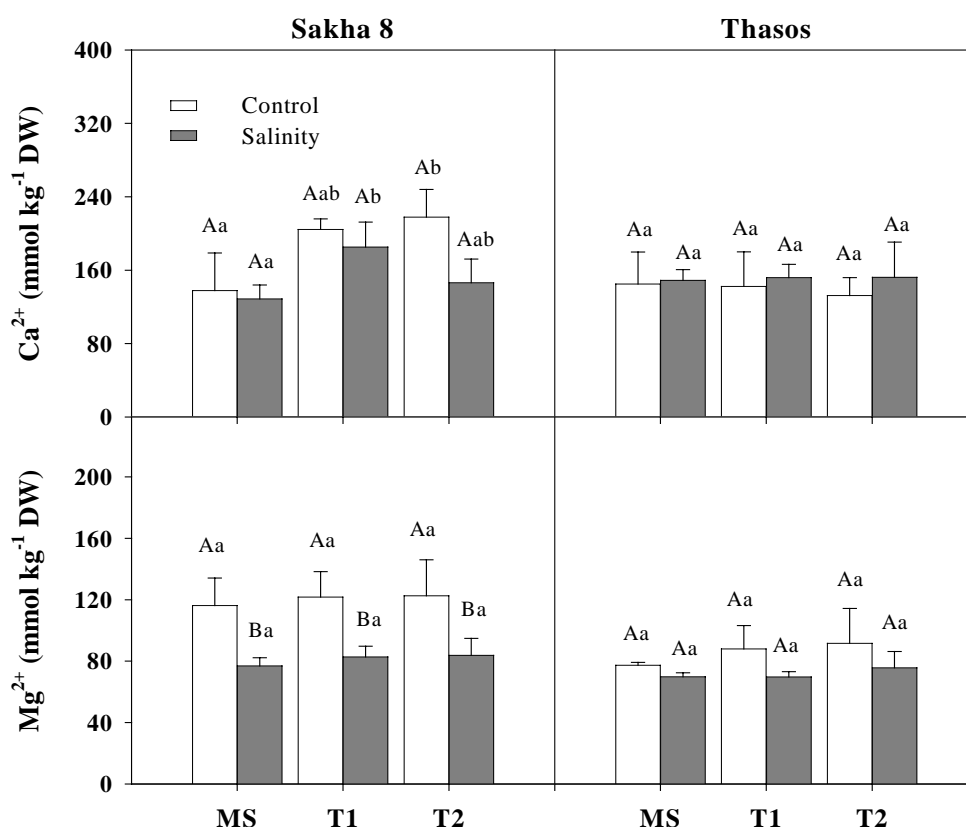


Figure 2.5 Effect of salinity on the Ca^{2+} and Mg^{2+} concentrations of the two youngest fully developed leaves of the mainstem (MS), T1 and T2 for Sakha 8 and Thasos. The same upper case letters in the different colour bars or lower case letters in the same colour bars are not statistically different ($P \leq 0.05$).

Similar to Na^+ , Cl^- concentrations in the young leaf tissue of all tillers of both cultivars increased significantly under salinity. However, all tillers in Sakha 8 accumulated less Cl^- than those in Thasos (Fig. 2.6). Chloride concentrations in T1 and T2 in both Sakha 8 and Thasos were higher than those in the mainstem, although this difference was only significant between the mainstem and T1 in Sakha 8. Salinity greatly decreased the NO_3^- concentrations in all tillers for both cultivars (Fig. 2.6), with a greater reduction in Sakha 8 being observed. Similar to the findings with K^+ and Mg^{2+} , NO_3^- concentrations in T1 and T2 were higher than those in the mainstem regardless of the cultivar and treatment. However, the mainstem tiller showed a smaller reduction in NO_3^- concentration than did the subtillers in both cultivars. This trend is identical with Ca^{2+} in Sakha 8 and Mg^{2+} in Thasos.

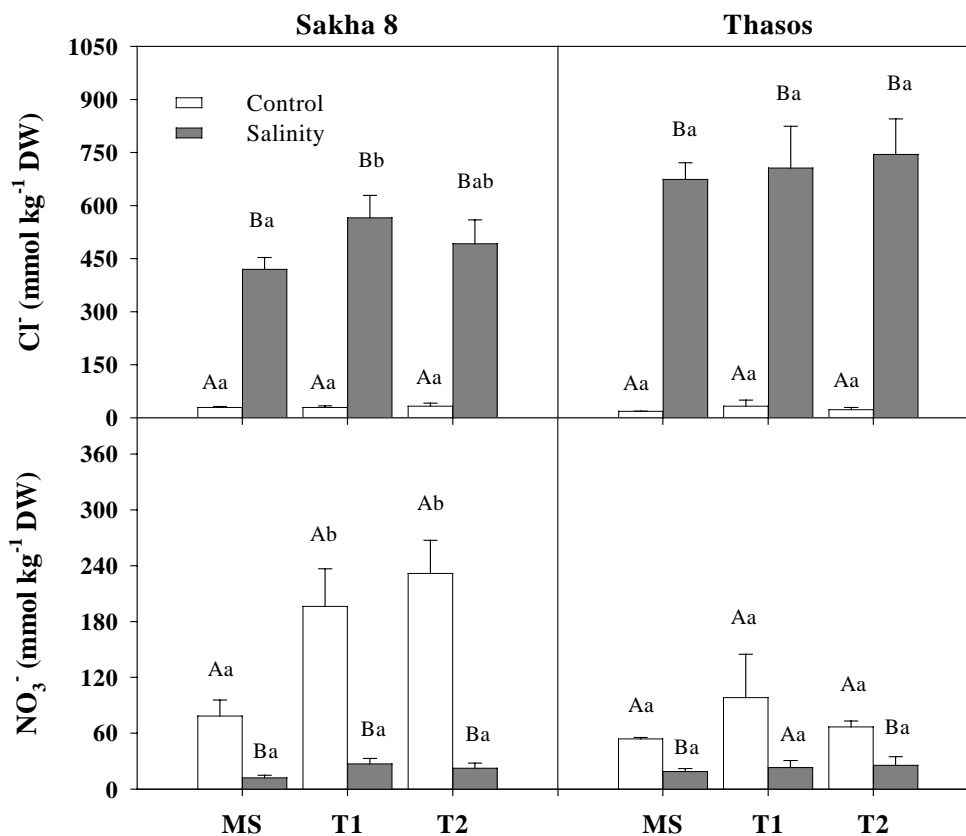


Figure 2.6 Effect of salinity on the Cl⁻ and NO₃⁻ concentrations of the two youngest fully developed leaves of the mainstem (MS), T1 and T2 for Sakha 8 and Thasos. The same upper case letters in the different colour bars or lower case letters in the same colour bars are not statistically different ($P \leq 0.05$).

2.4 Discussion

The yield potential of wheat under saline conditions is highly dependent upon the number of tillers per plant, the number of grains per spike and thousand-grain weight (Maas and Grieve 1990; Salam *et al.* 1999; El-Hendawy *et al.* 2005a). Hu *et al.* (1997) found that the yield of the mainstem is less affected by salinity compared with that of the sub tillers under saline conditions, which was confirmed here for both the salt-tolerant Sakha 8 and the salt-sensitive Thasos. The less influence of salinity in grain yield in the mainstem of both cultivars could be related to the reduced effect on the growth (i.e. above-ground dry weight and leaf area) in the mainstem at the vegetative stage compared to the sub tillers (T1 and T2). It is well known that the reduction in plant growth by salinity is related to the uptake of salt and nutrients, which sequentially result in ionic imbalance within the cell. Therefore, under saline

Differential effect of salinity in mainstem and sub tillers

conditions, the transport and accumulation of salt and nutrient within the plant could be associated with the inhibited growth of the mainstem and sub tillers.

As reported, the roots of some species regulate the rate of salt transport into the xylem, but the retranslocation of salt in the phloem could also help regulate the build-up of salt in the fully expanded leaves (Munns *et al.* 1986; Wolf *et al.* 1990; Lohaus *et al.* 2000; Watson *et al.* 2001; Davenport *et al.* 2005). The ratio of phloem/xylem Cl^- flux has been reported to be approximately 20% in both lupin (Jeschke *et al.* 1992) and maize (Lohaus *et al.* 2000). As a result, the regulation of the Na^+ and Cl^- concentrations between the mainstem tiller and sub tillers under saline conditions could occur through the retranslocation of Na^+ and Cl^- via the phloem. We hypothesize that the mainstem tiller might shunt salt into the sub tillers T1 and T2 and/or compete with them for nutrients (e.g. NO_3^- , K^+ , Ca^{2+}) as a strategy for increasing the salt tolerance under saline conditions. However, the sub tillers accumulated more Na^+ and Cl^- in the young leaves not only in the salt treatment but also in the control as compared with the mainstem. In addition, the concentrations of NO_3^- , K^+ , Ca^{2+} and Mg^{2+} in T1 and T2 of Sakha 8 and those of NO_3^- , K^+ and Mg^{2+} in T1 or T2 of Thasos were higher than those in the mainstem regardless of the treatment. This indicates that the sink strength in the sub tillers at day 55 might still be stronger than that in the mainstem. Jeschke *et al.* (1995) reported that 72% of K^+ uptake in the whole plant was utilized for tiller growth in *Leptochloa fusca* due to the vigorous production of tillers. Therefore, there is no evidence in this study to prove that the exclusion of Na^+ and Cl^- from parent tillers could be one of the strategies to improve wheat salt tolerance under saline conditions, perhaps due to other established mechanisms providing for the increase of salt tolerance: the restriction of xylem loading and delivery from the roots to the shoots (Shone *et al.* 1969; McCully *et al.* 1987; Gorham *et al.* 1990; Santa-María and Epstein 2001), the recirculation of salt from the shoots to roots by the phloem (Munns *et al.* 1988; Lohaus *et al.* 2000), a reduction in transpiration (Ball 1988; James *et al.* 2002; El-Hendawy *et al.* 2005b), and the reallocation of ion content between the different leaves (Munns 1993; Salam *et al.* 1999; Rashid *et al.* 1999). On the other hand, compared with the mainstem, the higher accumulation of Na^+ and Cl^- in the sub tillers under saline conditions results in the deficiency and/or imbalance of nutrients, with higher Na^+/K^+ , $\text{Na}^+/\text{Ca}^{2+}$ and $\text{Na}^+/\text{Mg}^{2+}$ ratios and greater reduction in NO_3^- , Ca^{2+} or Mg^{2+} being observed in the

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leaves of sub tillers, especially in the salt-sensitive cultivar. Thus, there was a greater reduction in the growth of sub tillers. The higher selectivity of K^+ and Ca^{2+} over Na^+ in the mainstem under salinity could be an efficient system contributing to its salt tolerance, with restricting the access of Na^+ at cellular membranes in cells (Jeschke 1984; Muhammed *et al.* 1987). The increased uptake of Cl^- in salt-stressed sub tillers could further be responsible for the general reduction in the growth by depressing the uptake of other anions such as NO_3^- (Hu and Schmidhalter 1997; Hu *et al.* 2005).

The effects of salinity on growth at day 55 after sowing and on yield at the final harvest recorded in this study confirm that the growth in the later, mature stages of wheat plants is less sensitive to salinity than that in the vegetative growth stages (Francois *et al.* 1994; Hu *et al.* 1997). In wheat, the vegetative growth stage is characterized by the appearances of tiller and leaf and the shoot growth. Salt stress during the vegetative growth stage can inhibit the emergence of tiller and leaf as well as shoot growth by water deficit, reduction of photosynthesis and nutrient imbalance (Maas and Poss 1989; Nicolas *et al.* 1994; El-Hendawy *et al.* 2005a). In addition, the requirement of plants for water and photosynthesis is gradually decreased during the growth, which could diminish the effect of salinity on plants. Surprisingly, the salt-tolerant Sakha 8 became increasingly tolerant to salinity in the mature stages compared to the salt-sensitive Thasos, especially for grain yield and grain number of mainstem and T2. Under saline conditions, the slightly increased grain yields and grain numbers in mainstem and T2 for Sakha 8 indicated that their reduction in the whole shoot is mainly related to the secondary or later primary tillers, while for Thasos, those reductions could be related to all tillers. The increased salt tolerance for Sakha 8 could be due to its high ability to exclude Cl^- and Na^+ . At day 55 after sowing, the Na^+ concentration in the leaves of Thasos was about six-fold higher than that in Sakha 8, with the Cl^- concentration similarly being about 20-40% higher (Figs 4, 6). These higher levels of Na^+ and Cl^- in the leaves of the sensitive cultivar might represent the cause of the greater inhibition of growth observed in its later growth stages. The apparent ability of Sakha 8 to exclude Na^+ is in agreement with our previous studies (El-Hendawy *et al.* 2005a, 2005b) and is likely related to its increased salt tolerance. Indeed, various reports in literature have demonstrated that the ability of plants to exclude Na^+ represents an effective marker for screening for the more salt-tolerant genotypes and to breed more tolerant plants of durum wheat

(Munns and James 2003; Lindsay *et al.* 2004). It is noticeable that the concentration of K^+ or Ca^{2+} was slightly increased by salinity in the young leaves of both cultivars or Thasos, respectively, which confirmed the observation of Salam *et al.* (1999) in the young leaves of wheat. Rashid *et al.* (1999) found that the K^+ concentration was higher than the Na^+ concentration in two weeks old leaves under saline conditions, and decreased with increasing the order of leaf age. However, the young leaves under non-saline conditions showed much lower Na^+/K^+ and Na^+/Ca^{2+} ratios compared to saline conditions. As pointed out by Tester and Davenport (2003), high level of Na^+ or high Na^+/K^+ ratio could disrupt protein synthesis in the cell, given that Na^+ competes with K^+ for binding sites essential for cellular function but cannot substitute for K^+ to activate functional enzymes (Bhandal and Malik 1988). As such, sodium generally disturbs the ionic balance of the plant and causes ion-specific damage.

In conclusion, the growth of the subtillers T1 and T2 was reduced by salinity to a greater extent than it was in the mainstem during the vegetative stages for both the salt-tolerant and salt-sensitive cultivars. The reduced effect of salinity on grain yield in the mainstem as compared to the subtillers could be related to the less reductions in plant growth at the vegetative stage for both the salt-tolerant cultivar and the salt-sensitive cultivar. Furthermore, in contrast to the salt-sensitive cultivar, the salt-tolerant cultivar is characterized by its ability to both exclude more Na^+ and Cl^- and to accumulate more K^+ in the tillers under saline conditions. The higher accumulations of Na^+ and Cl^- in subtillers under saline conditions may impair their growth by causing larger nutritional deficiency and imbalance compared with the mainstem tiller regardless of the cultivar. It is not proved that the mainstem tiller in particular is able to exclude Na^+ or Cl^- into the subtillers and to select NO_3^- , K^+ , Mg^{2+} or Ca^{2+} over them as a strategy to improve the survival of wheat plants under saline conditions.

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3

Insights in the Role of Tillering in Salt Tolerance of Spring Wheat from Detillering

Abstract Tillering is reduced by salinity, with the primary and secondary tillers being more affected than is the mainstem. To understand the importance of tillering in the salt tolerance of wheat plants, two contrasting cultivars of spring wheat (*Triticum aestivum* L.) were grown in a greenhouse under saline or non-saline conditions and were subjected to five progressive levels of detillering. Regardless of the cultivar and salt stress, dry weight, grain yield and grain number per plant were all significantly decreased in the treatments where only one or two tillers per plant remained compared with the untouched treatment (more than 3 tillers), whereas these same parameters per tiller tended to be increased on a per tiller (mainstem or subtiller) basis. The increased grain yield per tiller observed with tiller reduction may be attributed to their enhanced spikelet fertility. Under saline conditions, the reductions in dry weight, grain yield and grain number per plant for the salt-tolerant cultivar Kharchia were of a greater magnitude in the treatments where only one or two tillers per plant were present compared with the untouched treatment, whereas the magnitude of this reduction in the salt-sensitive cultivar Sakha 61 was decreased. Thus, the results suggest that the salt tolerance for the tolerant genotype might be further enhanced with more tillers per plant, whereas it might be increased with fewer tillers per plant in the sensitive genotype.

3.1 Introduction

Tillers are important for grain yield in wheat (*Triticum aestivum* L.) as well as in other cereals, but are also sensitive to environmental stresses. Under saline conditions, the growth of the mainstem and subtillers is reduced, which usually results in losses in yield. However, to complicate matters, salinity affects the growth of mainstem and subtillers differentially in wheat. To improve the salt tolerance of wheat plants, therefore, a better understanding of these differential effects of salinity is required.

In the plant, the early growth of subtillers is supported entirely with photoassimilates and nutrients from the mainstem (Kemp and Whingwiri 1980; Lauer and Simmons 1985). Even during the grain filling stage, subtillers are still supplied with assimilates from the flag leaf and other leaves on the mainstem (Ong and Marshall 1975). There is also evidence to support the existence of competition between the mainstem and subtillers (Kirby and Jones 1977; Martinez-Carrasco and Thorne 1979). Compared with the subtillers, the mainstem is less susceptible to salt stress during the period of growth (Maas *et al.* 1994; Hu *et al.* 1997). Several hypotheses exist to explain these observations: (1) the carbohydrate supply is reduced more by salinity in the subtillers than in the mainstem, thereby inhibiting the growth of the former to a greater degree (Grieve *et al.* 1992); (2) the mainstem may retain more photosynthates for its own growth rather than exporting source reserves to the subtillers under saline conditions; and 3) the mainstem competes with the subtillers to obtain more nutritional ions to lessen nutrient deficiency and/or imbalance caused by toxic ions. Recently, Zeng *et al.* (2002) and El-Hendawy *et al.* (2005a) have demonstrated that different wheat and rice cultivars exhibited various responses to salinity according to the three agronomic parameters, tiller number, leaf number and leaf area per plant. In all cases, the salt-sensitive cultivars showed a greater reduction in tiller number and biomass than did the salt-tolerant cultivars. This result suggests that the mainstems and subtillers of salt-tolerant and salt-sensitive cultivars have different mechanisms in response to salinity. Thus, by comparing the growth of the mainstem and subtillers in the contrasting cultivars, the effects of salinity on tillering may be clarified.

Only a few studies have investigated the relationship among cultivar, tiller and grain yield. Papadakis (1940) reported that high-tillering cultivars have a greater potential to increase grain yield on poor soil conditions than do low-tillering ones.

Huel and Baker (1991) supported this conclusion, finding that increasing the tillering capacity of cultivars could increase grain yield in semiarid environments. Within a given cultivar, however, Jones and Kirby (1977) indicated that restricting tiller number of plants in semiarid environments tended to increase grain yield compared to free-tillering plants due to the enhanced water-use efficiency. Benbelkacem *et al.* (1984) found that breeding for high-tillering cultivars increased efficiency (i.e., added spike-bearing tillers) by only 36%.

All the above studies investigated the relationship between tiller number and grain yield only. No information exists about the effects of tillering on salt tolerance and grain yield in contrasting cultivars under saline conditions. Yet, exactly this information may be of great benefit to breeding programs designed to improve salt tolerance in wheat. However, according to Kirby (1973), morphological development was found to be similar in the artificial and mutant unculms in barley. Therefore, tillering was manipulated in the present study by artificial tiller removal, which proved to be a superior method in the absence of isogenic lines.

The objectives of this study, therefore, were to investigate how the tiller number affects the growth both of the individual tiller and of the whole wheat plant under saline conditions at different growth stages, and to understand the impact of different tiller number of plants on salt tolerance in two contrasting wheat cultivars.

3.2 Materials and Methods

3.2.1 Plant materials and growth conditions

Thirty seeds of two contrasting spring wheat cultivars Kharchia (salt-tolerant) and Sakha 61 (salt-sensitive) that were pre-germinated at room temperature for 2 days were sown in pots containing 10 kg of dry soil with or without salt stress. Soil collected from the soil surface (0-15 cm) was air-dried, ground and passed through a 5-mm mesh screen. The initial soil was filled layer-wise in pots with four layers. For the salt-stressed pots, the first three soil layers starting from the bottom was salinized by adding 120 mM NaCl solutions layer-wise, and the top layer was salinized at day 10 after sowing. The final electrical conductivity in soil solution was about 5.6 dS m⁻¹, respectively, matching the value measured before the experiment started. Based on a set of the preliminary experiments, only a moderate saline stress was applied in this study because it was found that high salinity could cause the plant to skip tiller

formation. Soil water content was maintained at a 25% dry soil basis by replacing any water lost through evapotranspiration. The plants were grown in a growth chamber at 23/18°C (day/night) with 16-h light period of photon flux density 550 $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$ (PPFD) until the first tiller emerged, and then transferred to a greenhouse. At the two-leaf stage, the seedlings were thinned to 22 plants per pot.

The preliminary experiments also showed that the optimal soil nutrient was obtained by applying 0.57 g NH_4NO_3 per pot initially, with an additional 0.57 g NH_4NO_3 per pot being added at the third, sixth, eighth and tenth weeks after sowing. In addition, 0.1 g KH_2PO_4 and K_2SO_4 per pot were added at the sixth and eighth weeks after sowing, respectively.

3.2.2 Experimental design and sampling

The experiment was arranged as a randomized complete block design and consisted of five detillering treatments each replicated three times. The processes of tillering in wheat can be summarized generally as: (1) the mainstem produces the primary tillers (T1, T2, ..., TN) from the buds of its leaf axils; (2) the primary tillers likewise bear secondary tillers (T10, T11, ...) from their leaf axils; and (3) the secondary tillers can occasionally also produce tertiary tillers from their leaf buds. All primary tillers, secondary tillers, tertiary tillers, and so on, are named subtillers. According to this scheme of tiller appearance, the detillering treatments we employed were:

1. Untouched: Mainstem and all subtillers were left intact;
2. MS: Mainstem was left intact; all subtillers were removed;
3. MST1: Mainstem and the primary tiller from leaf 1 on the mainstem (subtiller T1) were left intact; all other subtillers were removed;
4. T1: Subtiller T1 was left intact; the mainstem and all other subtillers were removed;
5. T1T2: The primary tillers from the first two leaves on the mainstem (subtiller T1 and subtiller T2) were left intact; the mainstem and all other subtillers were removed.

The subtiller buds were broken off by a blunt needle at the base of the leaf as soon as they became visible; the mainstem was excised by a surgical scalpel from the plant when the second leaf of the youngest subtiller that was left intact was fully

expanded (Kirby and Jones 1977; Alaoui *et al.* 1988). The mainstem and subtiller buds were removed two times according to the growth of the meristem.

During plant growth, leaf numbers of the mainstem and sub tillers T1 and T2 were recorded. Leaves of the mainstem and sub tillers T1 and T2 were harvested from ten plants at the first harvest (flag-leaf stage) and from the remaining plants at the final harvest (mature stage). At the first harvest, after leaf area was measured in the leaves of the mainstem, subtiller T1 and subtiller T2, respectively, the plant materials were dried at 65°C for 48 h to determine dry weight for them and whole-plant. At the final harvest, dry weight, grain yield, grain number and spikelet number were measured in the mainstem, subtiller T1, subtiller T2 and whole-plant, respectively, after the plant materials were dried as above.

3.2.3 Statistical analysis

Data were analyzed by analysis of variance (ANOVA) using the PROC GLM procedure of The SAS System v9.1. Means separation on the data was conducted using LSD multiple range tests. Terms were considered significant at $P \leq 0.05$.

3.3 Results

3.3.1 Association of mainstem growth with tiller number

Effects of tiller number on mainstem leaf growth under saline conditions were observed in both salt-tolerant and salt-sensitive cultivars (Table 3.1). Regardless of salinity, mainstem leaf areas in the MS or MST1 treatments were increased compared with the untouched treatment for both cultivars. However, a larger reduction compared with the untouched treatment under saline conditions was observed for the MS treatment of Kharchia and for the MS and MST1 treatments of Sakha 61 (Table 3.1). Furthermore, the number of leaves in the MS and MST1 treatments was the same as in the untouched treatment for both cultivars regardless of salinity (Table 3.1).

The effects of salinity on dry weight, grain yield, grain number and spikelet number on the mainstem are presented in Figs 3.1-3.5. Regardless of salt stress, dry weight, grain yield and grain number on the mainstem at maturity were increased greatly compared with the untouched treatment in the MS treatments of both cultivars, but only slightly in the MST1 treatment of Kharchia (Figs 3.1-3.4).

Table 3.1 Effect of detillering on leaf number and leaf area of the mainstem and subtillers in the salt-tolerant (Kharchia) and salt-sensitive (Sakha 61) cultivars subjected to salinity.

Treatments		Kharchia						Sakha 61					
		Mainstem		T1		T2		Mainstem		T1		T2	
		Leaf number	Leaf area (cm ²)	Leaf number	Leaf area (cm ²)	Leaf number	Leaf area (cm ²)	Leaf number	Leaf area (cm ²)	Leaf number	Leaf area (cm ²)	Leaf number	Leaf area (cm ²)
Untouched	No salt	10	18.9	6	14.6	5	14.1	10	14.8	7	12.8	6	13.0
	Salt	9	17.0	6	12.8	5	11.6	9	13.7	6	12.6	5	10.1
MS	No salt	10	23.2					10	19.2				
	Salt	9	20.7					9	16.5				
T1	No salt			6	20.8					7	17.0		
	Salt			6	18.2					6	15.1		
MST1	No salt	10	20.1	6	17.2			10	16.2	6	16.0		
	Salt	9	18.9	6	15.7			9	14.5	6	14.0		
T1T2	No salt			6	16.2	5	15.0			7	16.1	6	15.6
	Salt			6	15.0	5	14.0			6	14.9	5	12.7
LSD ($P \leq 0.05$)		-	1.7	-	2.2	-	2.4	-	1.7	-	1.6	-	2.1

Role of tillering in salt tolerance of spring wheat

Under saline conditions, the reductions in dry weight, grain yield, grain number and spikelet number on the mainstem were observed in both the salt-tolerant and salt-sensitive cultivars, with the magnitude being cultivar dependent (i.e. there was a smaller reduction in these parameters on the mainstem of the salt-tolerant cultivar than in the salt-sensitive cultivar regardless of treatments). At the final harvest, the reduction in dry weight on the mainstem of Kharchia under saline conditions was greater in the detilled treatments as compared to the untouched treatment, with the

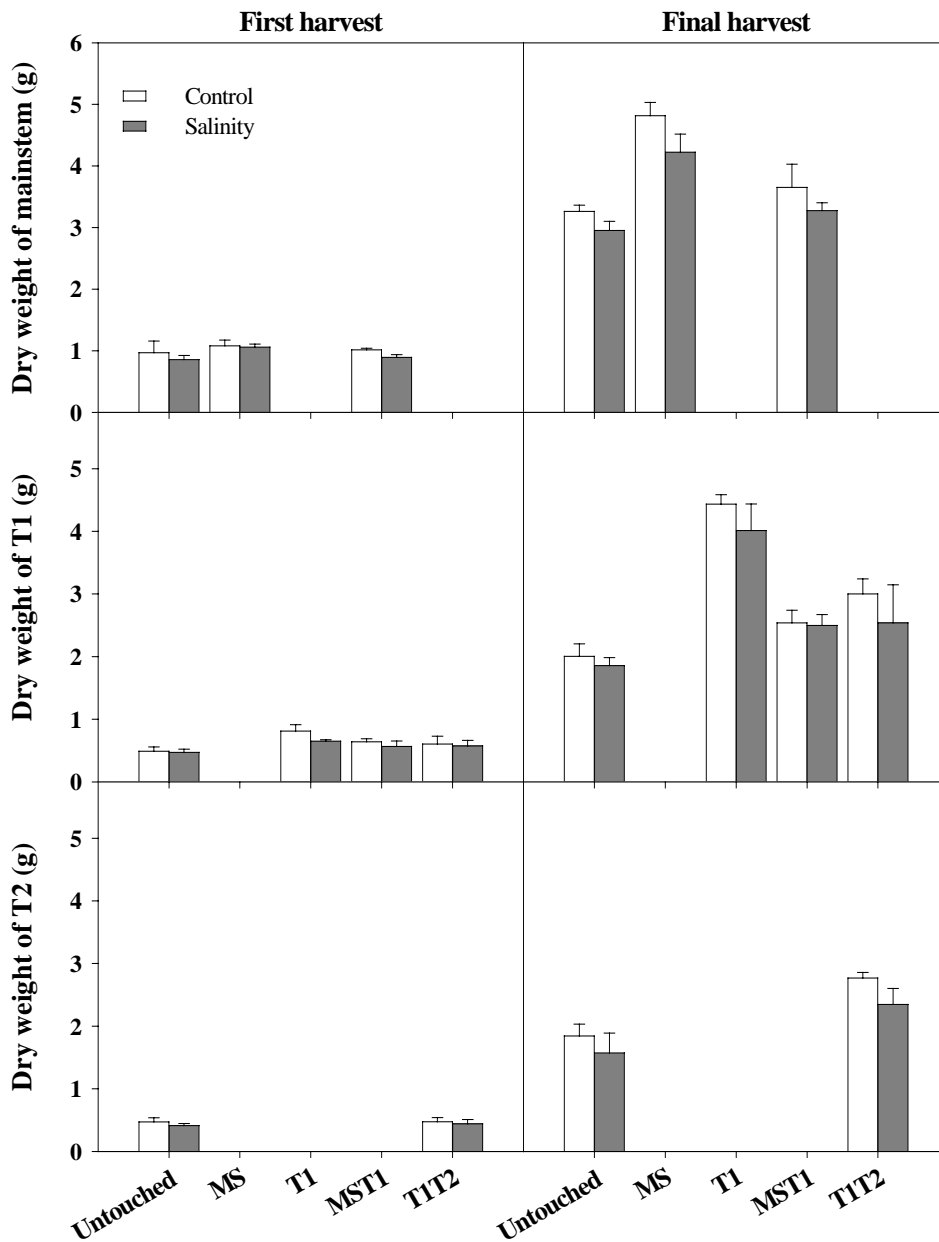


Figure 3.1 Dry weights of mainstem, T1 and T2 in the different detillering treatments of Kharchia with or without salinity at two harvest times.

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largest reduction occurring in the MS treatment. For Sakha 61, a greater reduction was found in the MST1 treatment, with there being no change relative to the untouched treatment in the MS treatment (Figs 3.1 and 3.2). Similarly, the reduction in grain yield on the mainstem resulting from salinity was comparatively greater in the MS treatment of Kharchia and the MST1 treatment of Sakha 61, but smaller in the MS treatment of Sakha 61 and the MST1 treatment of Kharchia, when compared with the untouched treatment (Fig. 3.3).

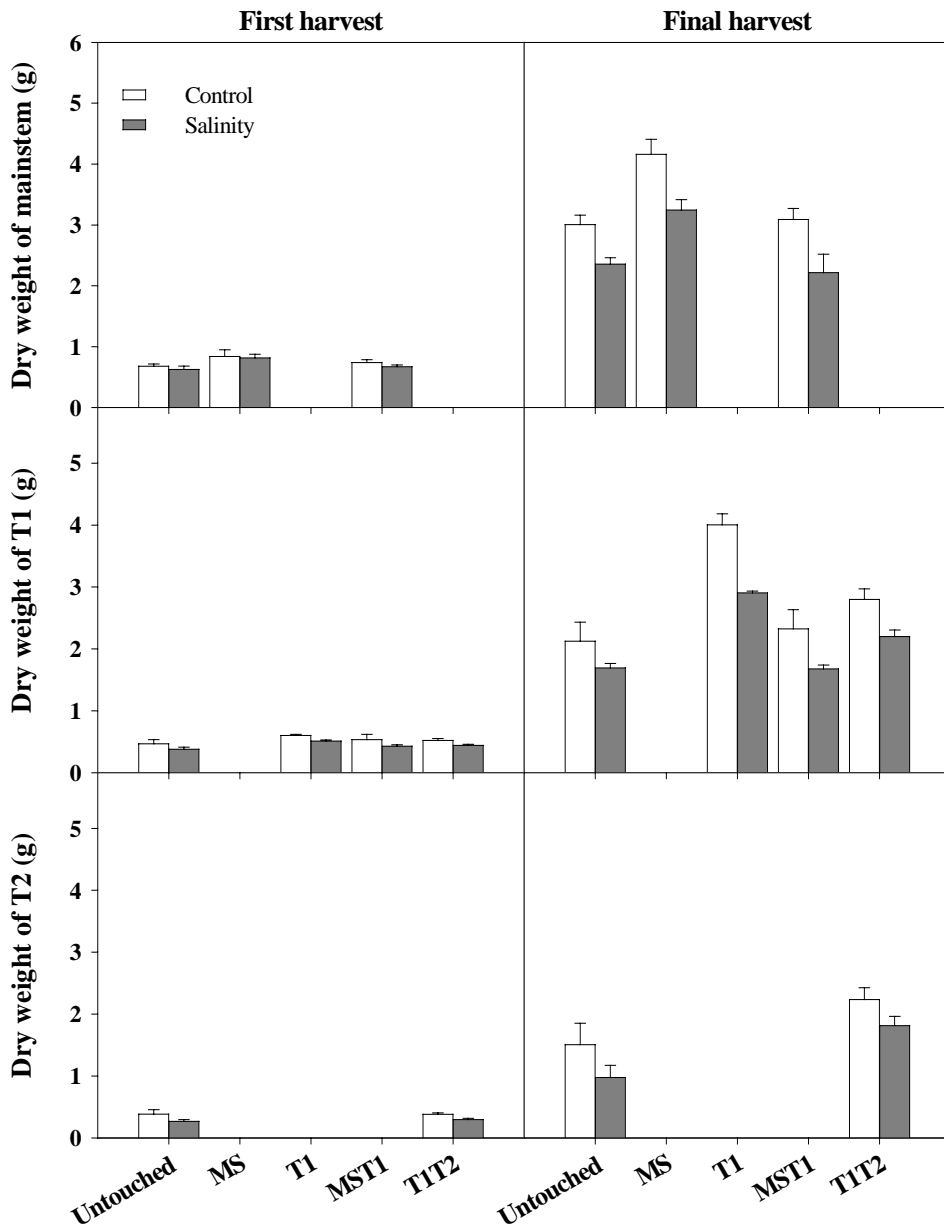


Figure 3.2 Dry weights of mainstem, T1 and T2 in the different detillering treatments of Sakha 61 with or without salinity at two harvest times.

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Compared with the salt-tolerant cultivar, salinity always resulted in a greater reduction in grain number on the mainstem of the salt-sensitive cultivar (e.g., 23% higher in the untouched treatment; Fig. 3.4). For both cultivars, however, decreases in tiller number also increased spikelet fertility of the mainstem under saline conditions compared to the untouched treatment as witnessed, for example, by the comparatively lower spikelet number and higher grain number on the salt-stressed Sakha 61 mainstem in the MS treatment.

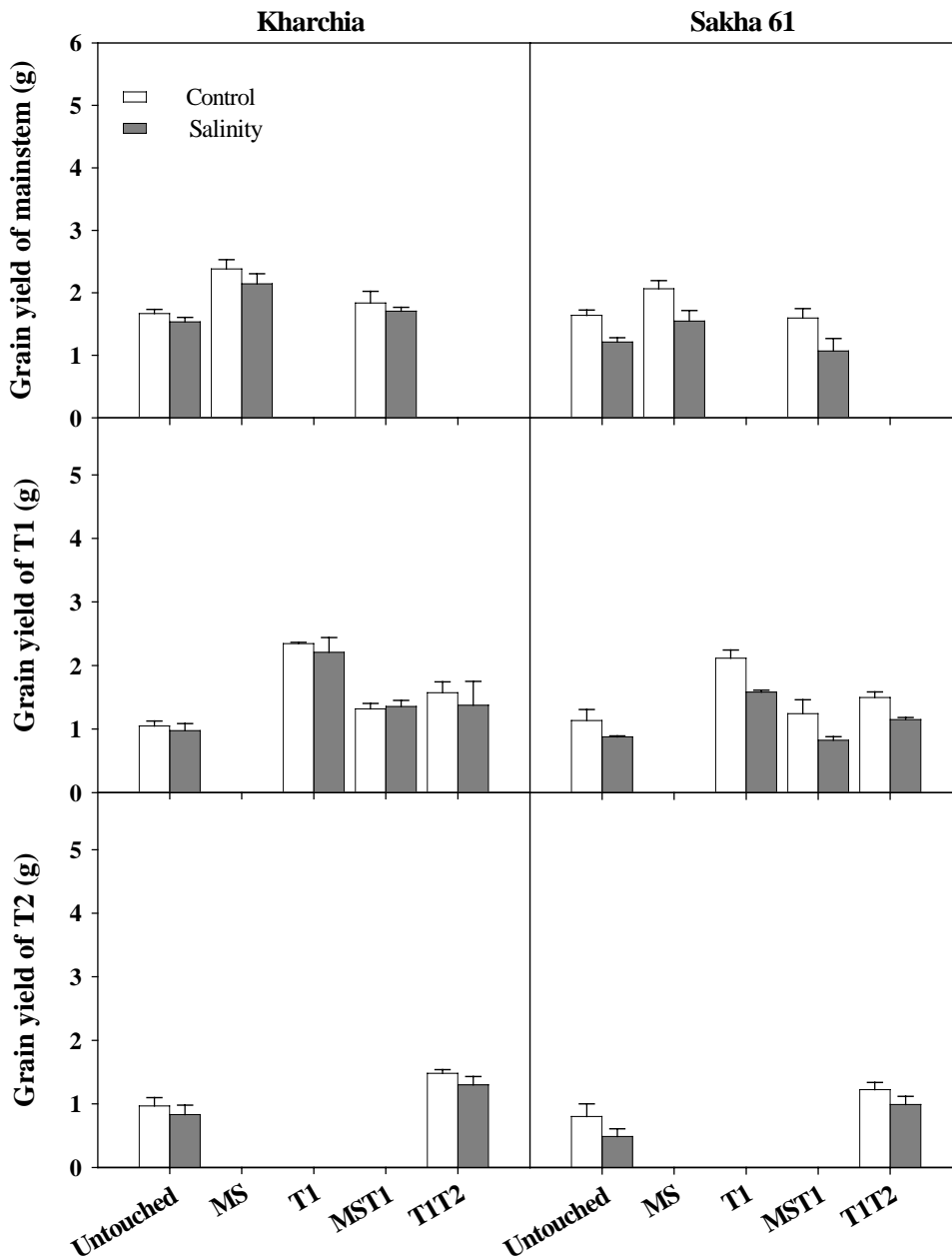


Figure 3.3 Grain yields of mainstem, T1 and T2 in the different detillering treatments of Kharchia and Sakha 61 with or without salinity.

3.3.2 Association of subtiller growth with tiller number

Compared to the non-saline plants, salinity reduced leaf numbers of sub tillers T1 and T2 in the untouched treatment in Sakha 61 only (Table 3.1) and no reduction in the leaf number of Kharchia was observed even in the detillered treatments. Furthermore, the detillered treatments did not change the leaf number of the salt-stressed sub tillers of either cultivar compared to the untouched treatment (Table 3.1). Interestingly, under the moderate salt stress used, sub tiller T1 was able to show a comparatively

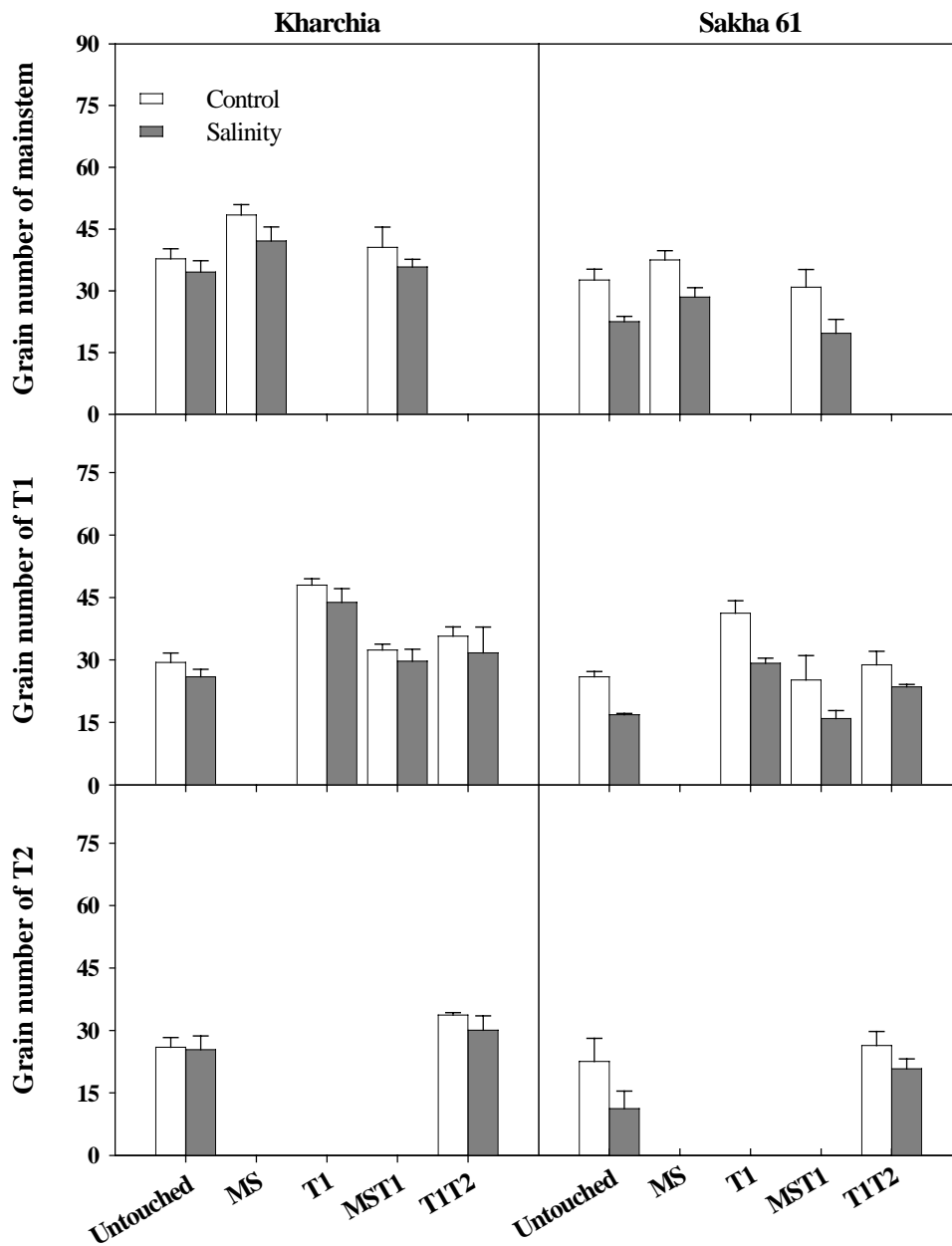


Figure 3.4 Grain numbers of mainstem, T1 and T2 in the different detillering treatments of Kharchia and Sakha 61 with or without salinity.

Role of tillering in salt tolerance of spring wheat

smaller reduction in dry weight than the mainstem during the period of growth (e.g. the vegetative growth) in the salt-tolerant, but not in the salt-sensitive cultivar. For example, the reduction in dry weight of Kharchia due to salinity in the untouched treatment was about 11% for the mainstem, 3% for the subtiller T1 and 12% for the subtiller T2 at the first harvest; the analogous values for Sakha 61 were 8%, 19% and 30%, respectively (Figs 3.1 and 3.2).

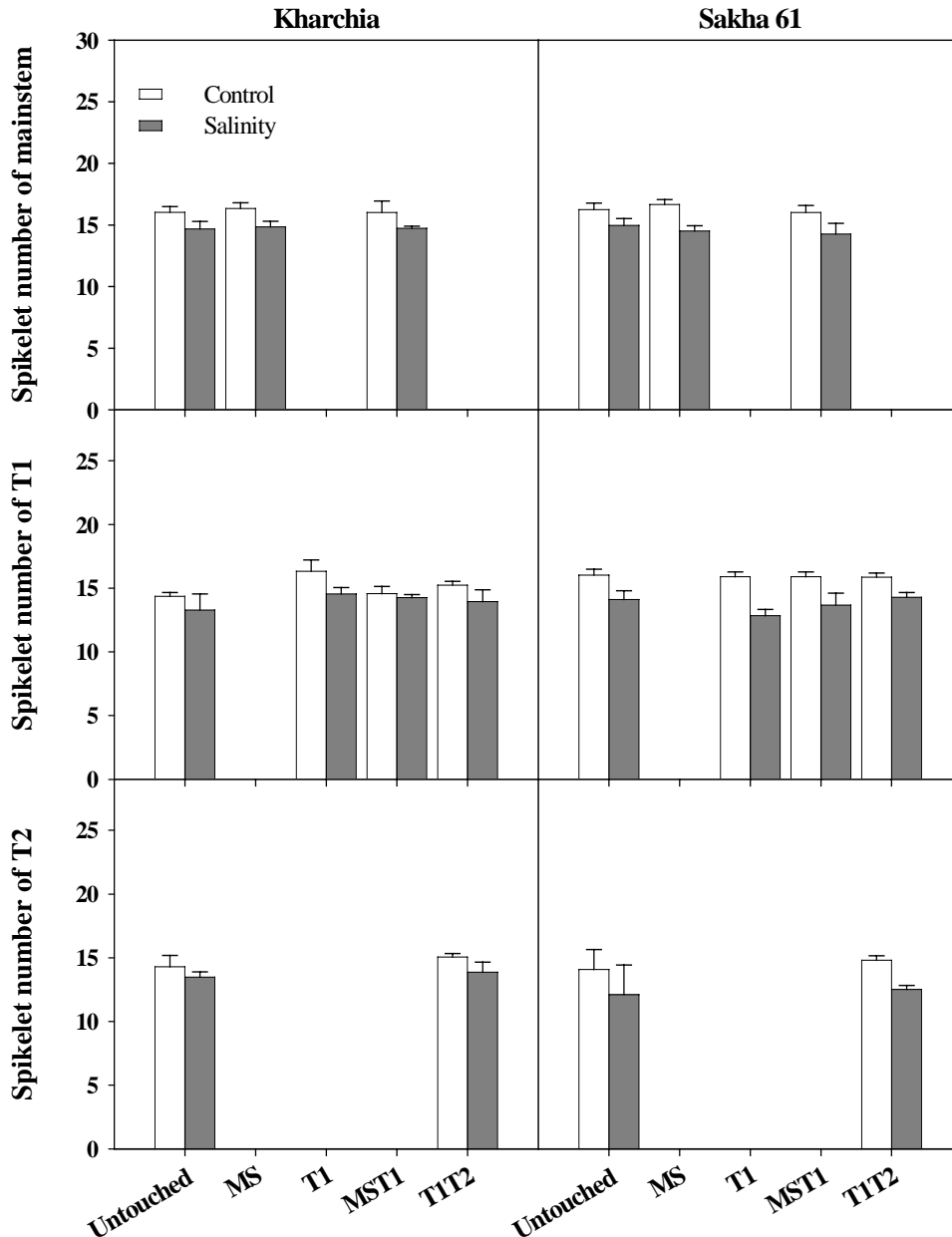


Figure 3.5 Spikelet numbers of mainstem, T1 and T2 in the different detillering treatments of Kharchia and Sakha 61 with or without salinity.

With a similar pattern on the mainstem, the detillered treatments significantly increases leaf area, grain yield and final dry weight on the subtillers compared to the untouched treatment in both cultivars under saline conditions, except for the MST1 treatment of Sakha 61. The increased grain yield in the detillered treatments was related more to an increase in grain number than in spikelet number (Figs 3.4 and 3.5). Under saline conditions, salinity inhibited the subtiller growth of both Kharchia and Sakha 61 by reducing both dry weight and leaf area, which, in turn, resulted in a reduced grain yield, grain number and spikelet number on the subtillers (Figs 3.1-3.5). However, compared with the untouched treatment, the reductions in final dry weight, leaf area and grain yield on subtiller T1 for Kharchia under saline conditions tended to be comparatively smaller in the detillered treatments, the exception being the T1T2 treatment; for Sakha 61, the reductions were comparatively greater in all treatments. For the subtiller T2, the T1T2 treatment resulted in comparatively smaller reductions in final dry weight, leaf area and grain yield for both cultivars under saline conditions, especially for the salt-sensitive cultivar.

3.3.3 Association of whole shoot growth with tiller number

The effects of salinity on dry weight, grain yield and grain number per plant are presented in Figs 3.6 and 3.7. Except for the T1 treatment at the first harvest, dry weight per plant was less affected by salinity in Kharchia than in Sakha 61 over all treatments at either harvest. Our results (Fig. 3.6) further show that the comparative reduction in dry weight per plant under saline conditions for Sakha 61 became continually greater over time until the mature stage, whereas a comparatively smaller reduction for Kharchia was found at the mature stage, a difference that might stem from their differential salt tolerance during the reproductive growth stage. For example, the reduction in dry weight per plant of Sakha 61 at the final harvest compared to the first harvest was about 10% greater in the untouched treatment, 19% in the MS treatment, 12% in the T1 treatment, 14% in the MST1 treatment and 2% in the T1T2 treatment. By contrast, the comparative reduction in dry weight per plant of Kharchia at the final harvest was dependent on tiller number per plant. Furthermore, the enhanced salt tolerance of Kharchia during reproductive growth may be associated with its greatly reduced loss in grain yield and grain number per plant (regardless of treatments) compared with Sakha 61.

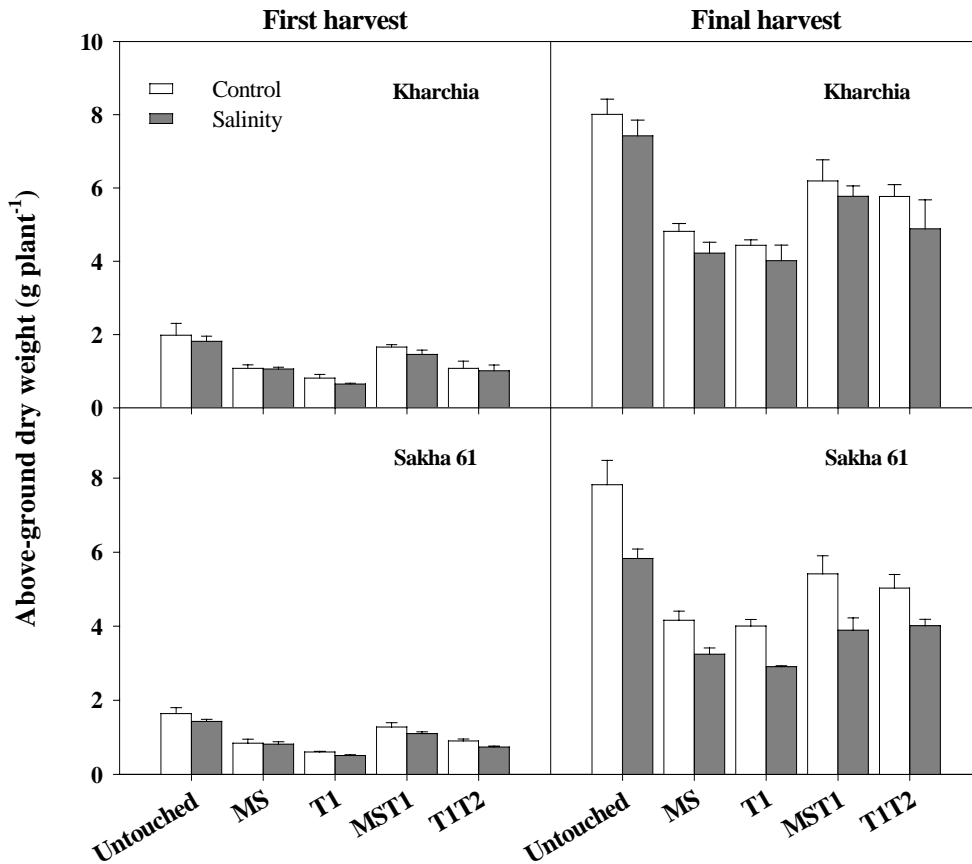


Figure 3.6 Above-ground dry weight of the whole plant in the different detillering treatments of Kharchia and Sakha 61 with or without salinity at two harvest times.

Compared with the untouched treatment, the detillered treatments significantly decreased dry weight (both harvests), grain yield and grain number per plant regardless of the cultivar and salt stress, with the MS and T1 treatments showing comparatively greater reductions than did the MST1 and T1T2 treatments (Figs 3.6 and 3.7). Under saline conditions, however, the manipulation of tiller number could alter the apparent salt tolerance of both the salt-tolerant and salt-sensitive cultivars. Overall, the reductions in final dry weight, grain yield and grain number per plant for Kharchia under saline conditions tended to be comparatively greater in the detillered treatments (one or two tillers per plant) compared with the untouched treatment (more than three tillers per plant), whereas they tended to be comparatively smaller for Sakha 61.

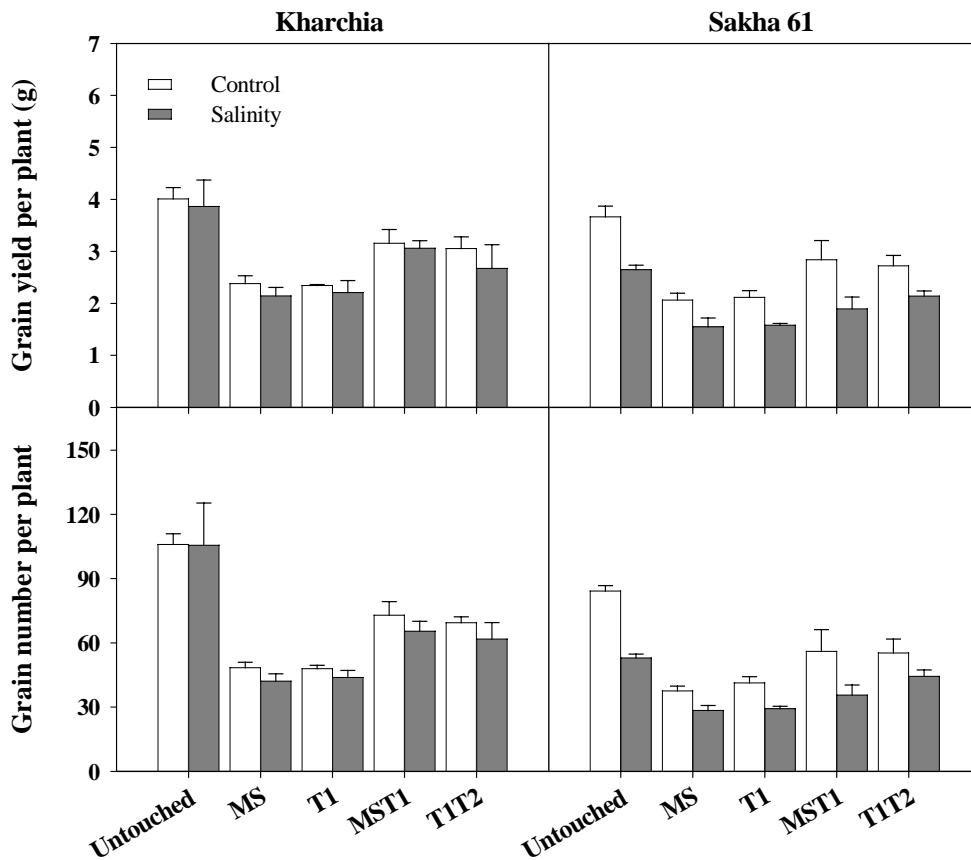


Figure 3.7 Grain yield and grain number of the whole plant in the different detillering treatments of Kharchia and Sakha 61 with or without salinity.

3.4 Discussion

Regardless of the cultivar and salt stress, a reduction in tiller number is associated with increasing in leaf area, dry weight, grain yield and grain number per tiller on both the mainstem and subtillers in spring wheat, which is in agreement with the observation of Kirby and Jones (1977) in barley. Alaoui *et al.* (1988) point out that the enhanced leaf area may increase the photoassimilate supply needed for the greater tiller grain yield. For the whole plant, however, reduced tiller numbers significantly reduced dry weight, grain yield and grain number. It is apparent that tiller number is a very important contributor to the total grain yield of plant in wheat, despite grain yield being increased in the mainstems or subtillers of the detillered treatments. It has been reported previously, that the increased grain yield on the mainstem resulting from tiller removal does not compensate for the loss of the subtillers (Kemp and Whingwiri 1980; McDonald 1990). The present study further suggests that the enhanced grain

yield on the subtillers when only they were left intact did not compensate for the loss of the mainstem.

Under saline conditions, however, knowledge about how difference in tiller number affects the salt tolerance of the mainstem and subtillers as well as of whole-plant could prove to be more crucial in terms of improving wheat yield under salt stress. Hu *et al.* (1997) have pointed out that, under moderate salinity, nutrient deficiency and salinity may equally limit plant growth, and they may not interact. Thus, the nutrient activities and ratio of salt ions in the mainstem and subtillers could be an important determinant of plant salt tolerance. Mainstem-to-subtiller relationships are naturally complex, but can be summarized as follows: (1) under adequate nutrient supply, the mainstem translocates nutrients to the subtiller to support its growth and development, and the subtiller also translocates nutrients to the mainstem to compensate its losses (Lauer and Simmons 1985; 1988); (2) under poor nutrient supply, the mainstem monopolises the available nutrients, greatly decreasing their translocation to the subtiller, which may also decrease or stop the nutrient translocation to the mainstem (Gu and Marshall 1988); and (3) the translocation of nutrients from the mainstem to the subtiller is high in early plant development and decreases subsequently, with the nutrient translocation from the subtiller to the mainstem only occurring before the later reproductive growth stage (Lupton 1966; Lauer and Simmons 1988). Therefore, under low nutrient activities and high toxic-ion ratios, the respective capacities of the mainstem and subtillers to obtain nutrients or exclude toxic ions contribute to their own salt tolerance, thereby affecting that of the whole-plant in turn.

In this experiment, one tiller per plant (i.e. the MS or T1 treatments) greatly increased the dry weight, leaf area, grain yield and grain number on the mainstem or subtiller T1, respectively, compared with the corresponding values in the untouched treatment regardless of the cultivar and salt stress, indicating the high potential of both mainstem and subtiller to take up nutrients under saline conditions. By contrast, the mainstem and subtillers could have the differential responses to salinity due to the competition for nutrients and the accumulation of toxic ions, for which both tiller number and cultivar could play a key role. For example, the salt tolerance of the mainstem was decreased in two tillers per plant (i.e. the MST1 treatment) compared with one tiller per plant (i.e. the MS treatment) in the salt-sensitive, but not in the salt-

tolerant cultivar. Lauer and Simmons (1985; 1988) reported that, as nutrient competitors within the plant, the subtillers could act as either a sink or a source of nutrients associated with the mainstem. As a result, the export and import of nutrients from the subtillers to the mainstem could be one of the possible reasons to diminish or enlarge the specific effects of toxic ions on the mainstem, with the exact effect depending on the genotype in question. The comparatively lower reduction in grain yield on the mainstem of the salt-tolerant cultivar Kharchia in the plants where both the mainstem and T1 were left intact than where only the mainstem was further indicate the advantage that the subtiller can provide in improving the salt tolerance of the mainstem. However, a similar advantage was not observed in the salt-sensitive cultivar Sakha 61.

As such, the comparatively smaller inhibition of growth on the subtiller T1 than on the mainstem in the salt-tolerant cultivar under moderate salinity in the untouched plant indicates that the subtillers (or at least for the primary tillers) may be by either receiving more assimilates and minerals from the mainstem and growth medium (Kirby and Jones, 1977) or translocating less photoassimilate to the mainstem (Lauer and Simmons, 1988) to alleviate salt-specific effects on them compared to the salt-sensitive cultivar. Here, the manipulation of tiller number with or without mainstem provides the evidence to support that the export and import of nutrients from the mainstem to the subtillers could be of importance with respect to salt tolerance. Our results showed that the dry weight of subtiller T1 in two tillers per plant with the mainstem (i.e. the MST1 treatment) in the salt-tolerant cultivar Kharchia was less reduced compared to that of two tillers per plant without mainstem (i.e. the T1T2 treatment). For the salt-sensitive cultivar Sakha 61, by contrast, this reduction was higher in two tillers per plant with the mainstem than without.

Differences in nutrient transport from the mainstem to the subtillers or vice versa in the salt-tolerant and salt-sensitive cultivars could be associated with their different mechanisms of salt tolerance. El-Hendawy *et al.* (2005b) summarised that salt-tolerant and salt-sensitive wheat cultivars use ion exclusion and high accumulation, respectively, in response to saline conditions. Kirby and Faris (1972) pointed out the possibility that the adjustment of resources between the mainstem and subtillers could help the plant to recover from poor environmental conditions. Therefore, we considered that the exclusion of toxic ions in the salt-tolerant cultivar in

combination with the relatively weak competition to balance nutrient distribution between the mainstem and subtillers under saline conditions might diminish the negative effects of toxic ions on the growth of mainstem and subtillers. By contrast, the high accumulation of toxic ions in the salt-sensitive cultivar in combination with an increased competition for nutrients between the mainstem and subtillers may enhance the inhibition of growth of both the mainstem and subtillers. Importantly, our results show that the subtillers (or at least for the primary tillers) of the salt-tolerant cultivar express superior genetic traits in relation to the exclusion of harmful ion during the reproductive growth stage compared to that of the salt-sensitive cultivar. For example, the reduction in dry weight on the subtiller T1 during the reproductive stage was decreased by 13 % in the salt-tolerant cultivar Kharchia but increased by 12% in the salt-sensitive cultivar Sakha 61 in the T1 treatment. Therefore, at the whole-plant level, it appears that different strategies are needed to improve the salt tolerance in the two cultivars. In the salt-tolerant cultivar Kharchia, the comparatively greater reduction in the investigated parameters under saline conditions resulting from a reduction in tiller number suggests that salt tolerance would be improved with more tillers (or at least for the primary tillers) per plant. By contrast, the comparatively smaller reduction in these parameters for the salt-sensitive cultivar Sakha 61 suggests that fewer tillers per plant could increase its salt tolerance.

3.5 Conclusions

With a reduction in the number of tillers to one or two per plant, dry weight, grain yield and grain number per plant were significantly decreased in both the salt-tolerant and salt-sensitive cultivars compared with the untouched plant (more than 3 tillers) regardless of salinity, while these same parameters tended to be increased on a per tiller (mainstem or subtiller) basis. The increased grain yield per tiller apparent upon tiller reduction could be due to the increased spikelet fertility of tillers under such conditions.

Our manipulations of tiller number could not alter the inherent genotypic effects on the differential salt tolerances between the cultivars. In other words, the salt-tolerant cultivar remained more tolerant to salinity than did the salt-sensitive cultivar under all the treatments performed. However, the plant salt tolerance with each cultivar could be improved by the manipulations of tiller number. For example,

whereas the salt tolerance of the salt-tolerant cultivar was improved with more primary tillers per plant, it was increased in the salt-sensitive cultivar when fewer or no primary tillers per plant were present.

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4

Influence of Tiller Number on Ion Accumulation in the Mainstem and Subtillers of Contrasting Wheat Cultivars under Saline Conditions

Abstract Plant growth is adversely affected by the accumulation of toxic ions under saline conditions. To investigate the influence of tillering on the accumulation of ions in the mainstem and subtillers, two contrasting spring wheat (*Triticum aestivum* L.) cultivars were subjected to five levels of detillering treatments under saline or non-saline conditions in a greenhouse. Sodium, K^+ , Ca^{2+} , Cl^- , NO_3^- concentrations in the leaves of different tillers was determined at the vegetative stage and maturity. From the vegetative stage to maturity, the mainstem or subtiller under saline conditions had a lower increase in Na^+ and Cl^- and a lower decrease in K^+ and NO_3^- in the salt-tolerant cultivar than in the salt-sensitive cultivar. Regardless of tiller number per plant, the salt-tolerant cultivar showed a higher Na^+/K^+ ratio in the youngest leaves and lower Na^+/K^+ ratio in the older leaves under saline conditions at plant maturity compared with the salt-sensitive cultivar. Furthermore, the accumulation of ions in the one-tiller plant showed that the mainstem or subtiller in the salt-tolerant cultivar had the better salt exclusion under saline conditions, and maintained higher K^+ accumulation and lower ratios of Na^+/K^+ and Na^+/Ca^{2+} in the older leaves at maturity, while for the salt-sensitive cultivar, the mainstem or subtiller showed lower K^+ accumulation and higher ratios of Na^+/K^+ and Na^+/Ca^{2+} . Especially for the salt-sensitive cultivar, the less tiller number per plant resulted in the higher Na^+ accumulation and higher Na^+/K^+ and/or Na^+/Ca^{2+} ratios in the mainstem or subtiller under saline conditions, indicating that there may exist the collective defense for resisting the salt toxicity in tillers.

4.1 Introduction

The inhibition of plant growth under saline conditions may involve osmotic stress, specific ion toxicity and ionic imbalance (Grattan and Grieve 1999), which are commonly related to the accumulation of either toxic or nutrient ions. Adverse effects of salinity on plant growth are mainly due to the increase in toxic ions, e.g. Na^+ and Cl^- , to suppress the uptake of nutrient ions, e.g. K^+ , Ca^{2+} , NO_3^- , Mg^{2+} , (Hu and Schmidhalter 1997; El-Hendawy *et al.* 2005). The reduced K^+ and Ca^{2+} levels could impair the integrity and selectivity of cell membrane under saline conditions (Kramer *et al.* 1977), while the reduction in NO_3^- , proposed as a plant growth regulator (Trewavas 1985), could affect the metabolism and development of plant. Therefore, the ion toxicity and nutrient balance are vital aspects under salt stress.

In wheat, sodium and Cl^- preferentially accumulate in the leaves and stem (Greenway and Munns 1980; Boursier *et al.* 1987; Hu and Schmidhalter 1997). Salt tolerance has been found to be associated with the low uptake of Na^+ and Cl^- in the leaves, with consequent high selectivity for K^+ , Ca^{2+} or NO_3^- over Na^+ or Cl^- (Schachtman and Munns 1992; Cerezo *et al.* 1999; El-Hendawy *et al.* 2005). It is reported that the supplementation of Ca^{2+} in the growth medium under saline conditions has shown to improve the shoot growth (Muhammed *et al.* 1987; Cramer 1992) and root elongation (Kurth *et al.* 1986; Husain *et al.* 2004), but restricting tiller number could improve shoot growth by increasing mineral nutrient level without additional supply, due to the modification of physiological and morphological traits of wheat plants (Mohamed and Marshall 1979; Gu and Marshall 1988). However, there is no information available about effects of tiller number combining with genotypes on mineral accumulation under saline conditions.

In recent decades, considerable attention has been focused on attesting the hypotheses of positive correlations between salt tolerance and the exclusion or sequestration of toxic ions (Munns 1993; Munns and James 2003). However, because the supports of these hypotheses were derived from the analysis of the whole plant, they may not reveal the potential factors responsible for the improvement of salt tolerance within the plant. We have previously observed that the growth of mainstem is less reduced by salinity compared to that of subtillers. This differential reduction in the growth may be associated with the altered ion uptake and accumulation in the mainstem and subtillers due to their competition for nutrients under saline conditions.

The subtillers are reported to function not only as the growth buffer under environmental stresses (Kirby and Faris 1972), but also as the sink and source of nutrients (Lauer and Simmons 1988). We can speculate that the plants could enhance their growth by adjusting tillering under salt stress. Therefore, knowledge of ion distribution among the tillers as varied by tiller number could provide a further understanding of relationships between tillering and salt tolerance.

The objectives of this study were to quantitatively evaluate the accumulation of ions, e.g. Na^+ , Cl^- , K^+ , Ca^{2+} and NO_3^- , etc., in the different tillers by the manipulation of tillering, and to understand internal regulation of ions in the plant in contrasting wheat cultivars under salt stress.

4.2 Materials and methods

4.2.1 Plant materials and growth conditions

Thirty seeds of two contrasting spring wheat cultivars Kharchia (salt-tolerant) and Sakha 61 (salt-sensitive) that were pre-germinated at room temperature for 2 days were sown in pots containing 10 kg of dry soil with or without salinity. Soils were salinized by adding 120 mM NaCl solutions to layer-built soils within the pot as the initial soil was filled layer-wise with four layers, but the top layer was salinized at day 10 after sowing. The final electrical conductivity in soil solution was about 5.6 dS m^{-1} , which was measured before the experiment started. Only a moderate saline stress was applied in this study because our preliminary experiments showed that high salinity could cause the plant to skip tiller formation. Soils (0-15 cm on the soil surface) were air-dried, ground and passed through a 5-mm mesh screen. Soil water content in pots was maintained at a 25% dry soil basis by replacing any water lost through evapotranspiration. The plants were grown in a growth chamber at $23/18^\circ\text{C}$ (day/night) with 16-h light period of photon flux density $550 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$ (PPFD) until the first tiller emerged, and then transferred to a greenhouse. At the two-leaf stage, the seedlings were thinned to 22 plants per pot.

The preliminary experiments also showed that the optimal soil nutrient was obtained by applying 0.57 g NH_4NO_3 per pot initially, with an additional 0.57 g NH_4NO_3 per pot being added at the third, sixth, eighth and tenth weeks after sowing. In addition, 0.1 g KH_2PO_4 and K_2SO_4 per pot were added at the sixth and eighth weeks after sowing, respectively.

4.2.2 Experimental design and sampling

The experiment was arranged as a randomized complete block design and consisted of five detillering treatments each replicated three times. According to the processes of tiller appearance, five detillering treatments were carried out such that plants consisted of: (1) MS: the mainstem only; (2) T1: the first leaf tiller on the mainstem only; (3) MST1: the mainstem and its first leaf tiller; (4) T1T2: the first and second leaf tillers on the mainstem; and (5) untouched: all culms left intact.

The procedures for removing the tiller bud were described as: the subtiller buds (T1 and T2) were broken off by a blunt needle at the base of the leaf as soon as they became visible; the mainstem was cut out by a surgical scalpel from the plant when the second leaf of the youngest subtiller left intact was fully expanded. The mainstem and subtiller buds were removed two times depending on the growth of meristem.

Leaves in the mainstem and sub tillers T1 and T2 were harvested from ten plants at the first harvest (flag-leaf stage) and from remaining plants at the final harvest (maturity), and separated into the flag leaf (the first leaf on the top) and two leaves below the flag leaf (the second plus third leaf on the top). After harvesting, leaves were dried at 65°C for 48 h and weighed in the mainstem, subtiller T1 and subtiller T2, respectively.

4.2.3 Analysis of ion contents

Oven-dried leaves of the mainstem and sub tillers at the first harvest and final harvest were ground into a fine powder by passing them through a 0.5-mm diameter sieve. To determine Na^+ , K^+ , Mg^{2+} and Ca^{2+} concentrations, 50-mg leaf samples were digested by adding 3mL concentrated HNO_3 and 2 ml H_2O_2 for 40 min at 2600 kPa (80 psi) in a MDS-2100 microwave oven (CEM Corp., Matthews, NC, USA). After digestion, each example was finally diluted to a volume of 25 ml with distilled-deionized water. The Na^+ , K^+ , Mg^{2+} and Ca^{2+} concentrations were determined with an Inductively Coupled Plasma Emission Spectrometer (ICP model Liberty 200, Varian Australia Pty. Ltd., Mulgrave Victoria, Australia). For determination of NO_3^- and Cl^- concentrations, 50 mg of ground leaf sample was extracted with 25 ml distilled water and shaken for 1 hour and then filtered. Chloride and NO_3^- were determined using an Ion Chromatography Analyser (Model LC20-1, Dionex, Sunnyvale, CA 94086, USA).

4.2.4 Statistical analysis

Data were analyzed by analysis of variance (ANOVA) using the PROC GLM procedure of The SAS System v9.1. Means separation on the data was conducted using LSD multiple range tests. Terms were considered significant at $P \leq 0.05$.

4.3 Results

4.3.1 Influence of tiller number per plant on ion accumulation in the mainstem leaves

Regardless of treatments, salinity greatly increased the Na^+ concentration of leaves (flag leaf and two leaves below the flag leaf) on the mainstem at the first and final harvests for both cultivars. There was a higher Na^+ concentration in the salt-sensitive cultivar Sakha 61 than in the salt-tolerant cultivar Kharchia (Figs 4.1 and 4.2). At the final harvest, the MS treatment compared with the untouched treatment significantly increased the Na^+ concentration of leaves on the mainstem under salt stress in both cultivars, while for the MST1 treatment, greater Na^+ content was observed in the flag leaf of Kharchia and two leaves below the flag leaf of Sakha 61. Regardless of cultivars, the Na^+ accumulation under saline conditions was increased with leaf age and plant age in the different treatments, i.e. the older leaves and plants accumulated higher Na^+ . For example, in the untouched treatment, the Na^+ accumulation in the mainstem of Kharchia due to salinity was 45% higher in the two leaves below the flag leaf than in the flag leaf at the final harvest, and 26% higher at the final harvest than at the first harvest in the two leaves below the flag leaf. For both cultivars, the accumulation of Na^+ in the detillered treatments under saline conditions was increased much more than that in the untouched treatment at the final harvest. For example, the Na^+ concentration of two leaves below the flag leaf on the mainstem under saline conditions compared to the non-saline conditions for Sakha 61 was about 5 times higher in the untouched treatment, 18 times in the MS treatment and 14 times in the MST1 treatment, while for Kharchia, it was about 5 times higher in the untouched treatment, 9 times in the MS treatment and 9 times in the MST1 treatment.

Influence of tiller number on ion accumulation in tillers

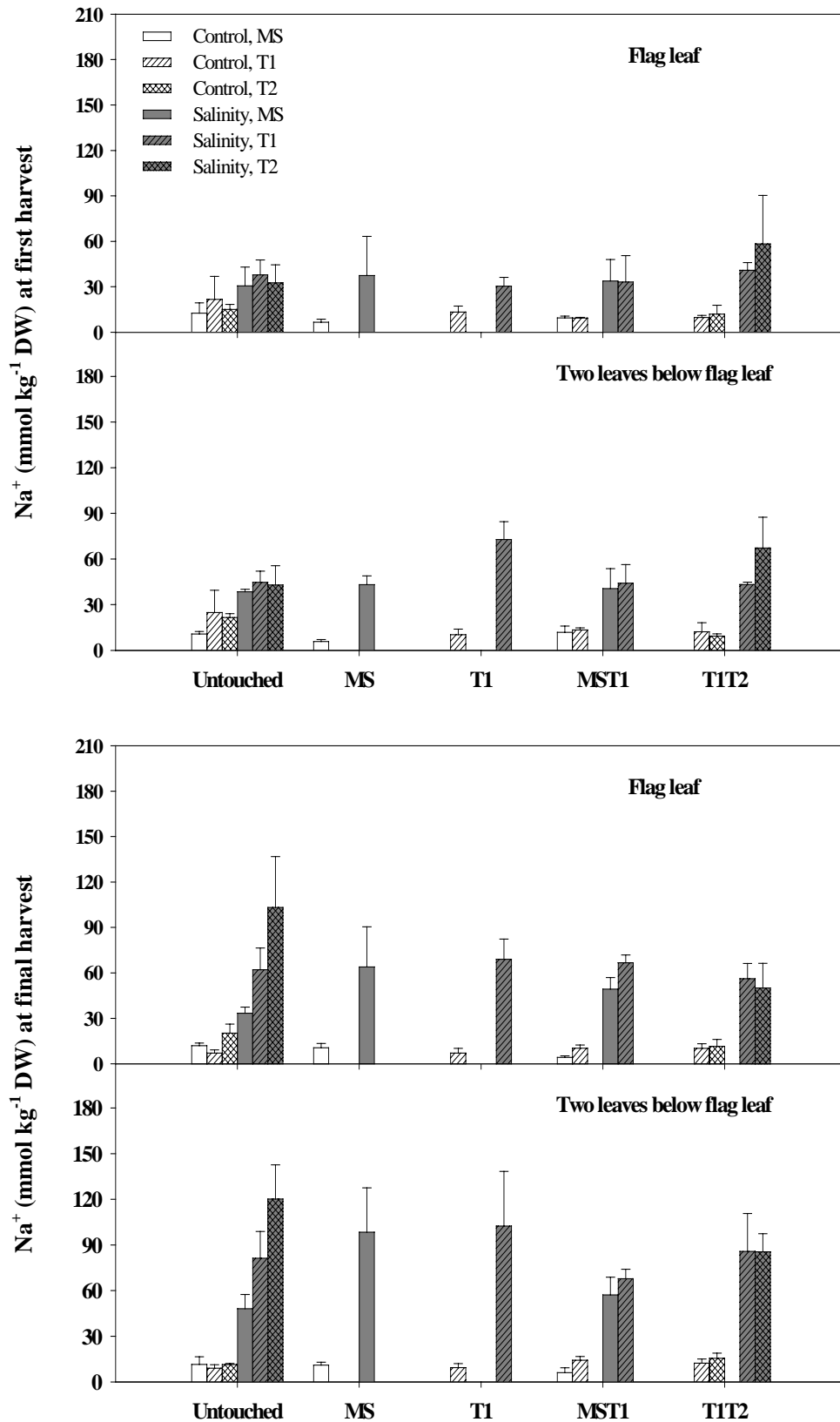


Figure 4.1 Sodium concentrations in the flag leaf and two leaves below the flag leaf in the mainstem (MS), T1 and T2 in the different detillering treatments of Kharchia with or without salinity at two harvest times.

Influence of tiller number on ion accumulation in tillers

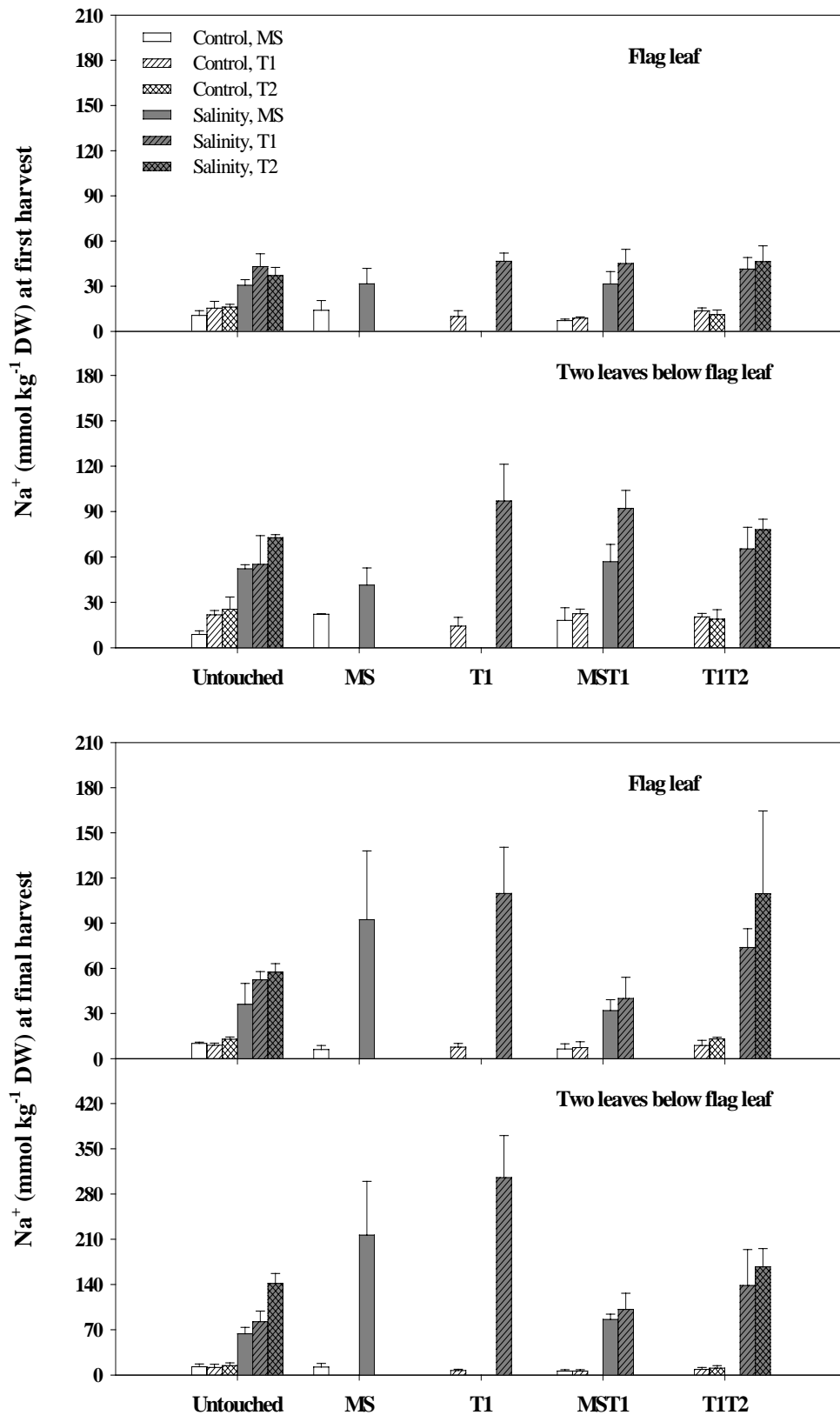


Figure 4.2 Sodium concentrations in the flag leaf and two leaves below the flag leaf in the mainstem (MS), T1 and T2 in the different detillering treatments of Sakha 61 with or without salinity at two harvest times.

Influence of tiller number on ion accumulation in tillers

Regardless of the salt stress and treatment, two leaves below the flag leaf had higher K^+ concentration at both harvests than the flag leaf on the mainstem of Kharchia, but only higher at the first harvest for Sakha 61 (Figs 4.3 and 4.4). Furthermore, the K^+ concentration of two leaves below the flag leaf at the final harvest compared to the first harvest was decreased in the mainstems of both cultivars. In contrast to Sakha 61, salinity greatly increased the K^+ concentration of two leaves below the flag leaf on the mainstem in all treatments of Kharchia at the final harvest. As one of physiological characters, the Na^+/K^+ ratio on the mainstem under saline conditions differs depending on tiller numbers and cultivars. For example, the Na^+/K^+ ratio of two leaves below the flag leaf for Kharchia at the final harvest was 0.10, 0.08 and 0.07 in the MS, MST1 and untouched treatments, respectively, while the analogous values for Sakha 61 were 0.36, 0.12 and 0.09, respectively.

In contrast to K^+ , the Ca^{2+} concentration of leaves on the mainstem had a greater increase at the final harvest than at the first harvest in the different treatments regardless of the cultivar and salt stress (Figs 4.5 and 4.6). Interestingly, salinity could further increase the Ca^{2+} concentration of leaves on the mainstem, with less tiller per plant showing more Ca^{2+} increase at the final harvest. Therefore, the Na^+/Ca^{2+} ratio compared with the Na^+/K^+ ratio at the final harvest became smaller in the mainstem leaves under saline conditions, with a smaller ratio in Kharchia than in Sakha 61. However, similar to Na^+/K^+ ratio, a reduction in tiller number in both cultivars increased the Na^+/Ca^{2+} ratio, which was observed at the final harvest, i.e. fewer tillers per plant had a higher Na^+/Ca^{2+} ratio than the untouched plant.

Compared to non-saline conditions, the accumulation of Cl^- in the leaves on the mainstem under saline conditions was greatly increased at two harvests in all treatments of both cultivars (Figs 4.7 and 4.8). However, compared with Sakha 61, the increase in the Cl^- concentration due to salinity became slower from first harvest to final harvest in Kharchia, resulting in lower Cl^- concentration at the final harvest. Interestingly, under moderate salt level used, there was no significant difference in the Cl^- accumulation between the detilled and untouched treatments in the two leaves below the flag leaf on the mainstems of both cultivars.

Influence of tiller number on ion accumulation in tillers

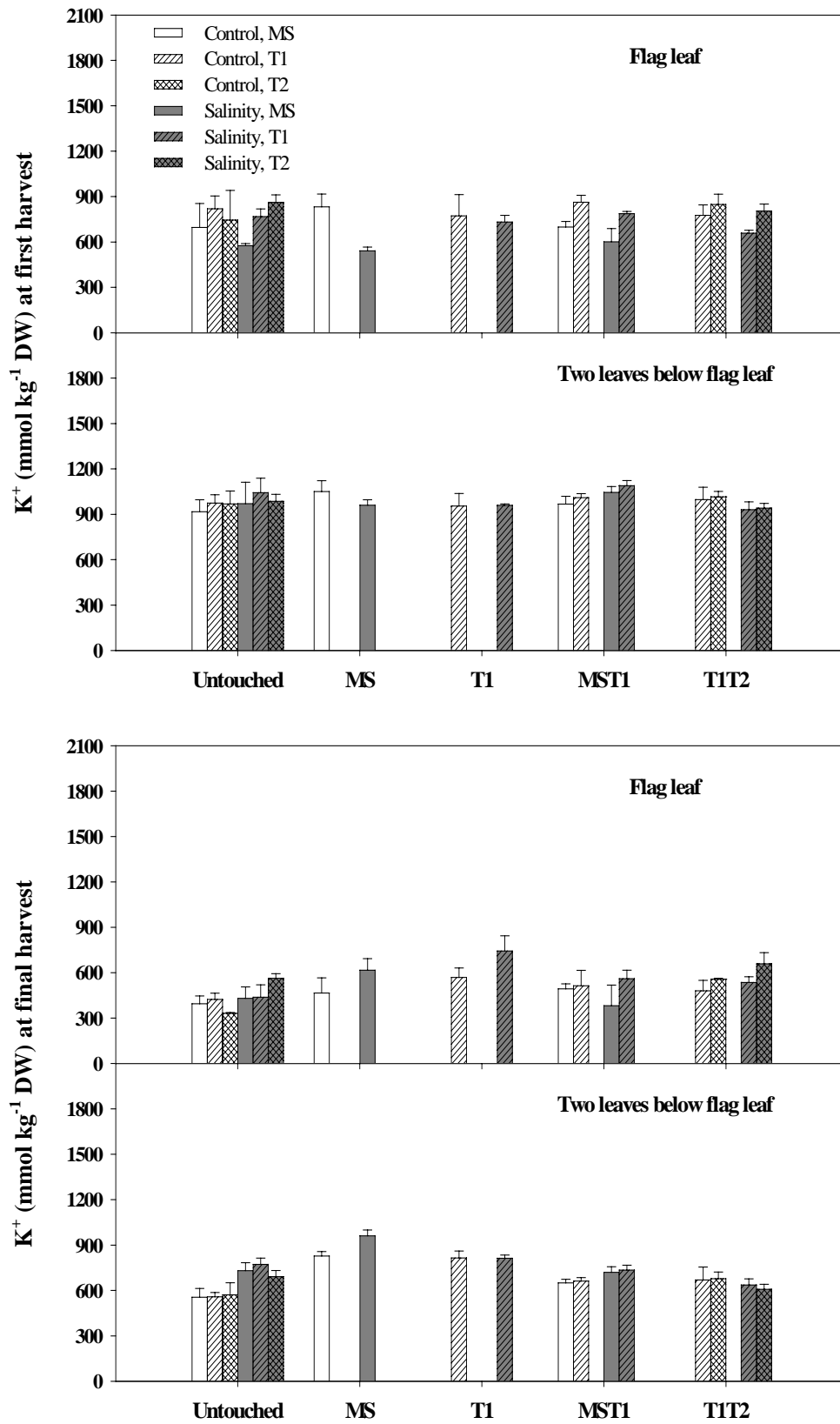


Figure 4.3 Potassium concentrations in the flag leaf and two leaves below the flag leaf in the mainstem (MS), T1 and T2 in the different detillering treatments of Kharchia with or without salinity at two harvest times.

Influence of tiller number on ion accumulation in tillers

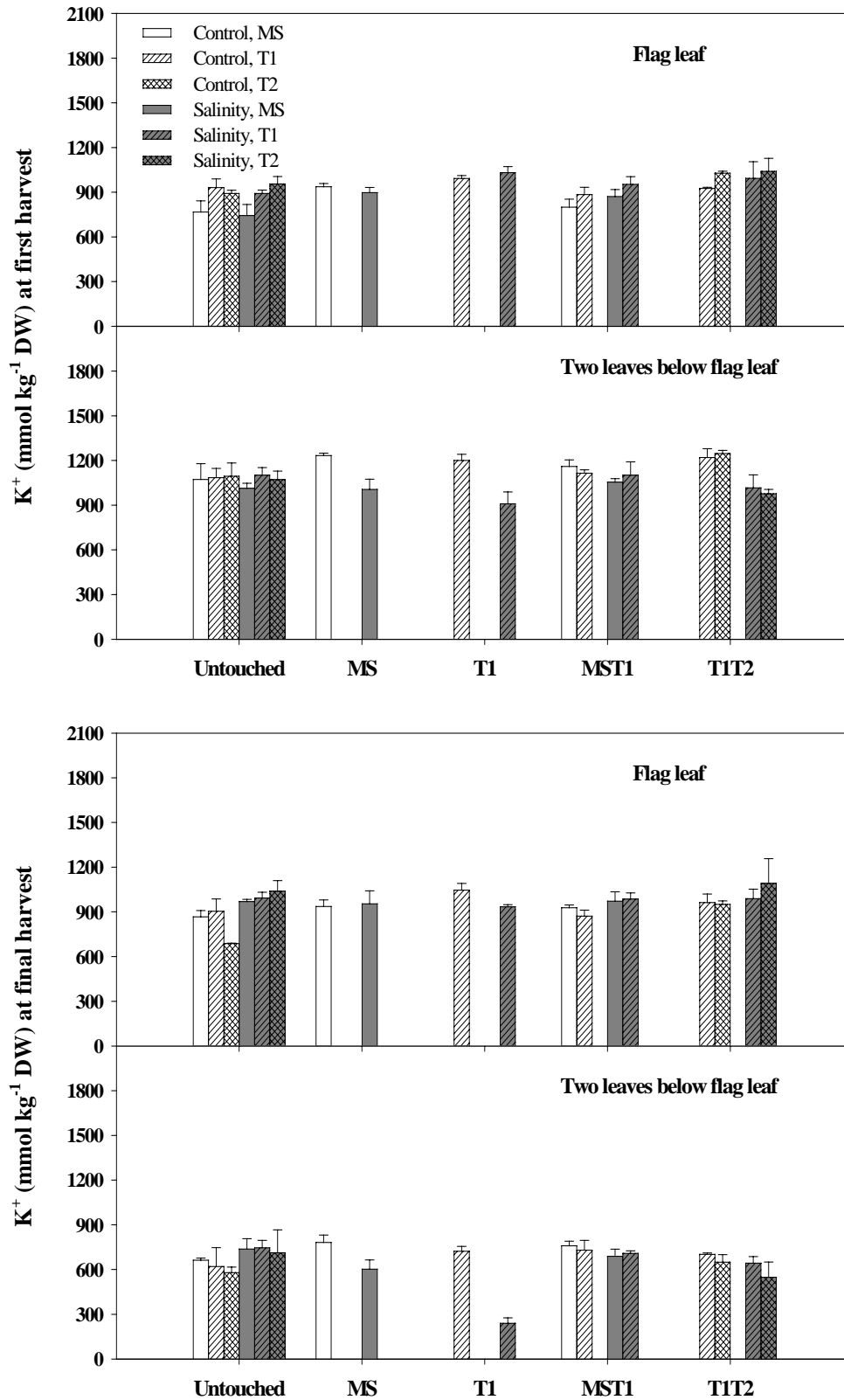


Figure 4.4 Potassium concentrations in the flag leaf and two leaves below the flag leaf in the mainstem (MS), T1 and T2 in the different detillering treatments of Sakha 61 with or without salinity at two harvest times.

Influence of tiller number on ion accumulation in tillers

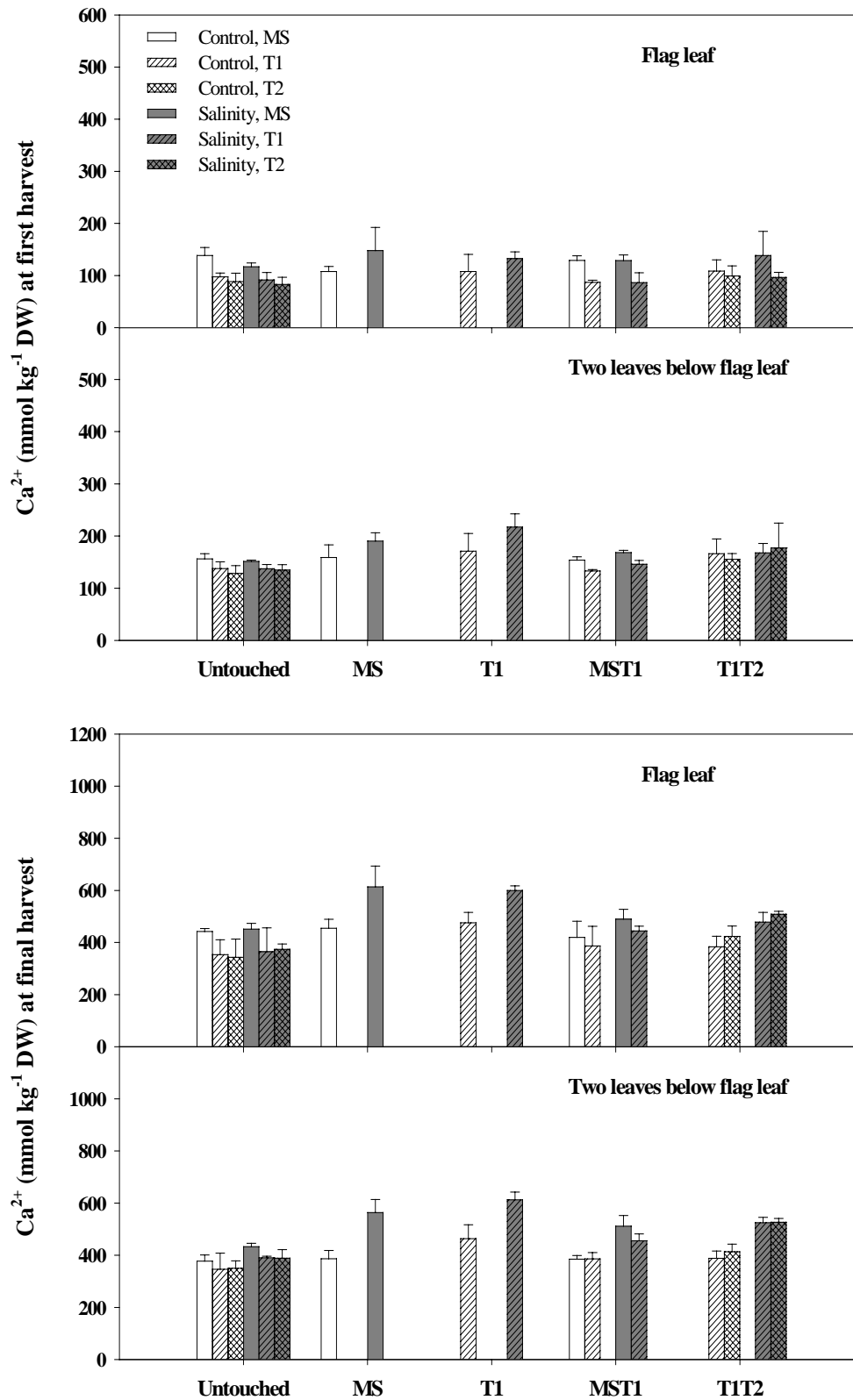


Figure 4.5 Calcium concentrations in the flag leaf and two leaves below the flag leaf in the mainstem (MS), T1 and T2 in the different detillering treatments of Kharchia with or without salinity at two harvest times.

Influence of tiller number on ion accumulation in tillers

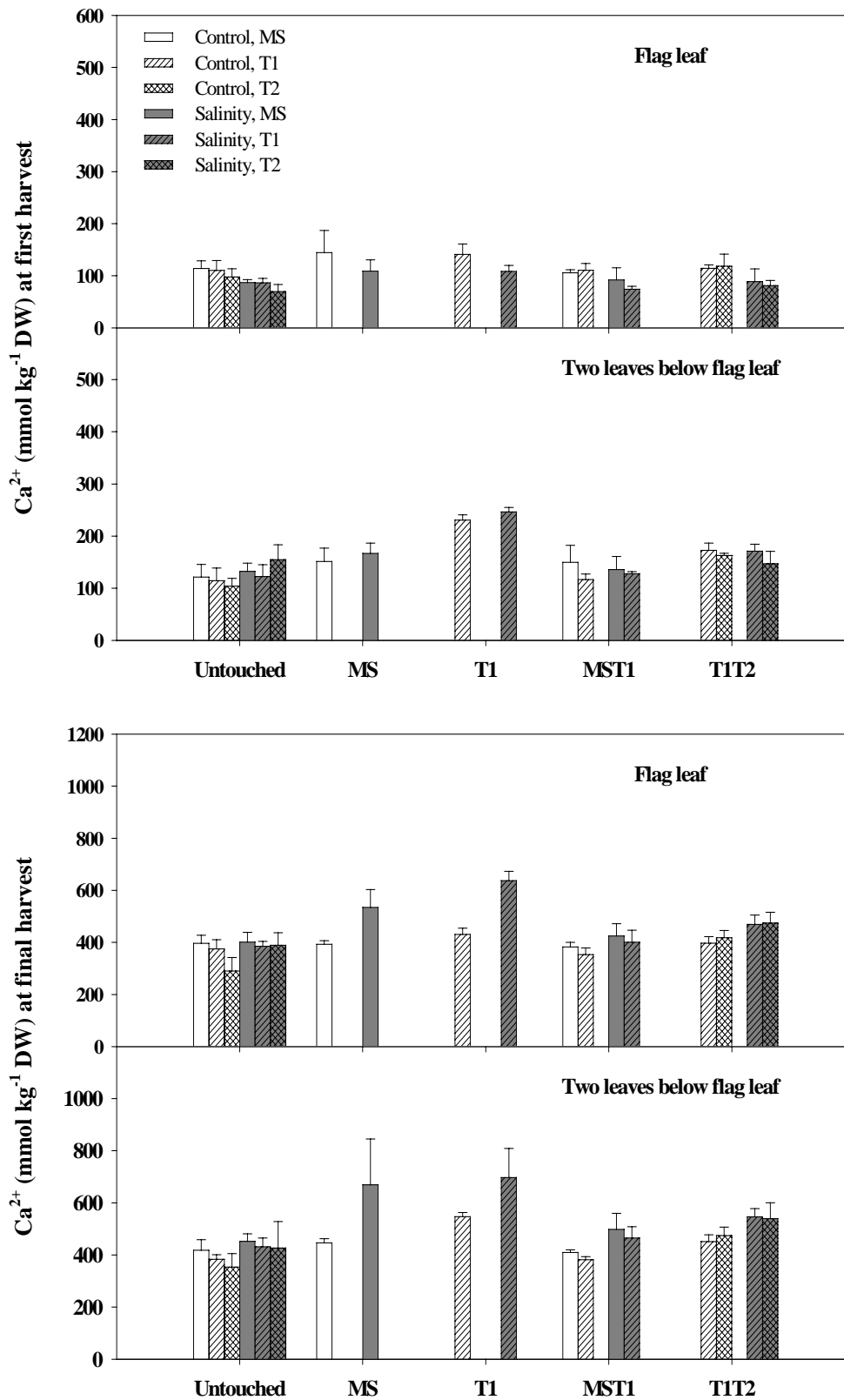


Figure 4.6 Calcium concentrations in the flag leaf and two leaves below the flag leaf in the mainstem (MS), T1 and T2 in the different detillering treatments of Sakha 61 with or without salinity at two harvest times.

Influence of tiller number on ion accumulation in tillers

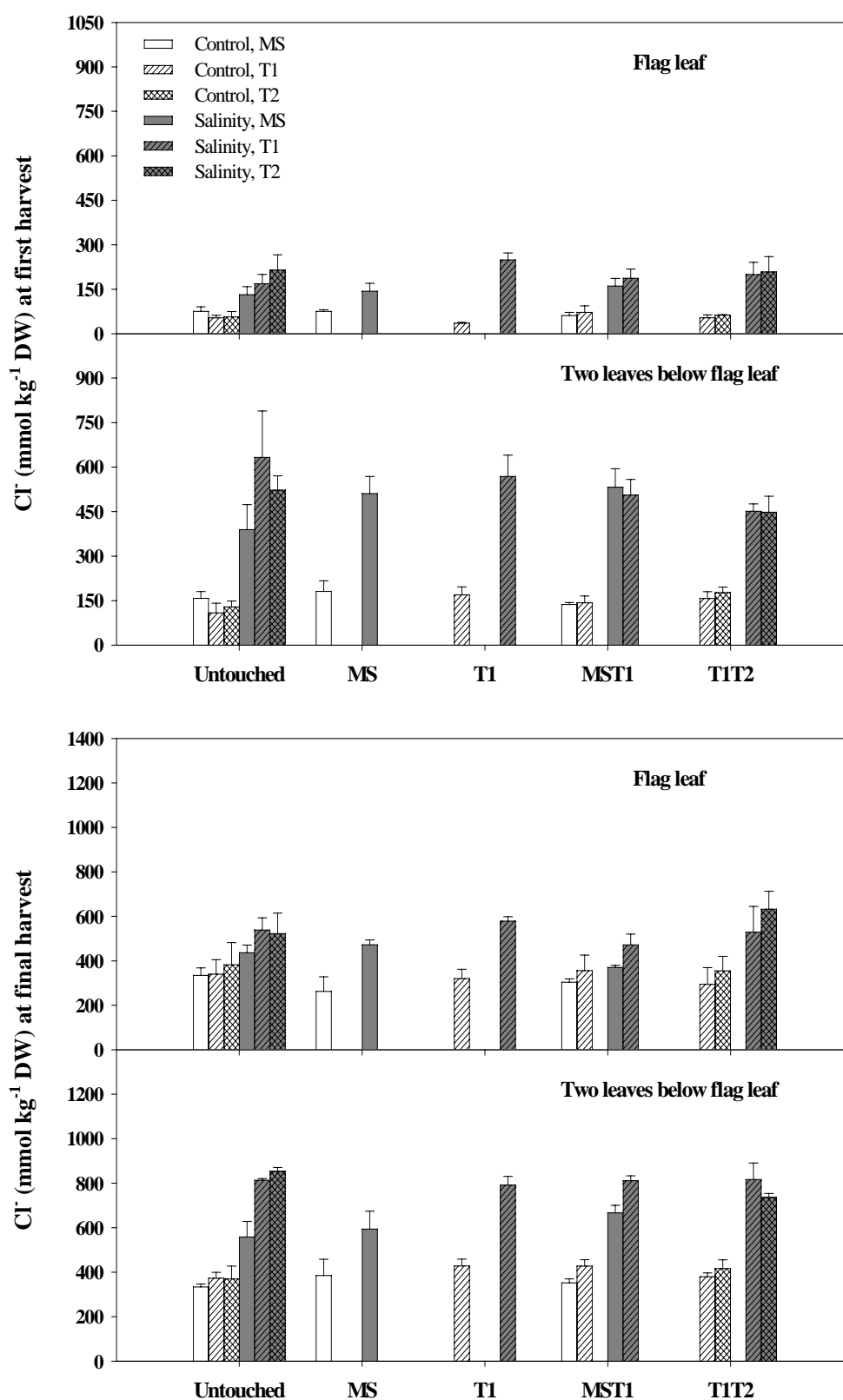


Figure 4.7 Chloride concentrations in the flag leaf and two leaves below the flag leaf in the mainstem (MS), T1 and T2 in the different detillering treatments of Kharchia with or without salinity at two harvest times.

Influence of tiller number on ion accumulation in tillers

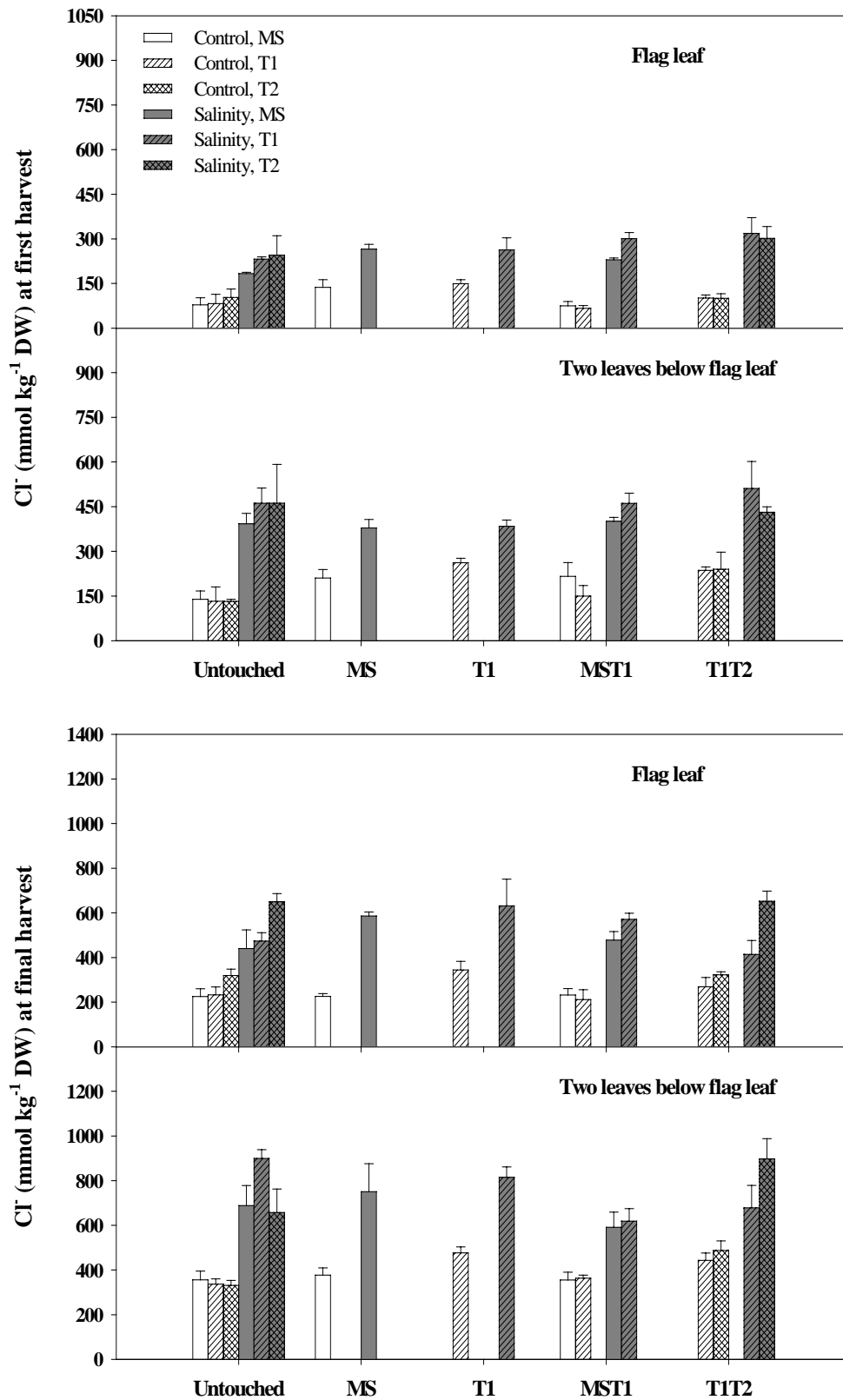


Figure 4.8 Chloride concentrations in the flag leaf and two leaves below the flag leaf in the mainstem (MS), T1 and T2 in the different detillering treatments of Sakha 61 with or without salinity at two harvest times.

Influence of tiller number on ion accumulation in tillers

Under non-saline conditions, the NO_3^- concentration at the final harvest compared to the first harvest in the mainstem leaves was greatly decreased regardless of cultivars and treatments. However, under saline conditions, the NO_3^- concentration was increased for the salt-tolerant cultivar Kharchia but decreased for the salt-sensitive cultivar Sakha 61, although salinity significantly reduced the NO_3^- concentration for both cultivars. Compared to the untouched treatment, therefore, the reduction in the NO_3^- concentration due to salinity in the mainstem leaves of Kharchia was decreased in the MS and MST1 treatments at the final harvest, while for Sakha 61, this reduction was increased (Figs 4.9 and 4.10).

4.3.2 Influence of tiller number per plant on ion accumulation in the subtiller leaves

In the leaves of sub tillers T1 and T2, the Na^+ concentrations under saline conditions for the salt-tolerant and salt-sensitive cultivars were increased in the different treatments, with higher Na^+ level in the salt-sensitive cultivar being observed (Figs 4.1 and 4.2). Similar to the leaves on the mainstem, the Na^+ accumulation in the leaves on the sub tillers of both cultivars was increased by following two directions: leaf age and plant age, i.e. the Na^+ concentration was higher in the two leaves below the flag leaf than in the flag leaf, and at maturity than at the vegetative stage. However, sub tillers accumulated more Na^+ than the mainstem in the treatments having mainstem for Kharchia and in all treatments for Sakha 61 under saline conditions. Compared with the untouched treatment, the Na^+ concentration of leaves on the subtiller T1 for Kharchia was increased by salinity more in the T1 treatment at either harvest, while for Sakha 61, it was increased more in the T1, MST1 or T1T2 treatments. In the leaves of subtiller T2, the Na^+ concentration for Kharchia was increased by salinity more in the T1T2 treatment compared to the untouched treatment at the first harvest, but less at the final harvest, while for Sakha 61, it was increased more in the T1T2 treatment for both harvests. Similar to the mainstem, the T1 treatment showed that the subtiller T1 of Kharchia compared to Sakha 61 had greatly lower increase in the Na^+ accumulation under saline conditions (Figs 4.1 and 4.2).

Influence of tiller number on ion accumulation in tillers

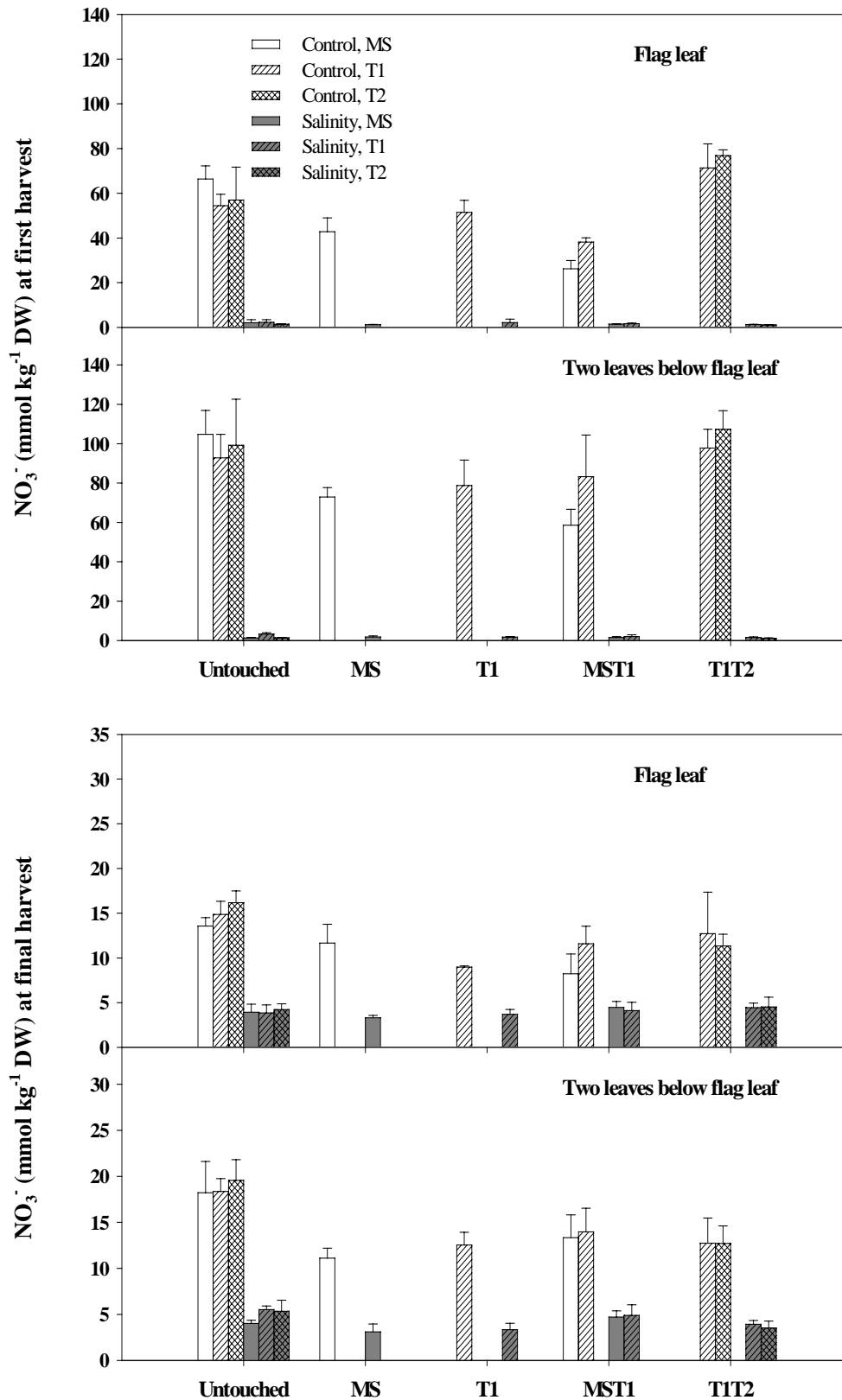


Figure 4.9 Nitrate concentrations in the flag leaf and two leaves below the flag leaf in the mainstem (MS), T1 and T2 in the different detillering treatments of Kharchia with or without salinity at two harvest times.

Influence of tiller number on ion accumulation in tillers

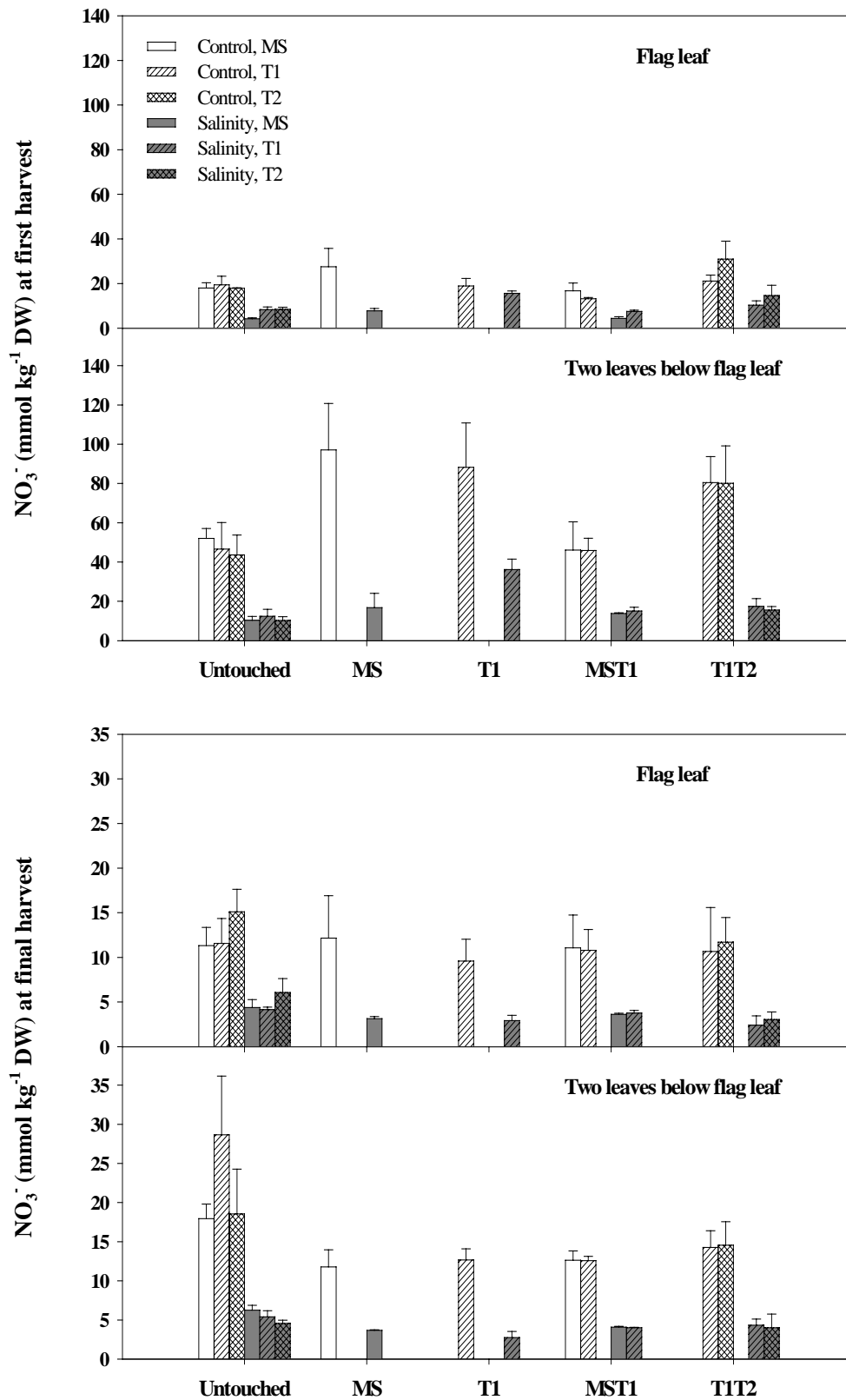


Figure 4.10 Nitrate concentrations in the flag leaf and two leaves below the flag leaf in the mainstem (MS), T1 and T2 in the different detillering treatments of Sakha 61 with or without salinity at two harvest times.

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Regardless of the salt stress and treatment, the K^+ concentration at the final harvest compared to the first harvest was lower in the two leaves below the flag leaf on the subtillers T1 and T2 of both cultivars (Figs 4.3 and 4.4). Different to Kharchia, the detillered treatments of Sakha 61 compared to the untouched treatment caused a comparatively greater reduction in the K^+ concentration of two leaves below the flag leaf on the subtiller T1 under saline conditions. Similar to mainstem, two leaves below the flag leaf of subtillers in the detillered treatments of Sakha 61, particularly in the T1 treatment, showed larger reduction in the K^+ concentration than the flag leaf under saline conditions, but not in Kharchia. At the final harvest, the Na^+/K^+ ratio in the subtillers under saline conditions became higher regardless of the cultivar and treatment. However, the detillered treatments showed higher Na^+/K^+ ratio than the untouched treatment in the two leaves below the flag leaf of both cultivars, especially for the salt-sensitive cultivar Sakha 61.

The accumulation of Ca^{2+} in the subtiller leaves of both cultivars was greatly increased with the growth stage in the different treatments (Figs 4.5 and 4.6). However, the reduction in tiller number combining with salinity enlarged this increase of Ca^{2+} accumulation that was observed at the final harvest. In contrast to the Na^+/K^+ ratio, the Na^+/Ca^{2+} ratio in the subtillers under saline conditions became smaller at the final harvest regardless of the cultivar and treatment. Compared with the untouched treatment, however, the detillered treatments further decreased the Na^+/Ca^{2+} ratio in the leaves of subtiller T1 for Kharchia under saline conditions, but increased this Na^+/Ca^{2+} ratio for Sakha 61. For the leaves of subtiller T2, the detillered treatments decreased the Na^+/Ca^{2+} ratio in both cultivars.

Regardless of the salt stress, the Cl^- concentration in the subtiller leaves at the final harvest was also greatly increased in all treatments of both cultivars, in which the older leaves had higher Cl^- accumulation (Figs 4.7 and 4.8). Although salinity greatly increased the Cl^- accumulation in the subtiller leaves regardless of cultivars and treatments, this increase became slower at the final harvest expect for the T1 treatment of Sakha 61. Similar to mainstem, the Cl^- concentration of leaves in the subtiller T1 or T2 of Kharchia did not show significant difference between the detillered and untouched treatments under saline conditions, while for Sakha 61, the significant difference was only observed in the two leaves below the flag leaf on the subtiller T1 in the MST1 and T1T2 treatments at the final harvest. In the same

treatment of tiller number, the subtiller leaves under saline conditions tended to accumulate more Cl^- than the mainstem leaves in both cultivars, especially for Kharchia. For example, in the one-tiller treatments (MS and T1) of Kharchia, the subtiller T1 accumulated higher Cl^- in the flag leaf than the mainstem.

Salinity greatly reduced the NO_3^- concentration of the subtiller leaves in the different treatments, with more reduction in Kharchia (Figs 4.9 and 4.10). From first harvest to final harvest, however, the NO_3^- concentration in the salt-stressed leaves of sub tillers was increased in Kharchia, but greatly decreased in Sakha 61 with the largest reduction in the T1 treatment. Therefore, at the final harvest, the reduction in the NO_3^- concentration in Kharchia was decreased with more in the detillered treatments, but this tendency was not observed in Sakha 61.

4.4 Discussion

It is generally accepted that salinity decreases the shoot weight of plant through all growth stages in the untouched plant of wheat, resulting in the reduction in yield and yield components. Similar to the untouched plant, salinity also largely decreases yield and yield components by inhibiting the plant growth in fewer tillers per plant in both cultivars (Chapter 3). In the different plant organs, the leaves are of importance due to supplying most of energy through photosynthesis to support the plant growth. Salam *et al.* (1999) reported that the inorganic ions in the leaves have the significant correlations with yield parameters under saline conditions, with the negative correlations between the concentrations of Na^+ and Cl^- and grain yield being observed. The increased Na^+ and Cl^- levels in the leaves by salinity limit the plant growth primarily by the nutrient disturbance, specific ion toxicities and osmotic stress (Hu and Schmidhalter 1998; Grattan and Grieve 1999). However, compared with the sub tillers, the mainstem always has the less reduction in the leaf growth and grain yield under saline conditions (Hu *et al.* 1997; Ruan *et al.* 2005), possibly due to its superior development to sub tillers (Fletcher and Dale 1977). The mainstem has been observed to compete with the sub tillers for a limited supply of nutrients, carbon and water resource in its development (Kirby and Jones 1977; Mohamed and Marshall 1979; Longnecker *et al.* 1993). In this study, the leaves of different order on the mainstem under saline conditions was found to accumulate lower Na^+ than those on sub tillers in the untouched plant with the significant difference being observed at

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maturity, suggesting that lower accumulation of toxic ions in the mainstem could be one of the reasons for less reduction in growth. Hu *et al.* (2000) reported that the length and width of leaves under salinity were reduced by about 20-30% due to the local high accumulation of Na^+ and Cl^- . The decrease in the length and width of leaves is related to the reduced size of meta- and protoxylem and number of small veins that result in the decreases in water deposition and nutrient retranslocation (Hu *et al.* 2005). El-Hendawy *et al.* (2005) suggested that the photosynthetic rate and leaf expansion that are associated with the water content and nutrients are two important growth-limiting factors in the salt-tolerant and salt-sensitive cultivars. Similarly, lower Na^+ concentration in the mainstem than in the subtiller in the two-tiller plant with mainstem further demonstrates the specific trait of relatively less buildup for toxic ions in the mainstem under saline conditions. We assumed that the mainstem might also exclude salt into the subtillers (T1 and T2) to increase its own inventory for mineral nutrients to improve plant growth under saline conditions. However, the further evidences are needed to prove this.

Compared with the corresponding one in the untouched plant, the mainstem or subtiller in the one-tiller plant accumulated higher Na^+ content in the leaves under saline conditions, especially in the salt-sensitive cultivar. The artificially adjusted tiller number in wheat cultivars appears to modify the normal regulatory influence for leaf expansion (leaf area; Chapter 3) that is enlarged by the activity of hormone (Mohamed and Marshall 1979), perhaps activating the uptake and accumulation of Na^+ in shoots. Due to the trait of ion toxicity (El-Hendawy *et al.* 2005), the tillers of the salt-sensitive cultivar could be more affected by decreasing tiller number under salinity. As much, the Na^+ increase due to salinity in the mainstem or subtiller T1 was greater in the one-tiller plant than in the two-tiller plant having mainstem, especially in the salt-sensitive cultivar. This tendency of the Na^+ accumulation related to tiller number may reflect that there is the collective defense for resisting salt toxicity in tillers, which may be achieved by: a) shrinking cell size to decrease leaf area, and b) retranslocating the nutritional ions among tillers to decrease the binding site of toxic ions in cell. On the other hand, if there is no competition of mainstem for resources (in T1 and T1T2 treatments), the subtillers of the salt-tolerant cultivar showed the significantly lower Na^+ accumulation than those of the salt-sensitive cultivar, suggesting their better ability to exclude toxic ions. The results indicate that, at whole-

plant level, improving salt tolerance in the cultivars might be mostly built on increasing salt tolerance of subtillers in the plants.

Under saline conditions, the increase in the Na^+ concentration of leaves is usually accompanied by a decline in the concentrations of K^+ and Ca^{2+} (Hu and Schmidhalter 1997). However, earlier studies under soil conditions focused on the effects of salinity on nutritional ions in all leaves. Our study showed that the K^+ and Ca^{2+} concentrations in the top leaves (flag leaf and two leaves below the flag leaf) of mainstem or subtillers could be increased under saline conditions compared to non-saline conditions in the untouched plant, especially at maturity. Salinity only decreased greatly the K^+ and Ca^{2+} concentrations in the bottom leaves of both cultivars (data not shown). Salam *et al.* (1999) also observed that salinity increased the K^+ level of young leaves grown under hydroponic condition compared to the non-saline control. In results, we identified that there were lower Na^+ accumulation in the flag leaf (relatively younger) under saline conditions than in the two leaves below the flag leaf (relatively older) for both cultivars, confirming that wheat plants may have the ability to restrict the Na^+ transport into younger leaves and concentrate it into older leaves to affect the accumulations of K^+ and Ca^{2+} (Rashid *et al.* 1999). Another possible reason is that the moderate salt level was used in this experiment. An increase in the K^+ concentration of leaves has been observed at 30-50mM NaCl level compared to the non-saline control depending on wheat cultivars (Hu and Schmidhalter 1997; El-Hendawy *et al.* 2005).

Through the ion competition, the high Na^+ concentration in saline environment strongly influences the uptake of cation such as K^+ and Ca^{2+} (Marschner 1995). El-Hendawy *et al.* (2005) reported that there is the significant genotypic variation in the accumulations of K^+ and Ca^{2+} , with higher K^+ and Ca^{2+} contents in the salt-tolerant cultivar than the salt-sensitive cultivar. This is suggested as a secondary result of genetic variation in the uptake of toxic ions under salinity (Munns and James 2003). Here, we found that the ion selectivity (e.g. K^+) under saline conditions depends on not only genotypes but also tiller number and leaf age order. Compared with the untouched plant, only one tiller per plant at plant maturity increased the K^+ accumulation of the mainstem or subtiller under saline conditions in the salt-tolerant cultivar, but decreased this K^+ accumulation in the salt-sensitive cultivar. As compared with the salt-sensitive cultivar, the K^+ concentration in the flag leaf under

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saline conditions for the salt-tolerant cultivar was significantly lower in the plants with different tiller number at maturity, while for the two leaves below the flag leaf, it was significantly higher in the one-tiller plant and similar in the two-tiller and untouched plants. The report by El-Hendawy *et al.* (2005) was only observed in the bottom leaves of plants with different tiller number, i.e. the salt-tolerant cultivar showed a higher K^+ accumulation than the salt-sensitive cultivar under salinity (data not shown). Therefore, the Na^+/K^+ ratio in the salt-sensitive cultivar is lower in the flag leaf than that in the salt-tolerant cultivar under saline conditions, and then becomes higher with the decrease in leaf age order, with the highest ratio being in the oldest leaf.

Potassium and Ca^{2+} play an important role in the essential physiological processes. The function of K^+ at the cellular level is involved in the maintenance of turgor and tissue rigidity, osmoregulation, protein synthesis and homeostasis (Chow *et al.* 1990), while for Ca^{2+} , it acts as secondary messenger on the signal transduction within the cell (Knight 2000), and protects the integrity of the cell membrane (Rengel 1992). Maathuis and Amtmann (1999) emphasized that the plant capacity to maintain a high cytosolic K^+/Na^+ (i.e. low Na^+/K^+) ratio play one of prominent roles for salt resistance. A large excess of Na^+ over K^+ under saline conditions causes the K^+ deficiency by the Na^+ competition at transport sites with K^+ entry into the symplast and inhibits the metabolic processes by the Na^+ substitution for K^+ binding sites in the cytoplasm (Epstein, 1966; Maathuis and Amtmann 1999). Cramer *et al.* (1985) reported that the displacement of Ca^{2+} by Na^+ impairs the membrane integrity in the cell. High Ca^{2+} during salt stress may prevent the net K^+ efflux from the cell (Shabala 2000), and hence influence the K^+/Na^+ ratio (Cramer 2002). Ca^{2+} is also considered to inhibit the major pathway for Na^+ influx, non-selective cation channels (Demidchik and Tester 2002). Up to now, there has been no report about the influence of tiller number on Na^+/K^+ and Na^+/Ca^{2+} ratios under saline conditions. Compared with the untouched plant, fewer tillers per plant depending on the cultivars caused higher Na^+/K^+ and/or Na^+/Ca^{2+} ratios in the mainstem or subtiller under saline conditions, particularly for one tiller per plant, suggesting that detillering increases the competition of Na^+ with K^+ and/or Ca^{2+} in the leaves, which further indicates the function of the collective defense in tillers to resist the salt-specific effects. However, at plant maturity, the Na^+/K^+ ratio in the mainstem or subtiller was higher in the plants

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without subtillers or mainstem, respectively, than with them in both cultivars, while for the $\text{Na}^+/\text{Ca}^{2+}$ ratio, this tendency was observed in the salt-sensitive cultivar. Lauer and Simmons (1985; 1988) reported that the mainstem-subtiller relationship could be mutual either sink or source of nutrients within the plant, i.e. the mainstem supplies the mineral nutrients to the subtillers, and the subtillers also retranslocate nutrients to the mainstem. Therefore, the mutual supply of mineral nutrient between mainstem and subtiller could alleviate the salt-specific effects within the plant. However, due to the different mechanisms of salt tolerance, the competition of Na^+ with K^+ or Ca^{2+} in either mainstem or subtillers is relatively weak in the salt-tolerant cultivar, but relatively strong in the salt-sensitive cultivar.

It has been reported that Cl^- is readily accumulated more in the leaves than Na^+ under saline conditions (Alam 1994; Hu and Schmidhalter 1997), hence it is a relatively sensitive indicator to detect the effect of salinity on plant growth (Hu and Schmidhalter 1997). However, compared with the Na^+ , plants have high tolerance to Cl^- . Hu and Schmidhalter (1998) has reported that the Cl^- level under saline conditions is about four times higher than Na^+ level in the growing young leaves, but this is not high enough to cause Cl^- toxicity. As much, in the different leaves, the Cl^- accumulation in the non-growing young leaves was reported to be about 10 times higher than that in the growing young leaves under similar conditions, which hardly affected the mainstem growth in wheat (Hu and Schmidhalter 1997). It is argued that the young leaves are able to regulate the Cl^- concentration by a combination of rapid growth and low transpiration, coupled with the continued uptake and minimal recycling of Cl^- in the old leaves to avoid an excessive Cl^- accumulation (White and Broadley 2001). The lower Cl^- level in the flag leaf than in the two leaves below the flag leaf in this study strongly supports the role of younger leaf in managing toxic ions in wheat genotypes, confirming that restricting the accumulation of Cl^- in the younger leaves is important for salt tolerance of wheat (Boursier *et al.* 1987). Interestingly, decreasing tiller number in both cultivars tended to not cause greater differences in the Cl^- level between the mainstems or subtillers of the detilled and untouched plants under saline conditions, indicating that the inhibition of tillering has little effects on the Cl^- accumulation of tiller, especially for the salt-tolerant cultivar.

Bernal *et al.* (1974) pointed out that the increased Cl^- level in the salt-stressed plants may suppress the uptake of other anions (e.g. NO_3^-), which is supported by that

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both cultivars had the lower NO_3^- level in the salt-stressed leaves regardless of tiller number per plant in this study. The lower concentration of NO_3^- in the leaves could be responsible for the inhibition of plant growth under saline conditions. The decreased NO_3^- uptake of plant was reported to associate with the inhibition of Cl^- on the NO_3^- reductase activity (Wilkinson and Crawford 1993). Due to genotypic difference, the NO_3^- concentration of leaves under salinity was reduced more in the salt-tolerant cultivar than in the salt-sensitive cultivar at the vegetative stage, suggesting that the nitrogen deficiency could be one of more important factors to reduce the growth of the salt-tolerant cultivar. However, during the reproductive growth stage, the NO_3^- level in the salt-stressed leaves was increased in the salt-tolerant cultivar, resulting in the decline of reduction in the NO_3^- level in the mainstem or subtillers in the detilled plants but this effect on the growth may be too small due to the great reduction in the NO_3^- level.

In conclusion, ion content in the leaves under saline conditions is depending on the tiller number, leaf age order and genotype. Compared with the salt-sensitive cultivar, the Na^+/K^+ ratio for the salt-tolerant cultivar at plant maturity varies from higher to lower with the leaf age order declined from top to bottom under saline conditions, indicating that low Na^+/K^+ ratio in older leaves is of importance for salt tolerance in the cultivars. Due to the genetic difference, one tiller per plant showed that the mainstem or subtiller in the salt-tolerant cultivar has the superior traits of salt exclusion and higher nutrient selectivity to alleviate the salt-specific effects on tillers, while for the salt-sensitive cultivar, high salt content in the mainstem or subtiller expedites the decline in the nutrients to cause larger nutritional imbalance and/or deficiency for the normal growth of tillers. Especially for the salt-sensitive cultivar, the accumulation of Na^+ and the Na^+/K^+ and/or $\text{Na}^+/\text{Ca}^{2+}$ ratios in the mainstem or subtiller under saline conditions were increased with the decrease in tiller number in both cultivars, indicating that there may exist the collective defense for salt accumulation in tillers to alleviate the salt toxicity.

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5

General discussion

The development of tillers is of considerable scientific and practical interest in understanding crop productivity and plant adaptation to environment. As yet, knowledge for the response of tillering to salinity has been limited. In particular, the growth of different tillers and the distribution of nutrients among tillers under saline conditions remain to be elucidated in contrasting cultivars for salt tolerance. Previous studies focused on the effects of salinity on the whole plant (all tillers) in the cultivars or the mainstem tiller of a single cultivar. The studies on the response of the tillers of different order to salinity in contrasting cultivars in this thesis help to understand mechanisms of tillering reduction, and provided information on improving crop salt tolerance by tillering under saline conditions.

5.1 Effect of salinity on growth of mainstem tiller and subtillers

5.1.1 Genotypes

The novelty of the present studies resides in identifying how salinity differently affects the mainstem tiller and subtillers in contrasting salt tolerant cultivars. The results in Chapters 2 and 3 showed that the negative effects of salinity on dry weights, grain yields and grain numbers of mainstem tillers and subtillers in the salt-tolerant cultivars were much less than those in the salt-sensitive cultivars. Under high salinity, the less reduction in agronomic parameters (e.g. dry weight and grain yield) in the mainstem tiller than in the subtiller has been observed in wheat in the previous studies (Mass *et al.* 1996; Hu *et al.* 1997). Our results further showed that, under moderate salinity, the reduction in dry weight and grain yield of mainstem tiller in the salt-sensitive cultivars became similar to that of T1 during the reproductive growth, but were lower than that of T2. The reduction in the growth of subtillers was observed to relate with the time of their emergence, i.e. the earlier emergence of the subtillers had the less reduction. However, this may not be observed in the salt-tolerant cultivars. In

any cases, the less growth in the subtillers than in the mainstem in contrasting cultivars as well as the much lower reduction in the growth of subtillers in the salt-tolerant cultivar than in the salt-sensitive cultivar under saline conditions suggest the importance of subtillers to improve the salt tolerance of cultivars due to their number.

5.1.2 Tiller number vs. salt tolerance

Regardless of saline levels, only one tiller per plant maximally increased leaf area, dry weight and grain yield in the mainstem tiller and/or subtillers in wheat cultivars, while these increases in two tillers per plant depended on genotypes and tiller orders (Chapter 3). Donald (1968) proposed that the most productive wheat cultivar should possess a large spike and a short, single culm (uniculm), because the unicum could provide a unidirectional drive toward spike and grain formation. The increased growth in the mainstem tiller or subtillers by reducing tiller number could be due to 1) the enhancement of the photoassimilate supply (Alaoui *et al.* 1988); and 2) the decrease of competition for nutrients among tillers to ameliorate nutrient availability under salinity (Lauer and Simmons 1985; 1988; Hu *et al.* 1997). Although the increased growth per tiller for the plants with fewer tillers could not compensate the loss of grain yield per plant compared with the plants with more tillers, adjusting plant density to an optimum number could compensate the yield loss per unit area. An appropriate plant density could produce the maximum productivity in the individual shoots to reach the maximum grain yield in field (Darwinkel 1978). Therefore, developing one-tiller cultivar (uniculm) with high salt tolerance could be one of approaches to improve the agricultural output in salinized area.

An important advance of this study is that the effects of tiller number on salt tolerance were effectively distinguished in contrasting salt tolerant cultivars. In general, the salt tolerance of a cultivar is depending on the salt tolerance of the mainstem tiller and subtillers. Either the competition of resource among tillers or the accumulation of salt ions in the plant tissue which is regulated by tiller number and genotype plays an important role in controlling salt tolerances of mainstem tiller and subtillers under saline conditions. In this study, the greater increase in leaf area, dry weight and grain yield on the mainstem tiller or T1 in the one-tiller plant showed that the mainstem tiller and subtillers could have the similar growth potential in contrasting cultivars under saline conditions. For the salt-tolerant cultivar, the results

showed that the salt tolerance of the mainstem tiller or subtiller was improved in the treatments with mainstem tiller and subtiller together, while for the salt-sensitive cultivar, this salt tolerance was improved in the treatments without sub tillers or mainstem tiller (Chapter 3). We speculate that the exclusion of toxic ions in the genotypes affects the accumulation of nutrients in tiller tissue and their distribution among tillers. Lauer and Simmons (1985; 1988) reported that the relationships between mainstem tiller and sub tillers are the reciprocal supplier and competitor for resource. Under saline conditions, the exclusion of toxic ions in the salt-tolerant cultivar could maintain relatively sufficient nutrients in tissue to either diminish competition or increase the mutual supply between mainstem tiller and sub tillers (Lauer and Simmons 1985; 1988). In contrast, higher concentration of toxic ions in the salt-sensitive cultivar increases the competition for nutrients among sub tillers and/or the monopoly of mainstem tiller to nutrients (Gu and Marshall 1988; Hu *et al.* 1997). Therefore, salt tolerance could be improved by breeding isogenic lines in the salt-tolerant cultivars with more sub tillers (at least primary tillers) and in the salt-sensitive cultivars with fewer sub tillers.

5.2 Effects of salinity on ion distribution among tillers

5.2.1 Genotypes

It is generally accepted that plant growth under saline conditions is regulated by the uptake, accumulation and distribution of salts and nutrients. The better exclusion of toxic ions from shoots has been previously observed at the whole-plant level in plants of the salt-tolerant cultivars (Poustini and Siosemardeh 2004; El-Hendawy *et al.* 2005). Our studies on the individual tiller (Chapter 2 and 4) showed that this is due to the better ion selectivity of the mainstem tiller and sub tillers of salt-tolerant cultivars. In contrast to salt-sensitive cultivars, the mainstem tillers and sub tillers in the salt-tolerant cultivars show a lower increase in the Na^+ and Cl^- accumulation and maintain lower Na^+/K^+ and $\text{Na}^+/\text{Ca}^{2+}$ ratios through the growth stages. The better ability of the salt-tolerant cultivars to exclude harmful ions in the mainstem tiller and sub tillers may be established on the mechanisms of the uptake and transport of ions from roots to shoots (Shone *et al.* 1969; McCully *et al.* 1987), and of the recirculation of ions from shoots to roots (Munns *et al.* 1988; Lohaus *et al.* 2000). However, the difference in the ion selectivity of contrasting cultivars varies in the different age of leaves (Chapter

4). In this study, we found that the salt-tolerant cultivar has an important trait, i.e. the older leaves have a lower Na^+/K^+ ratio under saline conditions. It may be used as an important trait to select the salt tolerance among wheat cultivars.

Furthermore, the salt-tolerant and salt-sensitive cultivars have a similar trait in the distribution of salt among tillers under saline conditions, i.e. the subtillers have higher accumulations of Na^+ and Cl^- than the mainstem tiller, resulting in their higher nutritional deficiency and/or imbalance, e.g. K^+ , Ca^{2+} , Mg^{2+} or NO_3^- , especially more severe in the salt-sensitive cultivar (Chapter 2 and 4). This could be due to: a) the retranslocation of Na^+ and Cl^- between mainstem tiller and subtillers via the phloem (Lohaus *et al.* 2000; Watson *et al.* 2001; Davenport *et al.* 2005); and b) the stronger sink strength in the subtillers than in the mainstem tiller (Jeschke *et al.* 1995). Therefore, the growth of subtillers is greatly inhibited under saline conditions.

5.2.2 Tiller number vs. ion distribution among tillers

The effects of tiller number on the ion accumulation are so far unclear in wheat. The studies here found that the effects of tillering on ion accumulation under saline condition are related to the genetic traits of cultivars. The results in Chapter 4 showed that the accumulation of Na^+ in the salt-stressed leaves in the mainstem tiller or subtiller was greatly increased by only one tiller per plant or with the increase of plant age, especially in the salt-sensitive cultivar. This tendency of the Na^+ increase under salinity became weak in two tillers per plant remaining mainstem tiller, especially in the salt-tolerant cultivar. Fewer tillers per plant could also increase the K^+ or Ca^{2+} concentrations in the salt-stressed leaves of the mainstem tiller or subtiller in either contrasting cultivar. However, the results from the detilled plants showed that the competition of Na^+ with K^+ or Ca^{2+} in either mainstem or subtillers was relatively weak in the salt-tolerant cultivar but it was stronger in the salt-sensitive cultivar. Therefore, the mainstem tiller and subtillers of the salt-tolerant cultivar could maintain lower Na^+/K^+ and $\text{Na}^+/\text{Ca}^{2+}$ ratios in the leaves than those of the salt-sensitive cultivar. Importantly, the leaves (except for the flag leaf) of the mainstem tiller or subtiller in the salt-tolerant cultivar showed that the less tiller number per plant had the higher Na^+/K^+ ratio under saline conditions at plant maturity, while for the salt-sensitive cultivar, it was observed that the less tiller number per plant had the higher Na^+/K^+ and $\text{Na}^+/\text{Ca}^{2+}$ ratios. The results indicate that there may exist the

collective defense for resisting the salt toxicity in tillers by ion selectivity. We speculate that this defense in plants may be achieved by: a) shrinking cell size (leaf area) to decrease the salt accumulation; and b) retranslocating the nutritional ions among tillers to reduce salt-specific effects. Interestingly, there was no difference in the Cl^- concentration between the mainstems or subtillers of the detilled and untouched plants under salt stress in both cultivars. In combination with tiller growth at maturity (Chapter 3), the better salt tolerance of tillers in the salt-tolerant cultivar suggests that more tillers are beneficial to alleviate nutritional deficiency and/or imbalance to increase the plant growth. In the salt-sensitive cultivar, only one tiller per plant produces higher dry weight and grain yield per tiller, even though there are higher Na^+ accumulation and higher Na^+/K^+ and $\text{Na}^+/\text{Ca}^{2+}$ ratios as compared with the untouched plant, indicating that fewer tillers per plant could increase tiller tissue tolerance to salinity. Greenway and Munns (1980) reported that plant salt tolerance could be improved by sequestering toxic ions into vacuoles away from cytoplasm and chloroplasts in cells. Furthermore, during the plant growth, the inhibition of tillering hardly affected the accumulation of NO_3^- in the salt-stressed leaves of mainstem or subtillers in the salt-tolerant cultivar but greatly affected it in the salt-sensitive cultivar.

5.3 Conclusions

Under saline conditions, the better salt tolerance in the salt-tolerant cultivars results from the better ion selectivity in both mainstem tiller and subtillers, i.e. lower Na^+ and Cl^- accumulations and lower Na^+/K^+ and $\text{Na}^+/\text{Ca}^{2+}$ ratios. Regardless of cultivars, the subtillers accumulate higher Na^+ and Cl^- than the mainstem tiller, which may cause larger injury of salinity in the growth of subtillers, especially in the salt-sensitive cultivar. In contrasting wheat cultivars, the less tiller number per plant results in the higher Na^+ accumulation and higher ratios of Na^+/K^+ or $\text{Na}^+/\text{Ca}^{2+}$ under saline condition, indicating that tillers may have the function of the collective defense to resist the salt toxicity by ion selectivity. Under saline conditions, the less growth of subtillers in contrasting cultivars and the greater difference in the growth of subtillers between salt-tolerant and salt-sensitive cultivars suggest that tillering could be important to regulate salt tolerance in wheat. In the salt-tolerant cultivar, more tillers may improve plant salt tolerance by increasing the supply of nutrients in the

mainstem-subtiller to alleviate salt toxicity, while in the salt-sensitive cultivar, fewer tillers may improve plant salt tolerance by increasing tissue tolerance to toxic ions.

5.4 References

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