Germination responses of endemic land-races of Saudi-Arabian cereal species

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Abstract

This investigation of endemic land-races of Saudi-Arabian cereals, *Triticum aestivum, Triticum durum*, *Hordeum vulgare*, *Panicum miliaceum* and *Pennisetum glaucum* was aimed at identifying and characterizing stress-tolerant populations appropriate for germplasm-banking. Native soils were saline, nutrient-poor, sandy and subject to seasonal drought and hot summers. Consequently, the work focused on responses of germination and early growth to salinity (0-1000 mM NaCl) and high temperatures, using mainly thermogradient plates and incubators. I examined germination rate, enforced dormancy and viability, in recently collected seeds and in material naturally or artificially aged under different storage conditions. Electrolyte leakage (measured as electrical conductivity) was evaluated as an indicator of deterioration of wheat seed quality. The germination responses to salinity of all five species showed remarkable tolerance, these cereal species are not normally regarded as halophytes but the behaviour of these land races suggested tolerance as high as that of many true halophytes of coastal salt marshes. None of these species showed significant dormancy, presumably as a result of previous domestication, and none showed critical temperature requirement for germination found in many wild species of adverse environments. It is additionally proposed that dry biomass can be an indication for quick inspection of crops under salinity stress. Salt stress similarly results in a significant reduction in the fresh and dry masses of leaves. Due to their response to salinity and significant positive correlation with germination and biomass these characters could be used to assess wheat genotypes under saline field environments. The germination response to artificial ageing was most influenced by the salt stress NaCl during the incubation period particularly at higher concentration of 500, 1000 NaCl mM. The initial moisture content of seeds resisted ageing when being low. The conclusion is that development of plants with increased resistance to inhibition of growth by the osmotic effects of external salinity is both feasible and desirable.

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Chapter 1

General introduction

Germplasm is the foundation of the genetic potential of living organisms. Among other things, diversified germplasm allows organisms to adapt to changing environmental conditions. No single individual of any species, however, contains all the genetic diversity of that species individually. Thus the total genetic potential is represented only in populations made up of many individuals. Such genetic potential is referred to as the gene pool. The potential represented in a gene pool is the foundation for our crop plants in both agriculture and forestry. Germplasm is only maintained in living tissue, most often preserved in the embryo of seeds in 'seed banks'. When the seed dies the germplasm is lost. The limited number of plants that has historically fed the human inhabitants is approximately one per cent of the flora of the world, and the numbers that have entered agriculture is a small fraction of that per cent. As our human inhabitants have grown in number over the last two thousand years, and especially since the development of the science of genetics, we have depended increasingly on a shorter list of the most productive and most easily stored and shipped crops. Today only about 150 plant species (Prescott-Allen and Prescott-Allen 1990) with about one-quarter million local landraces (Wilkes, 1991) are important in meeting the calorie and all nutrient needs of humans. Extinction of a species or a genetic line represents the loss of a unique resource. This type of genetic and environmental impoverishment is irreversible. All over the world people increasingly consume food, take medicine, and employ industrial materials that owe their source to genetic resources of biological organisms. Specified the desires of the future, genetic resources can be reckoned among society's most valuable raw material. Any reduction in the diversity of resources narrows society's scope to respond to new problems and opportunities (Altieri et al., 1987). To the degree that we cannot be certain what needs may arise for food security in the future (new plant diseases or pests, climatic change due to the greenhouse effect, and so forth), it makes sense to keep our options open. This conservation rationale for future generations of humans applies to the Earth's endowment of useful plants more than to almost any other category of natural resources. For many centuries wheat, for example, has been grown in Saudi Arabia. Being a vast area with small areas of fertile land scattered all over the country and in the absence of a unified agricultural policy – in the past – each

community has kept its own cultivars, growing them year after year for countless generations. These local 'cultivars' show a great diversity of types and in many cases they are rather mixtures so they are considered as traditional varieties. This is natural since wheat cultivars have never been bred in the area but introduced from the surrounding countries. Due to the social traditions, the Saudi people have favoured hard wheat for their own local recipes. This led to almost domination of hard red types of bread wheats in addition to amber durum types. However, some other types still existed in small quantities. In recent years the Kingdom of Saudi Arabia started programmes aiming towards the improvement of wheat production in the country. New Mexican cultivars were introduced for example cultivar, 'Super-X' was promoted to replace native bread wheat cultivars, while c.v. 'Jori' is an alternative for the native durums. This situation will eventually lead to the disappearance of old native land races or varieties, and even though their pure stands cannot match the new cultivars in productivity, they carry germplasm valuable for wide adaptation to tough growing conditions such as salinity, drought and maturing under adverse, hot conditions.

1.2.1 Crop production and salinity

Salinity is a major limitation to crop production in the arid and semiarid areas of the world, where low rainfall, high surface evaporation, irrigation with saline water, rising water tables and poor irrigation practices increase levels of soluble salts (Ashraf 1994; Hollington 1998). Salinity management and improved irrigation techniques are often prohibitively expensive and provide only short-term solutions to conquer salinity (Ashraf 1994; Shannon 1997). Plant breeding is a more attainable and permanent approach to minimizing the toxic effects of salinity, with the development of cultivars that can grow and produce economic yield under moderately saline conditions (Flowers and Yeo 1995; Shannon 1997). Selection is an essential part of the breeding programmes and several selection and selection schemes have been proposed for salt tolerance improvement in wheat and other crops (Dewey 1962; Kingsbury and Epstein 1984; Kelman and Qualset 1991; Karadimova and Djambova 1993; Pecetti and Gorham 1997). Field screening measures in saline soils are confronted by high spatial variation and unpredictability problems (Hajrasuliha et al., 1980; Richards 1983). Consequently, most screening attempts for salt-tolerant genotypes have been conducted under either *in vitro* or controlled environmental conditions (Kingsbury and Epstein 1984; Rawson et

al., 1988; Barakat and Abdel Latif 1996; Arzani and Mirodjagh 1999; Munns et al., 2000). Some researchers have recommended that screening for salt tolerance could be more efficient if the measurement was conducted under controlled environmental conditions and using physiological traits rather than selecting for yield and yield components under saline soil conditions (Shannon and Noble 1990; Flowers and Yeo 1995). For example, low Na^+ uptake and enhanced K^+/Na^+ discrimination, traits located on chromosome 4D of bread wheat (Gorham et al., 1987), had been projected as a decisive factor for selecting salt tolerant genotypes in bread wheat (Gorham and Wyn Jones 1993; Dvorak et al., 1994). These characteristics are controlled by a single locus (Kna1) and linked with RFLP markers on chromosome 4DL (Dubcovsky et al., 1996). However, (Munns et al., 2000) reported that low Na^+ increase and high K^+/Na^+ discrimination of similar magnitude to that of bread wheat have been found in durum wheat. Dry material production was used as a selection criterion for salt tolerance in controlled environments affected with salinity (Kingsbury and Epstein 1984; Meneguzzo et al., 2000). Despite the fact that in vitro screening of germplasm has been a successful approach to improve salt tolerance in wheat (Karadimova and Djambova 1993; Barakat and Abdel Latif 1996; Arzani and Mirodjagh 1999), reassessment of *in vitro* selected materials under field conditions has not been reported. This is not with standing the fact that genotypic differences observed under in vitro conditions may not match to those observed at the mature stages in the field. To facilitate and evaluate the efficiency of screening methods for improving salt tolerance in crops, re-examination should be carried out under naturally saline field environments (Richards et al., 1987; Kelman and Qualset 1991). The intention of this analysis was to evaluate the performance of selected salt-tolerant genotypes of durum wheat derived from indirect (*in vitro*) and direct (field) selection methods, using three growing conditions (saline field, non-saline field and saline hydroponic conditions). It should be mentioned that a similar but smaller range of germplasm was used for *in vitro* screening of salt-tolerant genotypes.

1.2.2 Salinity affecting soil

Information on soil salinity may assist in rapid identification of the required germplasm accessions and/or characteristics and may help to prevent excessive duplication of efforts, while showing where the gaps are. From a political and fairness perspective, information on the origin of the accessions is also relevant. Ease of access to the information may make it easier for both breeders (as is the case) and also farmers (as it should also be) to use the materials. Yet news from the Report from CGIAR (Consultative Group on International Agricultural Research) on what we know about the stored seeds is, again, not good: although 37% of national collections and nearly all the CGIAR gene bank accessions have passport data (the standard descriptors that characterize plants cultivars as well as recent field observations), in most collections these data refer only to the country of origin. Plant breeders and farmers often develop their own collections because of the lack of information on collections in the gene banks. The value of characterization and evaluation are also very low. In fact, the accessions of national collections are not fully utilized even by current gene bank clients: the breeders. The exceptions to poor characterization appear to be most countries in Europe, East Asia, North America, Ethiopia, India and the Philippines. Whereas some gene banks have their collections fully documented, computerized and even put in the Internet (as it is the case of the Vavilov Institute and the USA base collections), others have not documented any of their accessions. These factors clearly limit the use of the accessions stored in the gene banks. Nevertheless, a large number of accessions are exchanged around the world. For example, over the last three years the CGIAR centres have distributed an annual average of over 120,000 accessions to national programs all over the world. And in that time the USA has distributed 116,897 samples to 126 countries. However, Figure 1.1 and Table1.1 shows the distribution of salt-affected soils all over the world and show that continent on the plant are not isolated from this effect of soil to turn up as salt-effected soils (Szablose 1989).

Figure 1.1 Global distribution of salt-affected soils (Szablose 1989)

Table 1.1 Regional distributions of salt-affected soils (in million hectares).

Region	Total area	Saline soils		Sodic soils	
	Mha	Mha	%	Mha	%
Africa	1,899	39	2.0	1.8	1.8
Asia, the Pacific & Australia	3,107	195	34	249	8.0
Europe	2,011	7	6.3	73	3.6
Latin America	2,039	61	0.3	51	2.5
Near East	1,802	92	5.1	14	0.8
North America	1,924	0.2	15	0.8	0.8
Total	12,782	394.2	62.7%	389.6	17.5%

Source: FAO Land and Plant Nutrition Management Service (2000).

1.2.3 Traditional varieties salinity resistant and rich in genetic diversity

"A landrace of a seed-propagated crop is a variable population, which is identifiable and usually has a local name. It lacks 'formal' crop improvement, is characterized by a specific adaptation to the environmental conditions of the area of cultivation (tolerant to the biotic and abiotic stresses of that area) and is closely associated with the uses, knowledge, habits, dialects, and celebrations of the people who developed and continue to grow it."(Veteläinen et al., 2009) there are two forms of conservation:

in situ: Where the plant is grown, managed and harvested in its original agricultural environment.

ex situ: Were seeds, plants, plant parts, tissues or cells are preserved in an artificial environment. The most common form of ex-situ conservation is by the use of gene-banks. The seeds are typically stored in laminated packets which are placed in containers and kept frozen at -18°C.

Crop plants are one of the world's most valuable resources. Approximately 60 per cent of the human population directly or indirectly makes their living from agriculture (Wilkes 1992). However, the loss of the genetic diversity of some of the world's crops has accelerated in recent decades, with many crops becoming increasingly susceptible to diseases, pests, and environmental stresses (Plucknett et al. 1983). Human population around the world is increasing every year irrespective of the quantity and quality of the food production. Over the years people around the world had developed hundreds of crop plants through continuous selection and breeding (McMichael et al. 2007). Extensive and intensive breeding programmers, especially during the latter half of 20th century, have eventually resulted in replacing many of the age-old cultivars with high-yielding varieties (Paarlberg and Philip 2007). Large-scale production by these new varieties has, often, created problems for small-scale traditional producers, whose working resources were low inputs and marginal lands. The new high-yielding varieties are also often susceptible to pests and pathogens. The genetic constitution of many improved varieties is tolerant of only a few, specific contemporary problems (Buddenhagen 1983). New pests or pathogens, ozone depletion and consequent overexposure to ultraviolet rays, etc., may be serious problems the future farmers may

have to face. Future breeders therefore, cannot give up the genetic reserves holding the local races and wild relatives, in breeding for resistant varieties (Byrne 2001). Recent innovations in biotechnology, for example recombinant DNA technology, have made the transfer of genes across species much easier than before (Guanming et al., 2013).

Improved cultivars could offer a better-assured food supply, which might liberate most humans from the daily quest for food, particularly in less developed countries in Africa and elsewhere. However, in order to meet the current demands for agricultural modernization, efforts should be made to preserve indigenous agriculture along with the genetic diversity found in those areas associated with agricultural origins and development, such as the areas in south-western Arabian Peninsula and the northeast Africa. According to plant geneticists, the south-western region of the Arabian Peninsula together with northeast Africa are widely regarded as one of the centres of origin of some of the important cash crops and cereals such as wheat, millet and barley. A wide variety of vegetables like onion, okra, aubergine, potato, tomato, carrot, cabbage, turnip, different kinds of pumpkins, cucumbers and gourds, chilli, sugar beet, pulses and leaf crops, etc. are being cultivated in different parts of the country of Saudi Arabia, some of which are indigenous while others have found their way to Saudi Arabia in recent times from several Afro-Asian countries. Al-Qassim and Al-Baha region in the kingdom of Saudi Arabia are other areas where several crops are being cultivated, including an indigenous variety of wheat (Chaudhary and Al-Jowaid 1999)

The agricultural production from these areas has continuously contributed the food security of this region's inhabitants and the mode of cultivation was part of their culture and social expression. Unfortunately, the loss of genetic diversity of some of the Kingdom of Saudi Arabia's crops has accelerated in the past few decades. Over the years, the valuable germplasms of these cultivars has been subjected to genetic erosion as a result of the adoption of high yielding varieties, which has, finally resulted in abandoning the traditional varieties that were rich in genetic diversity (Hassan 1979).

Throughout the Middle East, due to the inflow of exotic cultivars, primitive crop varieties have been ignored and have been seldom cultivated in the past few decades, resulting in their gradual disappearance. In the mid-1960s, a number of plant breeders expressed concern about the accelerated displacement of primitive crop varieties. Germplasm development is vital for the conservation, management and recovery of

threatened cultivars. Conserved germplasm may be utilized for the establishment of *ex-situ* populations, which may then be used for the development of salinity-tolerant high yield/disease resistant varieties (Hassan 1979). Seeds of *Triticum aestivum* (Arabic local name, Haap), from Al-Baha region *Triticum aestivum* (Arabic local name, meyeh), *Triticum durum* (Arabic local name, logemei), *Hordeum vulgare* (Arabic local name, saear arubi), *Panicum miliaceum* (Arabic local name, mlessa) and *Pennisetum glaucum* (Arabic local name, sudany) from Al-Qassim region were collected for each species, The seed were brought to the U.K. and have been stored at the Millennium Seedbank, Wakehurst Place (Royal Botanic Gardens, Kew).

1.3.1 Seed viability and germination

Seed viability and germination tests are necessary for several reasons: to ensure that only good quality seed is banked for long-term storage; to inform workers of appropriate germination conditions when it is subsequently used; and as a further indication of the environmental tolerances of these races and therefore their potential contribution to breeding or genetic engineering programmes. Seeds of many agricultural crops as well as many wild plants in many countries of the world have been investigated (Vozzo 1978). Many researchers (Baskin and Baskin 1998) have pointed to the importance of the germination process in plant life histories in completing the plant life cycle successfully, especially for those plants that are native to dry, desert or saline environments. Plants that are endemic to the regions of desert areas depend on three important mechanisms: (1) dispersal to an appropriate microsite for germination, (2) germination in a timely manner, (3), and maintaining a 'bank' of seeds in the soil as insurance against unpredictable conditions. The germination process can be affected by many external environmental factors, such as temperature, salinity and light regime (Mayer and Poljakoff-Mayber 1989; Baskin and Baskin 1998; Carter and Ungar 2003). Furthermore, it is reported that temperature and salinity play an effective role in determining the timing of germination in most groups of plant and especially salt-tolerant plants, or halophytes (Rozema 1975; Ungar 1987; Woodell 1985). The thermal requirements for germination (including alternating or continuous temperatures) also may vary considerably between species (Crocker and Barton 1953; Berger 1985). For example, Robert (1988) has made comprehensive reference to the detailed

effects of temperature on the germination process. Also Steinbauer and Grigsby (1957) pointed out when they studied 85 plant species belonging to 15 families, using seeds collected from Europe, that the germination rates were high (80%) in alternating temperatures when compared to constant temperature. Thompson (1970) clarified that the geographical distribution of different types of plants of Caryophyllaceae in Europe was associated closely with the germination responses of their seeds to accumulated temperatures, expressed as heat-sums. Many seeds remain dormant even though they are viable.

1.3.2 Effects of salinity on plants

Plant growth and development in saline environments is one of the major themes that have been studied in soil science plant physiology. Salts, existing mainly in the rooting zone and affect all growth phases of the plant life cycle. Seed germination rate and percentage of growth tends to be reduced with salinity (Bernardo et al., 2000). A number of growth problem rose from the effects of salinity which can be in the form of reduced rates of net CO_2 acclimatization, cut-rate of leaf area, reduce leaf cell growth and leaf growth (Cramer et al., 2001). The reaction of a plant to salinity is complex and differs from one plant to another, depending on salinity, kind of salt, growth stage of plant at exposure, period of the day, and many other factors (Cramer et al., 2001). Excessive quantities of salts in the rooting area generate low (more negative) external water potentials and consequently decrease water availability to be up taking by the plants. It correspondingly decreases root pressure driven xylem transfer of water and solutes (Marschner 1995; Munns 1993). The outhers proposed a biphasic model to describe growth responses of plants to salinity. According to this model growth was primarily decreased by the reduction in soil water potential. This stage of the growth decrease was called water stress and is a consequence of salts external to the plants rather than internal ones. Furthermore, it is reported that the rate of leaf development responds more rapidly not like what happing in the ion concentration in the increasing cells or internally (Delane et al., 1982) and the decrease in leaf development can be correspondingly encouraged by additional osmotic (Delane et al., 1982; Termaat and Munns 1986; Yeo et al., 1991). Dissimilarities in genotypes occur because they vary in the period for salt to reach its maximum concentration in the vacuoles of mesophyll cells. Leaves normally die earlier among salt-sensitive genotypes because salts reach them more quickly, or because cells are less able to compartment alise salts in their

vacuoles than the tolerant genotypes at high concentrations. Other authors have also reorganized the growth response to salinity as two-stage process. Yeo et al., (1991) and Cramer and Bowman (1991) have proposed a well-defined separation between 'short term' and 'long term' consequences of salinity on plant growth. Sodium and chloride are mostly the major ions in saline environments, and their greater uptake leads to salt damage in salt sensitive plants (Serrano et al., 1999). Growth impairment and damage of the vegetation in many herbaceous crop species happens even at lower concentrations of NaCl salinity (Sykes 1992). Salt damage in plant leaves and stem occurs as a consequence of higher uptake of Na^+ and Cl^- associated with a decrease in K^+ supply (Sharma 1995). Definite ion controls have been connected to concentration of toxic ions, like Cl^- and Na^+ or to Ca^{2+} and K^+ reduction on leaf senescence (Yeo et al., 1991). Hu and Schmidhalter (1998) advocated that the accumulation of solutes under saline conditions arises both by increasing the net uptake rate and by decreasing growth. Salt accumulation in the leaf apoplast is an essential component of salinity toxicity; furthermore, there is growing support for the hypothesis of Oertli (1968), which depends on turgor loss and dehydration and eventually loss of leaf tissues and cells (Munns 1988; Flowers 1988). Sodium chloride toxicity is correspondingly believed to be accompanied by higher production of superoxide radicals and lipid peroxidation that imposes an oxidative strain at the mitochondrial level (Hernandez et al., 1993). The barrier of oxidative phosphorylation on exposure of mitochondria to NaCl has been mention by Flowers (1975) in salt-tolerant and also in salt-sensitive species. Higher concentrations of NaCl in the root zone lead to membrane depolarization (Shabala and Newman 2000). The superfluous of Na^+ causes membrane leakage (Epstein 1972) since the monovalent Na^+ might cause deterioration of the membrane structure by replacing divalent bridges provided by Ca^{2+} or other divalent cations (Leopold and Willing 1984). Conversely, Ca^{2+} might help to stabilize phospholipids and therefore, limit membrane permeability (Cramer et al., 1989). The ionic composition of saline soils is fairly different from that of normal soils, with Na^+ and Cl^- ions more predominant. As transport systems in plants are disturbed by exposure to NaCl, there are consequences for tissue ion distribution and thus nutrient status (Lauchli and Epstein 1990). At high salinity stress, inhibited nutrient uptake, consumption, and transport could lead to growth suppression (Marschner 1995). Uptake and transport of K^+ (Lynch and Lauchli 1984), Ca^{2+} (Lynch and Lauchli 1985), N (Pessarakli and Tucker 1988), and P (Martinez and Lauchli 1994) may be depressed. A reduction in K^+ uptake might be due

to external Na^+ blocking (Amtmann and Sanders 1999). Shabala (2000) discovered that net discharge of K^+ transpired under NaCl salinity. Salinity-induced Mn deficiency has also been postulate as a main cause of growth decrease in barley provided with low concentrations of manganese and high concentrations of NaCl (Cramer and Nowak 1992). The response to P under saline conditions is rather inconclusive. In natural conditions of high P availability, NaCl may improve P uptake and reduce plant growth by P toxicity (Roberts et al., 1984), while at low P concentrations, NaCl decrease uptake and translocation of P (Martinez and Lauchli 1991) and additional P amount on such sub traits increases the salt tolerance (Awad et al., 1990). Higher Na^+ concentrations in the saline zone may limit uptake and transport of Ca^{2+} and promote Ca^{2+} deficiency where Ca^{2+} concentrations are low or Na^+/Ca^{2+} ratios high (Lynch and Lauchli 1985). Sodium chloride induced Ca^{2+} efflux from cell walls has also been described (Shabala and Newman 2000). In saline soils, poor growth of wheat and barley could be attributed to Ca^{2+} deficiency, as there was greatly improved growth with addition of Ca^{2+} (Ehret et al., 1990). The efficiencies of nutrient usage are also reduced under saline conditions. Higher concentration of Cl^- is believed to decrease the uptake and consumption of NO^{3-} (John et al., 1977). The Cl^- induced NO^{3-} deficiency has been studied as a fundamental mechanism for growth decrease in wheat plants under salinity stress (Torres and Bingham 1973).

The production and transport of phytohormones is affected in saline environments and is increasingly thought to be part of the intricate plant responses to salinity. Helmy et al., (1994) stated that early leaf senescence in tomato plants could be due to increased production of ethylene (C_2H_4) in saline conditions. Incompatibility, among sensitive plant varieties inconsistent in salt tolerance, where in the tolerant varieties is more adept to produce C_2H_4 (Lutts et al., 1996). In saline environments, the level of cytokinins (CYT) is repressed (Kuiper et al., 1990), whereas abscisic acid (ABA) is increased (La Roza et al., 1985). The increased levels of ABA are correspondingly thought to assist with osmotic adjustment and consequently salt tolerance. (Amzallag et al., 1990) demonstrated enhanced salt tolerance in sorghum plants due to foliar sprays of ABA. A parallel enhancement in growth of sorghum under saline environments has also been described after application of CYT, especially if it was combined with gibberellic acid (Amzallag et al., 1992). Nabati et al., (1994) found better shoot and root growth in grass species in saline environments when different plant growth regulating mechanisms

(plant growth substances) were used. Nevertheless, there are occurrences where no relationships were found between salt tolerance and endogenous levels of ABA or CYT. Growth analysis is a key factor to understand effect of salinity on plant growth characteristics and to understand the raw data and the fitted regressions and growth-functions obtained for all selected species using (Hughes and Freeman 1967) programme. The details used are: relative growth rate and relative leaf area growth rate R and RL; leaf area ratio, LAR; unit leaf rate, E the values plotted are instantaneous fitted values for each harvest time.

1.3.3 Germination effects under salinity

Commonly, salinity inhibits seed germination (Jibury et al., 1986; Yaseen et al., 1989; Kumar et al., 1988; Mondal et al., 1988; Navetiyal et al., 1989; Alwan et al., 1989; Begum et al., 1992; Kabar 1986). Narele et al. (1969) found that salinity of 4.5 mmhos cm^{-1} usually did not affect germination, whereas salinity 8.9 mmhos cm^{-1} inhibited germination. Many other workers have reported reduced or delayed germination of wheat seed or Pepper (Babu and Kumar 1975; Kabar 1986 and Kadir et al., 2004) by salinity. Prakash and Sastry (1992) similarly discovered that germination and early growth phases in wheat were affected by salinity. Salinity and sodicity reduced the germination and root/shoot ratio in wheat (Ray and Khaddar 1992). Dell' Aquila and Spada (1993) detected a decline or loss of polypeptides under salinity stress of two salt tolerant genotypes at radical emergence stage. Additionally, whilst the seeds of the same genotypes were irrigated with water no new polypeptides. They likewise discovered a difference synthesis of polypeptides that are distinctive to every cultivar. Therefore they introduced the concept of salt stress proteins to be associated to the adaptive development of seeds to salinity in addition to the genetic composition of particular salt-tolerant genotypes. Rice seed germination and seedling growth are particularly sensitive to salinity (Alam et al., 2004). Dass and Jain (1988) found that *Ziziphus rotundifolia* was tolerant when irrigated with water of 4.5 - 6.5 mmhos EC throughout germination and seedling growth stages. *Ziziphus spinachisti* and *Z. mauritiana* cv Tikadi showed moderate tolerance at 2.5 mmhos, whereas *Z. hummularia* was sensitive to salinity. Poljakoff-Mayber et al., (1994) considered that the osmotic potential is the main effect of salinity on germination in nature. Nevertheless, the combined osmotic and ionic effects, particularly at high concentrations of NaCl, can prevent germination. Inhibition of germination at high NaCl concentrations is more

severe in scarified seeds than in intact ones, indicating that the seed coat can be something of a barrier to Na^+ influx, at least in *Kosteletzya virginica* (Malvaceae) (Somers 1982); they also reported that *K. virginica* is more resistant to salinity at the germination stage compared to the seedling stage. Shah et al., (1973) and Al-Ansari (2003) all have reported that in wheat increasing of the salinity reduces both germination and coleoptile length.

1.3.4 Strategic importance within the Kingdom of Saudi Arabia

The Kingdom of Saudi Arabia lies between 15°45' and 34°35' N and 34°40' and 55°45' E (Chaudhary and Al-Jowaid 1999). Occupying most of the Arabian Peninsula, the Kingdom, with an area of 2,200,000 km^2, contains significant diversity of arid vegetation. The diverse physiographic (Fig. 1.2) features coupled with their peculiar climates have influenced the vegetation remarkably. Though there is a vast expanse of desert in the Kingdom, Saudi Arabia is not totally a desert. The mountain ranges bordering the western seashore, rising from 500 m, run into escarpments as high as 3000 m (Chaudhary and Al-Jowaid 1999). This mountain system receives more rain than other parts of the country, and holds arborescent vegetation with high species richness. The shrubby and herbaceous life forms subtended by the arborescent vegetation also tend to be diverse. Numerous small and large interlaced wadis are distributed throughout the country. Because of the slow seepage of moisture preserved beneath the adjoining landmasses, the lower landscapes forming the wadis have a better soil moisture regime and shelter more species than the adjoining areas. These wadis provide shelter for a large segment of the flora of the country. The inland sabkhas, coastal salt marshes, and the littoral tidal zones host diverse halophytic communities. The relief features edging the Arabian shield are also peculiar niches for particular kinds of plants. The Rub' al- Khali, the Nafud and the Dahna are vast sand expanses, which experience extreme aridity (Abd El-Rehman 1986). Although sparse and species poor, the flora comprising species characteristically adapted to the extremes of the xeric climate inhabit these deserts. Surrounded by the Mediterranean, Near East, and Abyssinian (Ethiopian) and Indian centres, Arabia is an important plant diversity center (Al-Farhan 1991). The Peninsula provides some of the least man-modified landscapes and life forms within the Irano-Turanian phytochorion. Saudi Arabia is also an ancient cultural center housing many agricultural crops and practices. The climate of Saudi

Arabia are its sub-tropical latitude range of 16- 32°N, and its position both close to the circum-global latitudinal belt of generally high atmospheric pressure and sandwiched between the vast continental land masses of Africa and Asia. These aspects mark Saudi Arabia one of the hottest and sunniest countries in the world, with low humidity, except in summer along the coasts. Although Saudi Arabia is strictly a peninsula, the adjacent Red Sea and Arabian Gulf are narrow and close land. These waters become very warm in summer and limit the night-time drop in air temperature, making hot, humid nights an uncomfortable feature of summer along Saudi Arabia's western and eastern coastal plains. Along the coastal plains even typical night-time minima are as high as 29-30 °C through summer months, with relative humidity higher than in the daytime.

Rainfall is unreliable and annual average totals are typically around 100 mm or, especially inland, less; for instance 35 mm at Tabuk, inland in the north-west. The wettest area is the far south west in the region of Saudi Arabia's highest mountains, where most of the rain comes from spring and summer convection, raising annual totals to 199 mm at Khamis Mushait (about 2100 m above mean sea level (a.m.s.l.)) and 141 mm at Jizan on the adjacent coastline. In the northern half of the country, any rain falls mainly during November to April from weak weather systems moving eastwards from the Mediterranean or North Africa. In the southern half of the country away from the far south-west, what rain there is can fall in any season.

The temperature distribution across Saudi Arabia is controlled mainly by altitude and, to a lesser extent, proximity to sea. Temperatures are somewhat lower and more comfortable along the chain of mountains stretching from north-west to south-east along the western side of the country. To the west of these mountains is a very narrow Red Sea coastal plain and to the east is a vast high plateau that gradually descends to a broader eastern coastal plain. With the exception of the mountains, typical daytime temperature maxima from May to September are between 38°C and 43°C (several degrees higher on some days) in comparison to 30-32°C at 2100m (a.m.s.l) at Khamis Mushait. However, there is usually a sharp drop of temperature at night, especially in the interior, where, in addition to mountains in the northwest, frost and snow occur occasionally in winter. Annual mean temperatures range from 30-31°C at low-lying Dhahran, Makkah and Jizan to 25°C at more elevated Riyadh, 22°C at Tabuk (800m (a.m.s.l) in the north-west) and 20 °C at Khamis Mushait (2100 m (a.m.s.l) in the south-west).

In addition to extreme high temperature events in Saudi Arabia's long and very hot summer and the extreme combination of heat and humidity along the coasts on summer nights, climate hazards include sand/dust storms and localised floods. In late spring and early summer, a strong north-westerly 'shamal' wind blows almost constantly for almost three months, particularly in eastern Arabia, producing sand/dust storms that can decrease visibility to a few meters. However, strong winds and dust storms of briefer duration also occur in winter.

Two types of salt marshes are present in Saudi Arabia, namely inland and littoral (Coastal). Inland salty areas, which are usually far away from the seashore, are small to large depressions and are characterized by shallow underground water table. In certain areas, such as Al-Awshaziyah and Al-Hassa Oasis, the ground water reaches on the substratum and forms a somewhat perennial lake of saline water. Littoral or coastal sabkhas can be seen throughout the Arabian Gulf and the Red Sea coast. Coastal salty areas are characterized by fine clay soil mixed with humus, which are carried by flash floods from inland areas. Coastal lands are sparsely vegetated, mostly dominated by mangroves and some succulent plants of the families such as Chenopodiaceae, Zygophyllaceae, Plumbaginaceae, etc. The water for the growth of such plants growing in close proximity with sea mostly comes from seawater, whereas plants growing far away from the seashore are fed by rainwater. In certain areas of coastal salt marsh where the area is regularly inundated by tides, the composition and density of salt is low whereas in areas having high evaporation rate, the salinity level is very high. However, during rainy season, soil salinity will drastically drop due to flooding and runoff from the land coupled with heavy rainfall.

Present estimates show that Saudi Arabia contains over 100 species distributed in 33 families or so. These species are either strictly halophytes or having adaptations to survive in wider ecological amplitude. Among the halophytes recorded from Saudi Arabia, the highest number of from Chenopodiaceae followed by Poaceae, Zygophyllaceae and Tamaricaceae. In Saudi Arabia halophytes are regarded as good fodder during adverse climatic conditions; some of them rich in terms of nutritive value while others have plenty of water to quench the thirst of the domesticated animals. Some of the halophytes dominating in the Red Sea coastal regions are: *Avicennia marina*, *Rhizophora mucronata*, *Cressa cretica*, *Limonium* spp., *Zygophyllum coccineum*, etc. whereas the halophytic species dominating in the Arabian Gulf coast

are: *Suaeda vermiculata*, *Suaeda maritima*, *Salicornia europaea*, *Halocnemum strobilaceum, Arthrocnemum macrostachyum,* etc.

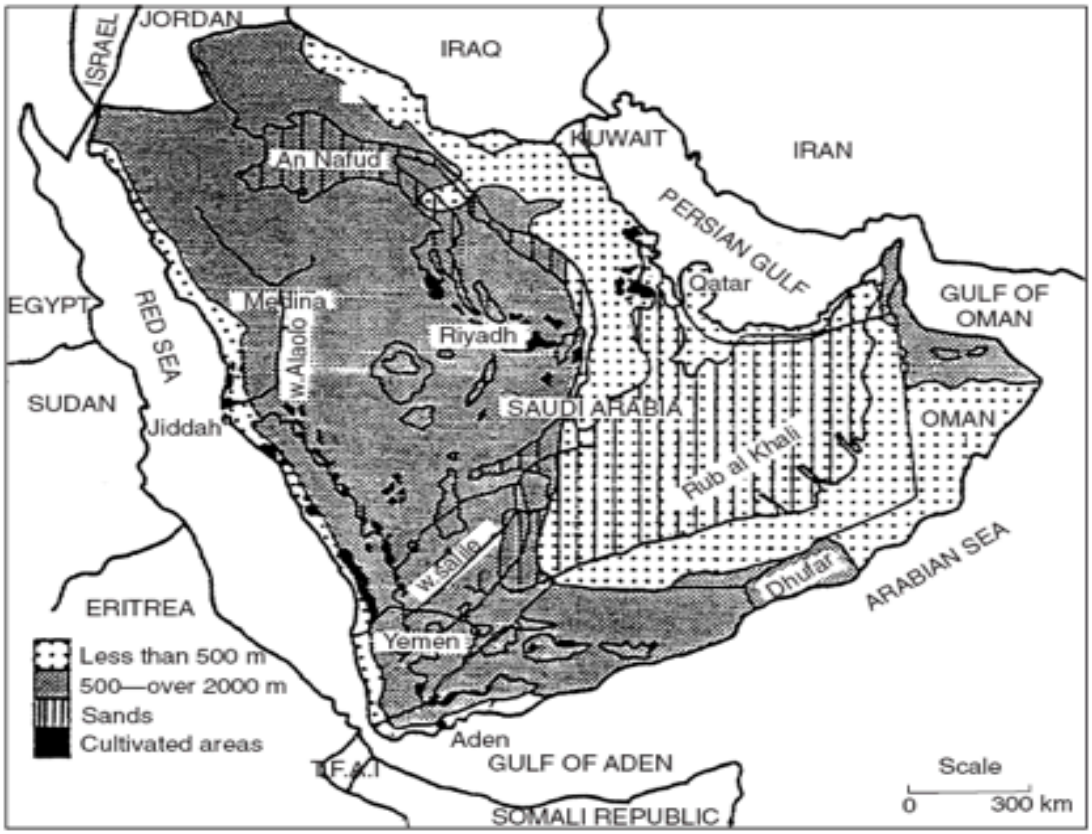


Fig. 1.2 Physiographic map of Saudi Arabia (after Abd El-Rehman, 1986).

1.4. Research aim and objectives

Wheat growing became the major objective of government encouragement in the early part of the 1980s in Saudi Arabia. Until 1981 the proportion of wheat to total cereal production was approximately 50%. The ratio jumped in 1981 to 63.7% and in 1982 to 85.3%. This high percentage was maintained for ten years. Throughout this phase, wheat production progressively increased to reach a record 3,741,229.86776 metric tonnes in 1992,3 well in excess of the self- sufficiency requirement of the country's 16.1 million population. Additionally, well beyond the storage capacity of the government's Grain Silos and Flour Mills Organization (GSFMO) of 2.38 million tons (Saudi Ministry of Planning 2003). In 1992 the government initiated emphasize in barley growing. Within one year, in 1993, production jumped by 340%, to 1.421 million tons from 417,000 tons in 1992. One year later, in 1994, barley production peaked to 2.01 billion tons. The number of farmers who sold their barley to GSFMO increased proportionately. In 1992, the number was 4,015 farmers. In 1993, it jumped to 11,804 farmers and in 1994, to a maximum of 12,637 farmers (SSY 2001). To achieve this enormous growth, the cereals producing surface was increased by a phenomenal 924,000 hectares (1.125 million hectares in 1992 – 201,000 hectares in 1973), or by 560%. The overall irrigated surface of all crops expanded by 1.198 million hectares, from 373,000 hectares in 1973 to 1.571 million hectares in 1992, or by 421%. The irrigated surface expanded further in 1993 to 1.596 million hectares. An arid Saudi Arabia was turned into the world's sixth largest wheat exporting country during the same period (1973 to 1992), the surface of the other agricultural irrigated area increased impressively as well, by 274,000 hectares (1.198 million hectares – 924,000 hectares), or 259%. However, production of the other crops increased sharply, vegetables; by 520% (to 2.073 million tons from 399,000 tons), fruits; by 253% (to 0.899 million tons from 355,000 tons) and alfalfa; by 705% (to 2.425 million tons from 344,000 tons). Starting 1993, however, financial pressures forced the Saudi government into a policy reversal. Combined with the cost of the 1991 Gulf War and persistent budget deficits since the early 1980s, caused the government a severe shortage of liquidity. In1992, subsidies to primarily wheat growing were reduced by 12%, from a peak of SR6 billions in 1991 (UK £1.02 billions) to SR5.28 billions (UK £0.89 billions). In August 1993, it was announced that the government would not buy wheat in 1994 from the six main commercial producers, while imposing quota restrictions on purchases from

smaller commercial farmers. In 1995, the subsidies dropped by 50% from 1994 [SR2.536 billions from SR5 billions]. The slide continued till the subsidies became about SR0.94 billions (UK £159.15 millions) in 2000.

Within 4 years, by 1996, the all-cereal growing land surface dropped by a dramatic 559,000 hectares (1.125 million hectares in 1992 – 566,000 hectares in 1996), or by 50%. Wheat production dropped even more steeply, by 70% (1.2 million tons in 1996/ 4,124 million tons in 1992). By 2000, however, wheat production recovered to a level sufficient for the country's domestic consumption, 1.787 million tons. Barley production's fall was similarly dramatic. Within one year, 40% of the 2.01 billion tons produced in 1994 was cut, to 794,000 tons in 1995. The number of farmers delivering barley to GSFMO dropped from 12,637 farmers in 1994 to 2,126 farmers in 1995 (SSY 2001). Since then, barley growing continued its sliding trend. In 1999 production was only 192,000 tons, with the number of barley growers who sold to GSFMO dropping to 368 farmers (MOP, 2003). In 2000, production became 118,000 tons. Meanwhile, barley imports in 2001 were 3.25 million tons. Barley production declined mainly due to problems of water supply (underground water) and salinity problems.

The main aim of this research is to investigate on the germination responses of endemic, locally preserved land-races of cereal-crop species *Triticum aestivum* (Arabic local name, Haap), from Al-Baha region *Triticum aestivum* (Arabic local name, meyeh), *Triticum durum* (Arabic local name, logemei), *Hordeum vulgare* (Arabic local name, saear arubi), *Panicum miliaceum* (Arabic local name, mlessa) and *Pennisetum glaucum* (Arabic local name, sudany) from Al-Qassim region from the varied climatic conditions found in Saudi Arabia. Specifically, in arid and semi-arid areas, it is important to understand adaptive responses to salinity at 0, 100, 200, 300, 400, 500, 600 and 700 mM sodium chloride and range of temperatures grids (6 up to 33 °C), using a surface thermometer controlled unit. Ultimately such work would provide information to help preserve the Kingdom's rare and indigenous crops for future generations, and provide plant breeders with the genetic resources necessary for developing stress-resistant cultivars in the future. In order to address these aims, a number of objectives needed to be met, which have been addressed in the chapters below:

Chapter2:

Plants growing in saline environments have developed a number of morphological, physiological and biochemical adaptive mechanisms, which qualify them to continue and grow in the presence of salts (Naseer et al., 2014). There is a great amount of literature accessible dealing with the responses of various plant species to external salinity however the precise mechanisms amended by different species is not yet well defined. Commonly plants prevent excessive amounts of toxic salts either by limiting ion uptake into the plant shoot, or by tolerating ion uptake and regulating high salt concentration throughout osmotic regulation (Mirza et al., 2014). In conclusion plants should reach and provide favorable water stability as well as a favourable ionic stability at the plant cell and entire plant levels to effectively grow under saline environments (Nadeem et al., 2014). This chapter will cover germination response to salinity and germination recovery, especially for those plants that are native to dry, desert or saline environments. Plants that are endemic to the regions of desert areas depend on three important mechanisms. Between 15 December 2012 to 7 January 2013, seeds from populations of five species of Poaceae, *Triticum aestivum*, *Triticum durum, Hordeum vulgare, Panicum miliaceum* and *Pennisetum glaucum*, were collected from Al-Qassim **(**Figure 4.1) The hypothesis, that local endemic land races of Saudi-Arabian cereal species will germinate in low concentrations of salt, and that some will germinate at higher concentrations.

Chapter 3:

Seed germination efficacy testing presents an integrative framework to explain and to predict biological changes achieved by different modes of treatment (Waller 2013). This *in vivo* examination of germination performance was made under different concentration of salinity and different range of temperatures as a major cause of decrease in seed germination as well as leaves and roots development. Temperature is one of the most important factors determining growth rates of plants in the field (Scherrer et al., 2011). Germination of non-dormant seeds under multiple temperature conditions can be predicted from constant temperature (Reyer et al., 2013). Thermal-response simulations of this type have not been confirmed under virtual field-variable

temperature conditions that vary in daytime form, diurnal range and longer-term trends in mean–daily temperature. The purpose of this experiment was to evaluate germination response of *Triticum aestivum* (Arabic local name, Haap), from Al-Baha region *Triticum aestivum* (Arabic local name, meyeh), *Triticum durum* (Arabic local name, logemei), *Hordeum vulgare* (Arabic local name, saear arubi), *Panicum miliaceum* (Arabic local name, mlessa) and *Pennisetum glaucum* (Arabic local name, sudany) from Al-Qassim region, under both constant and mutable temperature regimes in the laboratory. In addition, one commercial variety of winter wheat, *Triticum aestivum* (Istabraq), was used for comparison. The (1) to establish a protocol to investigate germination responses to temperature generally and, in particular, determine basal, optimal and ceiling temperatures for germination for these economically important Saudi Arabian cereal land-races; (2) to make broad preliminary comparisons of the effects of temperature, salinity and their interaction across a wide range of species and varieties of some of them.

Chapter 4:

Ecophysiology of seed germination, is the study of the interrelationship between a seeds physical functioning and its environment by analysing the physical and chemical characteristics of soils from the plant habitat as well as the climatically parameters (Angiolini et al., 2013). Plants are capable of absorbing and assimilating as many as forty or fifty different chemical elements. Sixteen of these chemical elements have been found to be essential to the growth of most plants (Stiles 2013). Knowledge of the chemical and physical characteristics of soil is essential to the successful growth of all plant life. Whether it is for the professional raising of crops for commercial purposes or an amateur enterprise such as the raising of grassland, shrubbery or a garden for decorative purposes. Soils are examined to define if important plant nutrients are available and if the soil result or pH value is accurate for growing the chosen plants (Chandra et al., 2014). If the appropriate elements do not exist, the soil tests tell what must be done to provide the correct balance of the necessary nutrients and to provide the proper soil reaction (Bird 2014). Subsequently certain known plant nutrients are essential; reasonably add the necessary plant nutrients mechanically to be sufficient amounts of these plant nutrients present. The determination of this experiment was to evaluate chemical and physical characteristics of soil and compare germination

response of *Triticum aestivum* (Arabic local name, Haap), from Al-Baha region *Triticum aestivum* (Arabic local name, meyeh), from Al-Qassim region, that taking from two different climatical region of Saudi Arabia, Seeds from a population of *Triticum aestivum* from Al-Qassim and a population from Al-Bahah, were collected between 15 December 2012 and 7 January 2013 (Table 4.2). It is hypothesized that the germination response of *T. aestivum* seeds collected from the two regions will differ under differing environmental stresses (temperature and salinity) and that this will be related to the climatic and soil conditions from where they originated.

Chapter 5:

Electrical conductivity (EC) has been evaluated as a possible method for measuring viability and seedling vigour in wheat and other crops (Suma et al., 2014). A study was conducted using dormant, stock, by artificial-ageing wheat seeds *Triticum aestivum* to evaluate the effect of different seed lots on electrical conductivity. All seed lots were subjected to the following tests: standard germination; speed of germination; germination rate; time to reach 50% germination; electrical conductivity test. There are variables that affect explanation of results based on the design of the vigor test. As, most vigour tests assess specific seeds/seedlings and then specify a composite value for example a percentage of the seed lot. This vigor test scheme has value since it is more rapid and less expensive to manage than individual seed analyses (Lazar et al., 2014). The overall understanding of conductivity outcomes it that it characterizes an average value functional to each seed. Another approach to vigor test design is based on identification that maximum vigor tests define specific facets of seed quality. Such as, the enhanced ageing test offers suggestion of the storage ability of a seed lot whereas the conductivity test evaluates membrane integrity. Together mechanisms are important determinants of seed vigor. Equally significant, it has been proposed that better knowledge about seed quality might be gained from conducting study of seed vigor tests and summarizing the results as a single vigor test index. This method is comprehensive but it is difficult to effectively implement. Seeds from a population of *Triticum aestivum* from Al-Qassim and a population from Al-Bahah, were collected between 15 December 2012 and 7 January 2013 (Table 4.2). The hypothesis was that aged seeds of poorer quality would be less salt tolerant. The second aim was to

investigate the practical value of EC measurements of electrolyte leakage in predicting relative seedling emergence for these land-races of *Triticum aestivum* under different salinity conditions, where average germination lay in the commercially conventional range. A supplementary aim was to measure electrolyte leakage from different parts of the seed and establish how it was affected by the different salinity treatments.

Chapter 6:

Growth is a fundamental role of plants and specifies the regular intensification in number and size of cells. The progressions of growth and development are painstaking to begin with germination, tailed with large composite sequence of physiological and morphological events (Ting 1982). Alongside with extra positive environmental situations, passable and also accessibility of crucial components help to increases the growth. The existence of salts in the irrigation systems of arid and semi- arid regions is among the critical reasons affecting the accessibility of water and important nutrients to plants by osmotic stress. Salinity forms the accessibility of nutrients and decreases plant growth (Zalba and Peinemann 1998). Growth parameter for example germination, leaf area, relative growth rate are very essential to evaluate the growth and are affected by salinity. Substantial decreases in vegetative growth on wheat genotypes have been detected in saline situations (Nassem et al., 2000). The reduction of growth may be due to slow cell division rate, reduction in seedling growth (Zeng and Shannon 2000). Growth of salt wheat genotypes that affected by salinity was mainly because of their failure in photosynthetic capability rather than a decrease in leaf area, (El- Hendawy et al., 2005). In his extensive work on the functional approach, (Hunt 1982) states 12 benefits of this technique. Among them are the following: (1) The functional approach provides a clearer perception of ontogenetic drift; (2) Assumptions involved in the calculation of mean values of NAR are avoided; (3) Statistical analyses may be integrated into the same analytical procedure as the calculation of the derived quantities. Effect of salinity on plant growth characteristics is to understand the raw data and the fitted regressions and growth-functions obtained for all selected species using Hughes and Freeman programme. The details used are: relative growth rate and relative leaf area growth rate R and RL; leaf area ratio, LAR; unit leaf rate, E the values plotted are instantaneous fitted values for each harvest time. Between 15 December 2012 and 7 January 2013, seeds from populations of five species of Poaceae, *Triticum aestivum*,

Triticum durum, Hordeum vulgare, Panicum miliaceum and *Pennisetum glaucum*, were collected from Al-Qassim **(**Figure 4.1). The hypothesis that the osmotic effects of salinity on water availability and/or the directly toxic effects of salt would inhibit the growth of the Saudi-Arabian land-races races of wheat. The objectives were (1) to compare the effects of salinity on them with those on known salt-sensitive and salt-tolerant varieties of wheat and (2) partition those growth effects using the methods of quantitative growth analysis to understand better their significance.

Chapter 7:

Presents general discussion of the preceding chapters and an evaluation of their results in order to draw conclusions and suggest recommendations for future research.

Chapter 2. Germination response to salinity and germination recovery

2.1. Introduction

Salinization is a very serious agricultural dilemma. Most of the crops have low salt tolerance. Isolation and identification of salt tolerant genes and cultivars are very important in salt-tolerant crop breeding (Li et al., 2003; Wang et al., 2004). Wheat (*Triticum aestivum* L.) is one of the most important nutritious crops, which ranks first in the production of nutritious crops in the world. Due to the increasing global population and limitation of cultivated lands, increasing the productivity of wheat, including the ability to exploit and utilize saline soils. However, more research is needed on the salt tolerance of potential crop species, particularly local cultivars of species that may be locally adapted to saline or droughted conditions.

Plants tolerance to salt is determined by a series of genes that have relevant direct or indirect effects to form a complex regulating network. However, it is impracticable to investigate all the genes of all potential salt tolerant plant cultivars simultaneously. Because of the numerous complications pathways such as the ion homeostasis, osmoregulation, antioxidant, hormonal systems and natural heterogeneity of soil, assessment of salt-tolerant cultivars frequently has occurred under controlled environments. Germination is a vital phase for plant establishment (Song *et al*., 2008) and most plants are vulnerable to ion stress at the germination stage (Catalan et al., 1994) or during seedling growth (Rogers et al., 1995; Carvajal et al., 1998). However, (George and William 1964) suggested that higher salinity tolerance at germination is associated with low respiration rates and the replacement of respiratory materials. (Saboora et al. 2006) investigated nine wheat cultivars at germination and initial seedling growth using six salt treatments, and found that differing salt treatments had significant effects on germination percentage, rate of germination, dry mass of shoot and root and total dry mass.

Salinity is deleterious to plant growth through various mechanisms that include induced nutrient deficiencies, osmotic effects and specific ion toxicities. There are strong negative associations between the absorption of Na^+ and Cl^- in leaf sap and fresh mass of a number of wheat genotypes (Saqib et al., 1999). In contrast, sodicity can inhibit plant growth predominantly due to abnormal pH, with high concentrations of bicarbonates, and frequently shows a negative correlation with Na^+; sodic soils may also have boron toxicity (Marschner 1995). In some regions of the world, salt-affected soils

are countered by heavy irrigation to leach soluble salts. This approach is applicable only for saline soils, excluding the sodic and saline-sodic soils (Muhammad 1983; Qadir et al., 1996). Additionally, for heavy irrigation good quality water is necessary in sufficient quantity for the leaching of soluble salts. However, high-quality water is rare and drought is more typical in many parts of the world.

Alongside the conventional approaches to cope with salinity and drought, researchers need to consider the technologies and methods needed to achieve greatest production under saline-sodic soils in a predominantly droughted environment. Breeding for salt-tolerant, as well as drought-tolerant, varieties could potentially help overcome the difficulties of these environmental stresses.

The desert climate and saline soils of the Kingdom of Saudi Arabia and other countries of the Arabian Peninsula mean that the native and agricultural flora have high potential to provide important salt tolerant cultivars and genetic plant materials. However, relatively few studies have examined the flora of these countries, particularly in regard to the germination of species in relation to salt. Little work appears to have been done on several key cereal species, including *Triticum aestivum, Triticum durum, and Panicum miliaceum*. The aim of this study was to investigate the germination of seeds of five Poaceae species, *Triticum aestivum* Arabic local name (Meyeh), *Triticum durum* Arabic local name (Logemei), *Hordeum vulgare* Arabic local name (saear arubi), *Panicum miliaceum* Arabic local name (Mlessa), *Pennisetum glaucum* Arabic local name (sudany), in differing saline conditions (0-700 mM sodium chlorine). The subsequent germination in freshwater of ungerminated *Triticum aestivum* seeds that were subjected to salt stress was also investigated. It was hypothesized that local endemic land races of Saudi-Arabian cereal species will germinate in low concentrations of salt, and that some will germinate at higher concentrations.

2.2. Methods

2.2.1 Seed Collection

Between 15 December 2012 to 7 January 2013, seeds from populations of five species of Poaceae, *Triticum aestivum*, *Triticum durum, Hordeum vulgare, Panicum miliaceum* and *Pennisetum glaucum*, were collected from Al-Qassim **(**Figure 4.1), Saudi Arabia (Table 2.1). For each species, the farmers collected a total of 2-3 kg of seed from a field of 1 hectare. Seeds were transported in paper bags to the laboratory, cleaned of impurities and particulates and left to dry in the lab rooms at a temperature of 22 °C. The seed was then brought to U.K. and were subsequently stored at the Millennium Seedbank, Wakehurst Place (Royal Botanic Gardens, Kew) (Fig. 2.1).

Table 2.1 Locations in Saudi Arabia and species names of plants from which seeds were collected.

Family	Scientific name	Arabic Local Name	Collectors	Location
Poaceae	*Triticum aestivum*	Meyeh	Sami Albarih	N 26,134,89 E 043,96864
	Triticum durum	Logemei		N 26,06349 E 440,63544
	Hordeum vulgare	saear arubi		N 25,91733 E 043,78138
	Panicum miliaceum	Mlessa		N 25,88129 E 043,80604
	Pennisetum glaucum	sudanyah		N 26,09399 E 043,96964

Figure 2.1 Millennium Seedbank, Wakehurst Place (Royal Botanic Gardens, Kew).

2.2.2. Germination response to salinity

For each of the five species, ten replicate Petri dishes each containing 20 seeds were used in each of seven treatments of differing sodium chloride concentrations (0, 100, 200, 300, 400, 500, 600, 700 mM sodium chloride). Seeds were placed on 9 cm filter papers (Whatman No. 1) and 10 ml of the appropriate sodium chloride solution was added per dish. This was topped up every four days, according to need and replaced every ten days. Dishes were wrapped around with a layer of Nescofilm, in order to reduce evaporation. Petri dishes were placed in temperature-controlled incubators with an 12 hour alternating temperature regime (15/25°C) and a 12/12 hour photoperiod. The choice of temperature range was based on the climatic information recorded in the study area, which were taken from the Meteorological Administration in Al-Qassim (see Chapter 4).

Germinated seeds (with emerged radicles) were counted in every dish every twenty-four hours, and the germinated seeds immediately removed. This was continued for a period of thirty days. The cumulative germination over time was used to calculate the t_{50}, the period of time taken for 50% of seeds to germinate. After this, seeds that had not germinated were washed with distilled water thoroughly several times, and then transferred to new Petri dishes with 10 ml distilled water, and incubated and counted as previously for a further 5 days until no further germination was observed.

In order to further investigate the recovery of seeds that have been subjected to salt stress, ten replicate Petri dishes each containing 20 *Triticum aestivum* seeds were subjected to treatments of four differing concentrations of sodium chloride, 400, 500, 600, 700 mM sodium chloride. Seeds were placed on 9 cm filter papers (Whatman No. 1) and 10 ml of the appropriate treatment solution was placed in the dish. This was topped up every four days, according to need. Petri dishes were placed in temperature-controlled incubators as for the germination experiment above. Germinated seeds (with emerged radicles) were counted in every dish every twenty-four hours, and the germinated seeds immediately removed. This was continued for a period of three and five days. After this, seeds that had not germinated were washed with distilled water thoroughly several times, and then transferred to new Petri dishes, and incubated for five days to make sure no farther germination occurs.

2.2.3. Seed viability

At the end of the experiment all ungerminated seeds from each species were tested for viability by the tetrazolium method, following the method recommended by the International Seed Testing Association (Anon 1999 a, b). The tetrazolium method was selected because it is widely used throughout the world as a highly regarded method of estimating seed viability and is a routine test in many seed testing laboratories. Furthermore, it is a quick test, completed in only a few hours, compared to regular germination tests that require as long as two months for some species. More important was to simulate the effect of re-irrigation in the field, an agronomic practice that will be explained in detail in section 6.2.2. Seeds to be tested were soaked in distilled water for about half an hour and were then opened into half and fully immersed in petri dishes filled with tetrazolium. Petri dishes were placed in a darkened incubator, to prevent photo-conversion of the solution, set to 30 °C. After 24 h, seeds were observed and diagnosed using a magnifying glass or microscope. Tetrazolium test is a biochemical test, which differentiates live from dead tissues of seed embryos on the basis of dehydrogenase enzyme activity (respiration enzymes). Upon seed hydration, the activity of dehydrogenase enzymes increases, resulting in the release of hydrogen ions, which reduce the colourless tetrazolium salt solution (2,3,5-triphenyltetrazolium chloride) into a chemical red compound called formazan. Formazan stains living cells with a red

colour, while dead cells remain without colour. The viability of seeds is interpreted according to the staining pattern of the embryo and the intensity of the colouration.

Proportion of seeds that germinated were arcsine and transformed prior to analysis. The differences in germination of a species between salinity treatments were examined using one-way ANOVAs and Tukey post-hoc tests using SPSS, version 21. The differing responses amongst species to salinity treatments were examined using a two-way ANOVA (R Core Team 2012).

2.3. Results

2.3.1. Germination response to salinity

Triticum aestivum

Seeds of *T. aestivum* germinated at all sodium chloride concentrations except 700 mM. However, the number of seeds germinating was reduced with increasing sodium chloride concentration (Fig. 2.2, Table 2.2), with significant differences between treatments ($df=7,72$, $F=1753$, $P<0.001$). There were no significant differences in the percentages of germination between 0 mM, 100 mM and 200 mM of sodium chloride (Fig 2.3). The speed of germination (t_{50}) was faster for *T. aestivum* than for the other species (Table 2.3). The speed of germination was reduced by increasing concentrations of sodium chloride (Fig 2.3, Table 2.3). Also seeds recovered from the salinity in both treatments, as was evident after three and five days, at 400, 500, 600 and even 700 mM NaCl (Figure 2.4, 2.5)

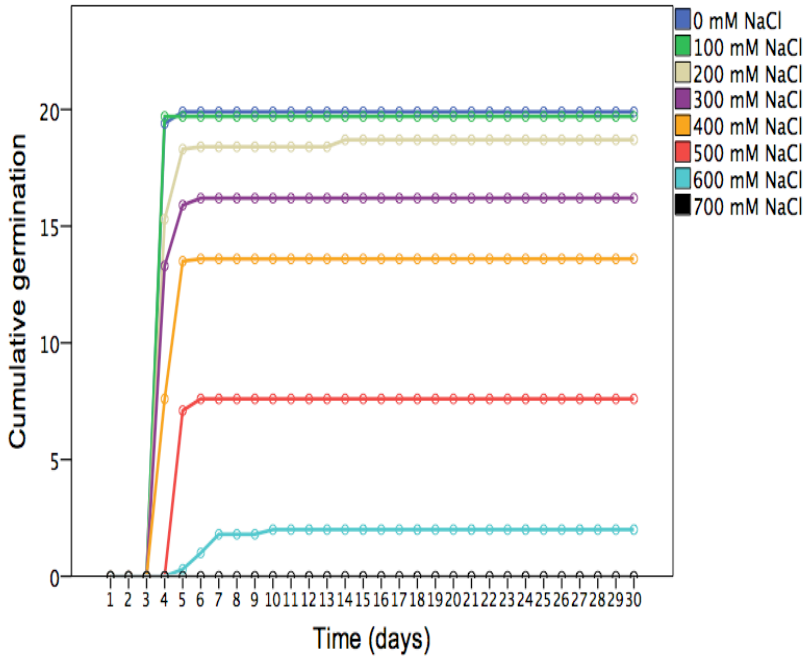


Figure 2.2. Mean cumulative number of *Triticum aestivum* seeds that germinated over time (10 replicates, each containing 20 seeds) in 0, 100, 200, 300, 400, 500, 600 and 700 mM of sodium chloride.

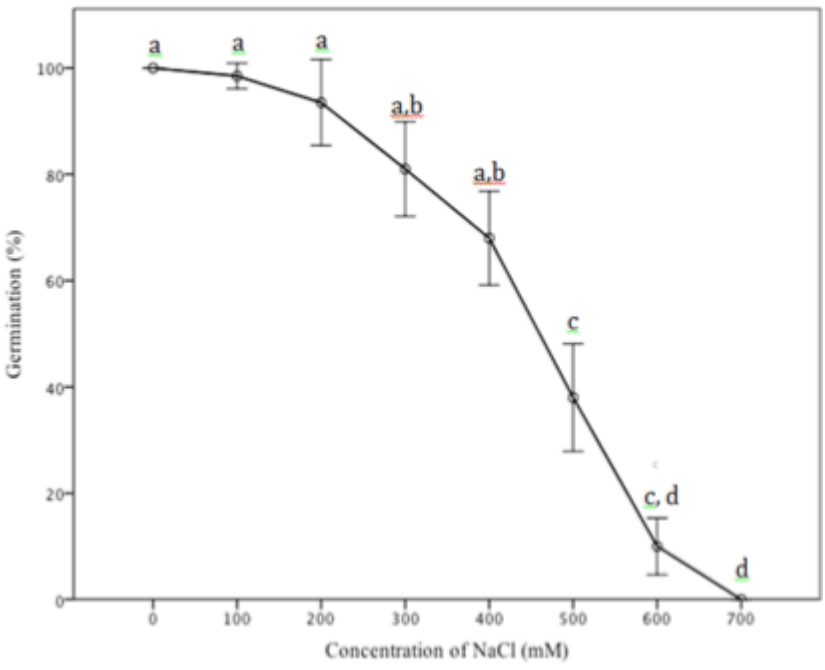


Figure 2.3. Mean (±SE) proportion (%) of *Triticum aestivum* seeds that germinated in different concentrations of sodium chloride. Differing letters denote significant differences (P<0.001).

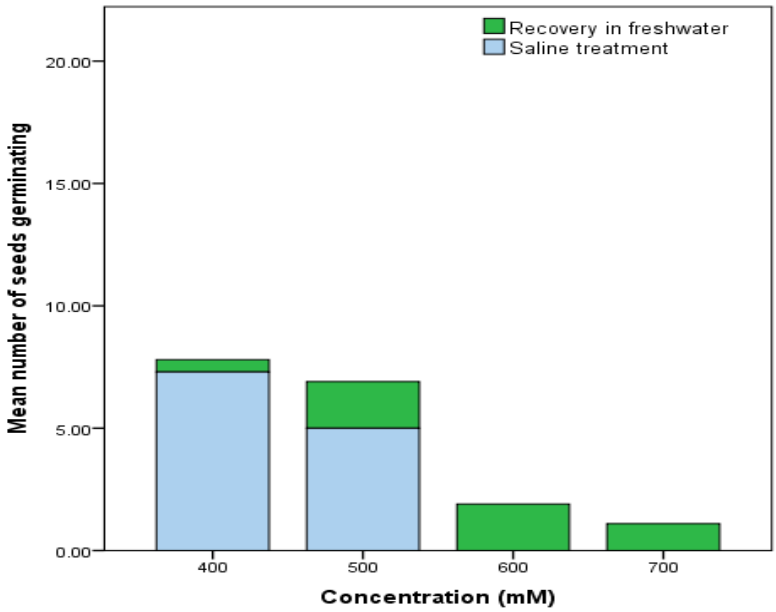


Figure 2.4. Mean number of *Triticum aestivum* seeds that germinated after three days in different concentration of sodium chloride (saline treatment) (10 replicates, each with 20 seeds), and the number of seeds that did not germinate under the salt treatments, but which subsequently germinated in 0 mM NaCl (recovery in freshwater) 400, 500, 600, 700.

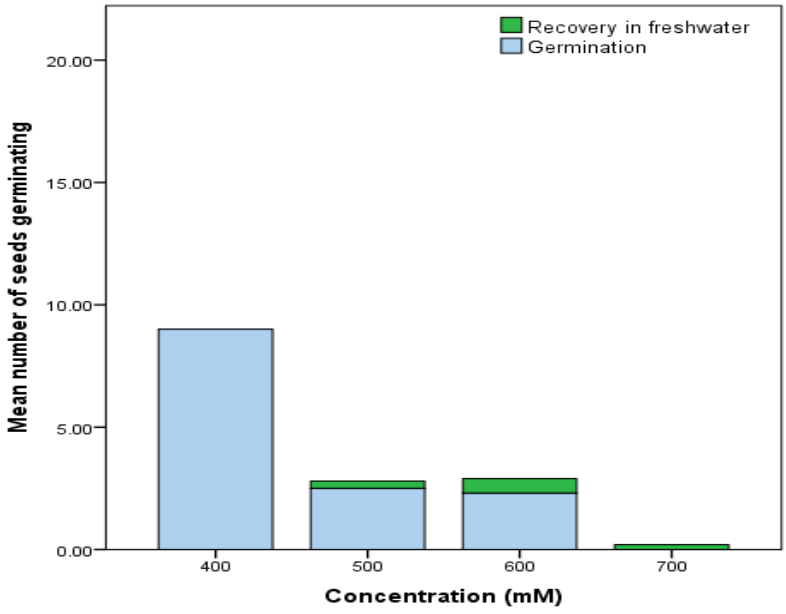


Figure 2.5. Mean number of *Triticum aestivum* seeds that germinated after five days in different concentration of sodium chloride (saline treatment) (10 replicates, each with 20 seeds), and the number of seeds that did not germinate under the salt treatments, but which subsequently germinated in 0 mM NaCl (recovery in freshwater) 400, 500, 600, 700.

Triticum durum

Seeds of *T. durum* did not germinate in sodium chloride concentrations of 600 or 700 mM and the number of seeds germinating was reduced with increasing sodium chloride concentration, with significant differences between treatment (df=7,71, F=206, P<0.001) (Fig. 2.5, Table 2.2). There was no significant difference in the percentages of germination between 0 mM, 100 mM and 200 mM of sodium chloride, and there was no significant difference in the germination between the highest concentrations (500, 600 and 700 mM) (p<0.001 in all cases). The speed of germination was reduced (t_{50}) by increasing concentrations of sodium chloride (Fig 2.6, Table 2.3).

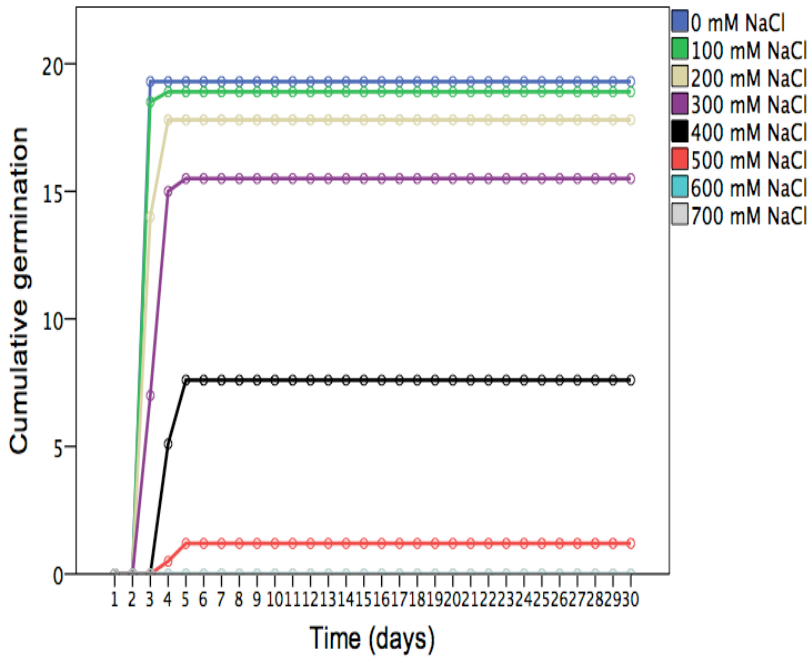


Figure 2.5. Mean cumulative number of *Triticum durum* seeds that germinated over time (10 replicates, each containing 20 seeds) in 0, 100, 200, 300, 400, 500, 600 and 700 mM of sodium chloride.

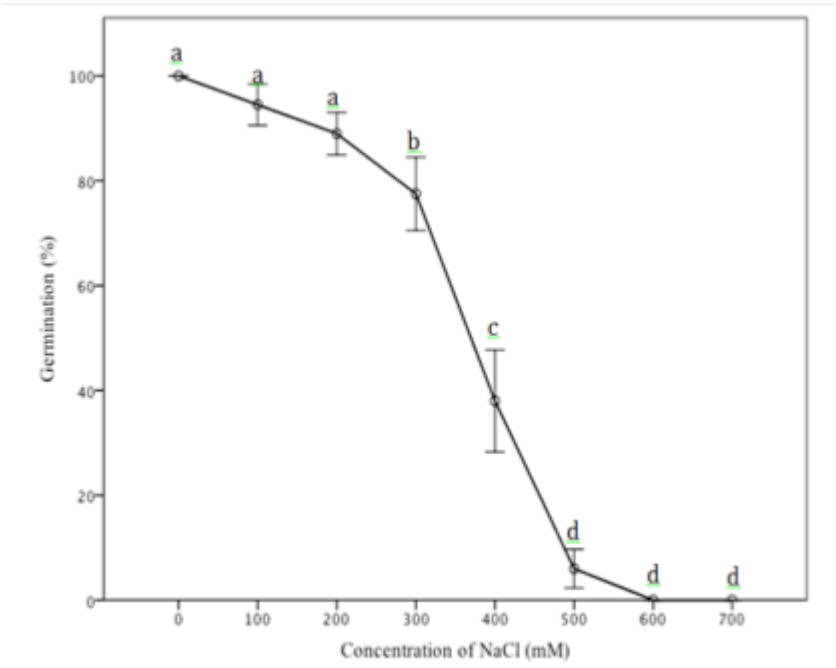


Figure 2.6. Mean (±SE) proportion (%) of *Triticum durum* seeds that germinated in different concentrations of sodium chloride. Differing letters denote significant differences (P<0.001).

Hordeum vulgare

Seeds of *H. vulgare* germinated in all sodium chloride concentrations except 700 mM and the number of seeds germinating were reduced with increasing sodium chloride concentration (Fig. 2.7, Table 2.2), with significant differences between treatments (df=7,72, F=130, p<0.001). There was no significant difference in the percentages of germination between the treatments 0 mM and 100 mM, but germination in the control treatment (0 mM) was significantly higher than in all other treatments (p<0.05 in all cases). The germination in all treatments greater than 300 mM were significantly different to those in all concentrations (P<0.001 in all cases), with the exception of 600 mM and 700 mM between which there was no significant difference. The speed of germination was reduced (t_{50}) by increasing concentrations of sodium chloride (Fig 2.8, Table 2.3).

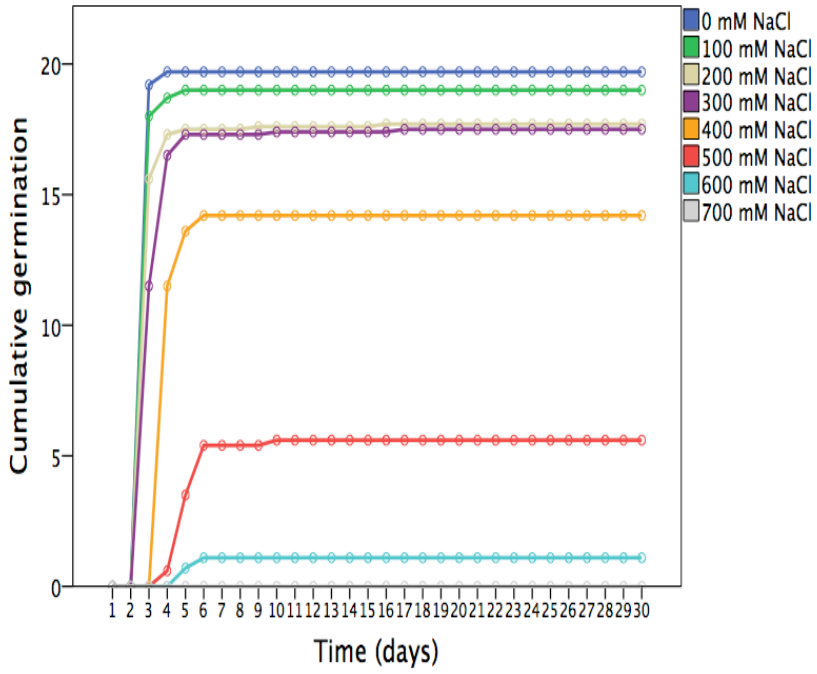


Figure 2.7. Mean cumulative number of *Hordeum vulgare* seeds that germinated over time (10 replicates, each containing 20 seeds) in 0, 100, 200, 300, 400, 500, 600 and 700 mM of sodium chloride.

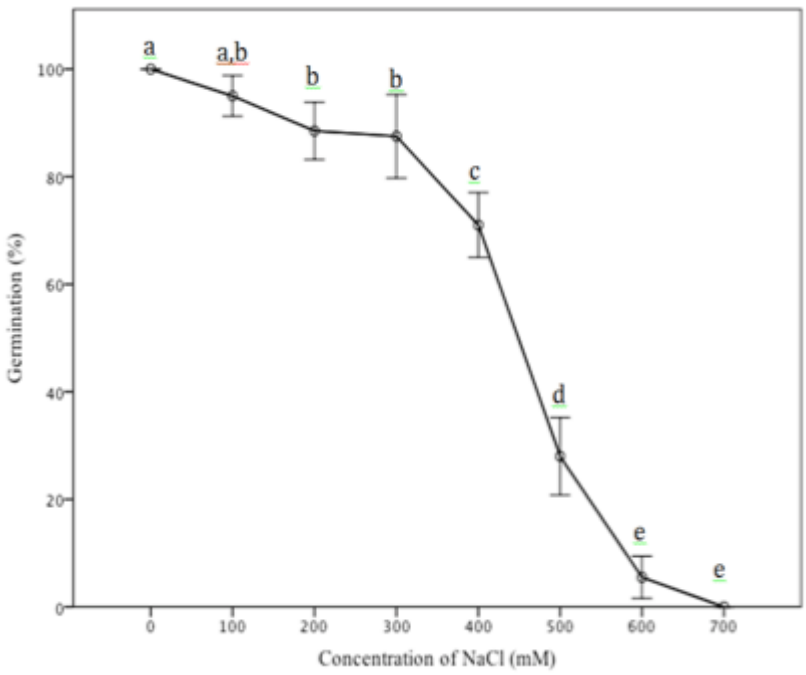


Figure 2.8. Mean (±SE) proportion (%) of *Hordeum vulgare* seeds that germinated in different concentrations of sodium chloride. Differing letters denote significant differences (P<0.001).

Panicum miliaceum

Seeds of *Panicum miliaceum* germinated poorly in 300 and 400 mM of NaCl and there was no germination in concentrations greater than 400 mM (Fig. 2.9, Table 2.2). There were significant differences in the percentage of seeds germinating between treatments (df=7,72, F=136, p<0.001). There were no significant differences in the percentage germination between 0 mM, 100 mM and 200 mM of sodium chloride, and there were no significant differences between the highest concentrations (Fig. 2.10, P<0.001 in all cases). The speed of germination was reduced (t_{50}) by increasing concentrations of sodium chloride (Fig 2.10, Table 2.3).

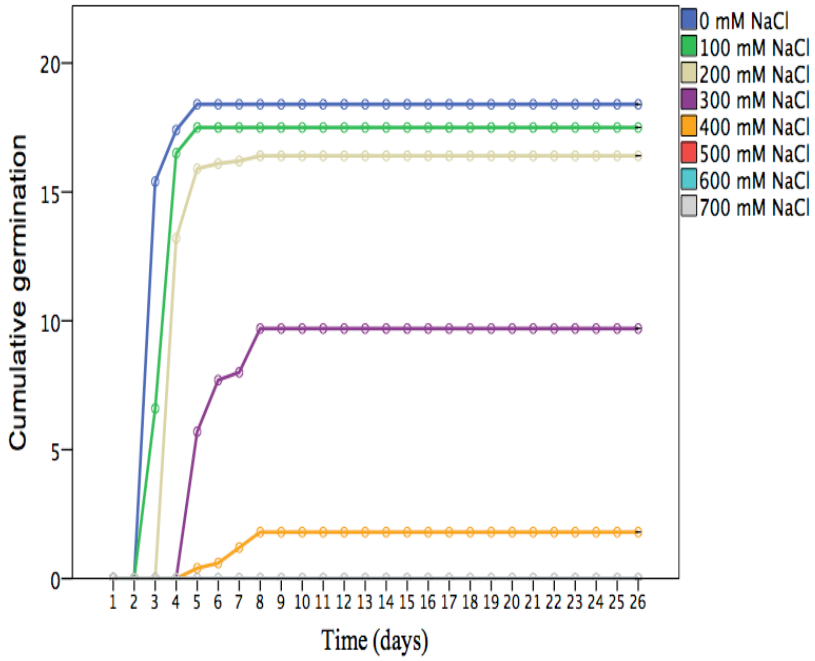


Figure 2.9. Mean cumulative number of *Panicum miliaceum* seeds that germinated over time (10 replicates, each containing 20 seeds) in 0, 100, 200, 300, 400, 500, 600 and 700 mM of sodium chloride.

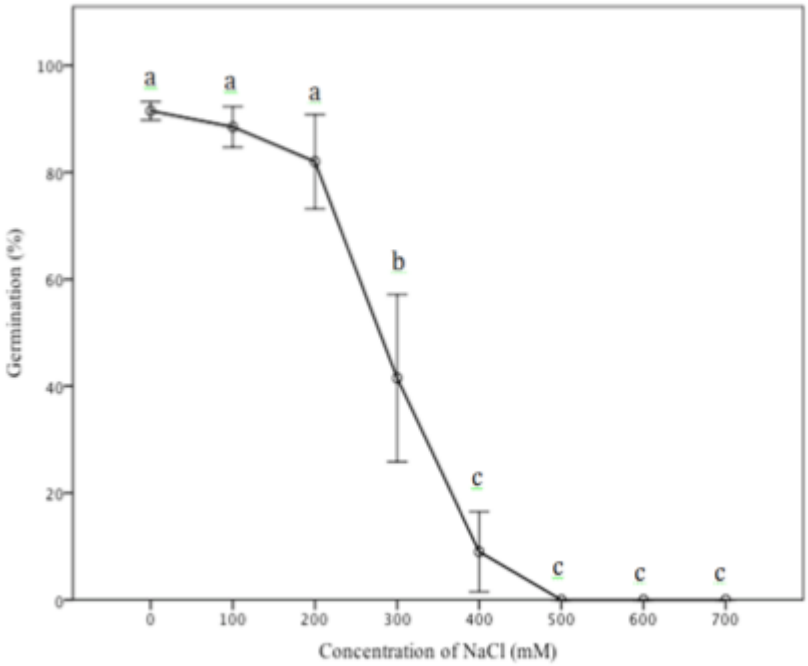


Figure 2.10. Mean (±SE) proportion (%) of *Panicum miliaceum* seeds that germinated in different concentrations of sodium chloride. Differing letters denote significant differences (P<0.001).

Pennisetum glaucum

Seeds of *Pennisetum glaucum* also germinated poorly in 300 and 400 mM of NaCl and there was no germination in concentrations greater than 400 mM (Fig. 2.11, Table 2.2). There were significant differences in the percentage germination between treatmetns (df=7,72, F=218, p<0.001). The percentage germination in the control treatment (0 mM) was significantly higher than in all other treatments (P<0.001 in all cases). Germination in the higher concentrations (≥400 mM) were significantly lower than in the other treatments (P<0.001 in all cases). The rapid speed of germination (t_{50}) was reduced with increasing concentrations of sodium chloride (Fig 2.10, Table 2.3).

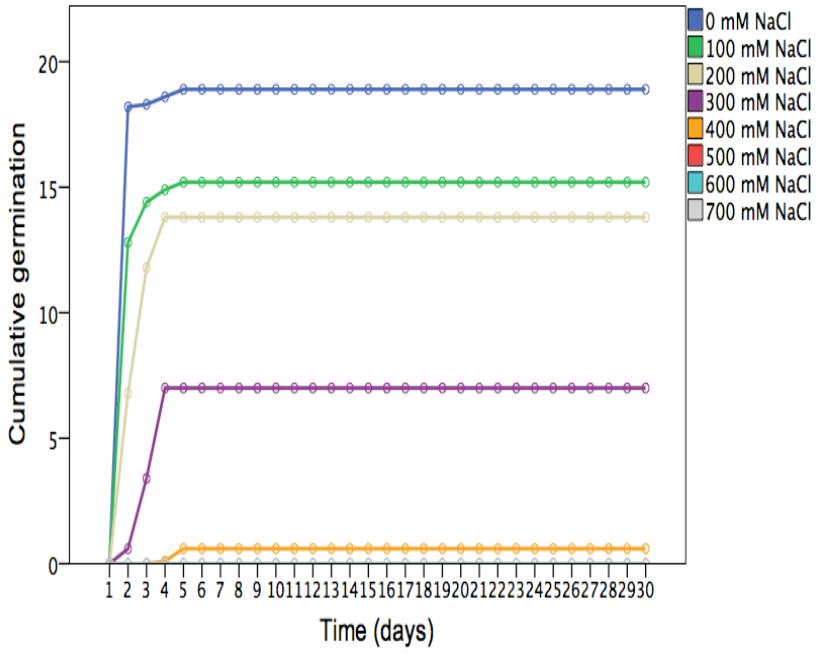


Figure 2.11. Mean cumulative number of *Pennisetum glaucum* seeds that germinated over time (10 replicates, each containing 20 seeds) in 0, 100, 200, 300, 400, 500, 600 and 700 mM of sodium chloride.

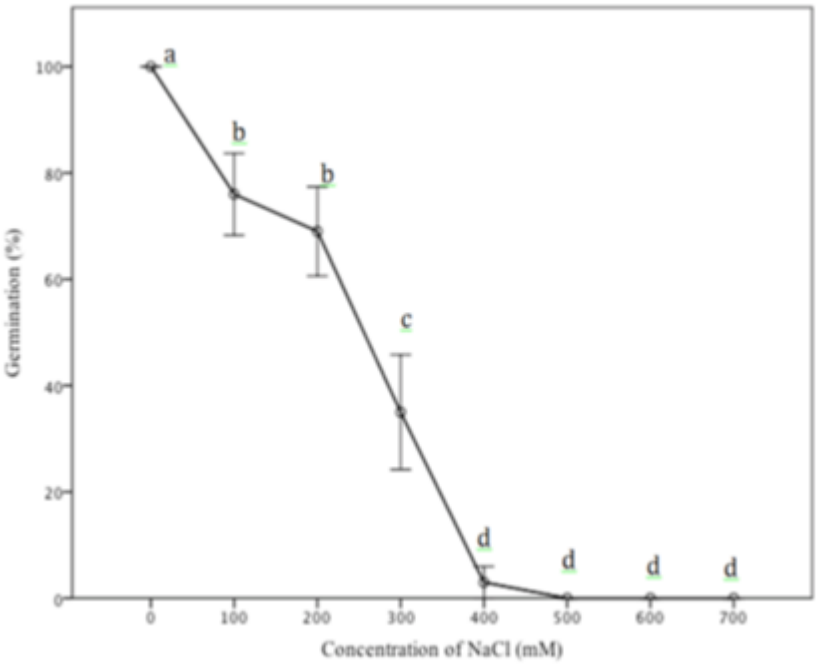


Figure 2.12. Mean (±SE) proportion (%) of *Pennisetum glaucum* seeds that germinated in different concentrations of sodium chloride. Differing letters denote significant differences ($P<0.001$).

Differing responses of species to salinity

Species differed significantly in their response to salinity treatments (significant interaction between concentration and species, $F=4.73$, $P<0.001$). *T. aestivum* and *H. vulgare* were able to germinate in higher concentrations compared to the other species. In contrast, whilst *Pennisetum glaucum* and *Panicum miliaceum* had slightly lower germination in the control (0 mM) compared to the other species, both had substantially lower germination rates that the other species in saline conditions (Table 2.2).

Table 2.2. Mean proportion (%) of seeds germinating of five Poaceae species under different concentrations of sodium chloride.

Plant species	Concentration of sodium chloride (mM)							
	0	100	200	300	400	500	600	700
T. aestivum	99.5	98.5	93.5	81	68	38	10	0
T. durum	96.5	94.5	89	77.5	38	6	0	0
H. vulgare	98.5	95	88.5	87.5	71	28	5.5	0
P. miliaceum	92	89	82	48.5	9	0	0	0
P. glaucum	94.5	76	69	35	3	0	0	0

Table 2.3. Time (days) taken for 50% of seeds of five Poaceae species to germinate (T_{50}) in differing concentration of sodium chloride (mM).

Species	Concentration of NaCl (mM)							
	0	100	200	300	400	500	600	700
T. aestivum	1	1	2	2	2	6	7	–
T. durum	1	1	2	3	4	7	8	–
H. vulgare	1	1	2	4	4	6	10	–
P. miliaceum	1	1	2	4	7	–	–	–
P. glaucum	1	2	4	6	6	–	–	–

2.2.4 Seed viability tests

There was little variation between species in the viability of seeds (Table 2.4), with germination rates being very high (≥95%).

Table 2.4. Seed viability (100 seeds), tested using tetrazolium (2,3,5-triphenyl-2H-tetrazolium chloride), for five Poaceae species collected in Al-Qassim, Saudi Arabia.

Species	Live seeds (%)	Dead seeds (%)
Triticum aestivum	98	2
Triticum durum	97	3
Hordeum vulgare	97	3
Panicum miliaceum	95	5
Pennisetum glaucum	96	4

2.3. Discussion

The germination responses to salinity of all five species showed considerable tolerance to high concentrations of NaCl other researchers have found what is very sensitive. All species, with the exception of *Pennisetum glaucum*, germinated equally well in 100 mM of NaCl as in distilled water. Furthermore, all species germinated at 400 mM and two species, *Hordeum vulgare and Triticum aestivum*, germinated in 600 mM solution, a concentration greater than full strength seawater. These cereal species are not normally regarded as halophytes but the behavior of these races suggested tolerance as high as that of many true halophytes of coastal salt marshes. For example, (Woodell 1985) found that several species considered true halophytes and frequently on intertidal salt marshes, such as *Triglochin maritimum* and *Plantago maritima*, failed to germinate in full strength seawater. Most halophytes normally germinate best in fresh water (Woodell 1985, Bakker et al., 1985), with increasing enforced dormancy and mortality as salinity increases (Pujol et al., 2000). *Triticum aestivum, T. durum* and *Hordeum vulgare* were particularly salt-tolerant and could be considered for more saline agricultural situations, as well as being especially suitable germplasms for breeding tolerance into future crop varieties.

The use of germination tests as the standard methods of assessing seed quality was highly effective and indicated clearly that the vitality of seeds of all plant species studied was high. The tetrazolium testing and germination studies showed that overall the seed samples were of high quality for germplasm banking.

None of the study species showed critical temperature, presumably as a result of previous domestication (See chapter 4) found in many wild species of adverse environments (Crocker and Barton 1953; Berger 1985). None of them should present problems for regeneration of plants from the stored seeds. However, replanting seed from good grain fields is a common practice on local farms. Nevertheless, cleaning seeds in the field from weed seed is needed, especially if the seed originated from another farm or from another region. Seed must be dried cautiously to 10 or 12% seed moisture content immediately following harvesting to ensure good later germination. At 50% atmospheric relative humidity, the equilibrium moisture content of wheat and rye seeds is about 12%, barley 11% and oats 10.5% (Navarro and Noyes 2010). The equilibrium moisture content of small grain seed that exposed to 70% relative humidity is approximately 15% and this remains too high for safe storage. At 90% atmospheric

relative humidity, the seed moisture content of several small grain crops augments to 20 - 23% Viability and vigor are lost rapidly under these conditions.

The most remarkable aspect of the findings of these experiments with these land races was the apparent salinity tolerance from seed to the establishment of seedlings. Germination of all species was high in relatively high salinities; although it is unknown how well these races would grow as adult plants in saline conditions, these results are positive and the effect on adult plants should be investigated as a priority. The performance at higher salinity of *Hordeum vulgare* and *Triticum aestivum* was particularly interesting. The germination result of *Pennisetum glaucum* in 100 mM of NaCl equally to distilled water was unexpected. Interestingly, growth of *Panicum miliaceum* in the salt was meaningful at 300 mM NaCl, which also was not predicted. Exposure to high concentration of salt might either begin the priming of seeds before germination or it might result in their death (Gulzar & Khan, 2002; Ungar, 1995). Furthermore, extreme salinity and destructive photoperiod together with thermoperiod that plant exposed too could inhibit seeds germination, strikingly in this land race *Triticum aestivum* have recovered from salt stress at 400, 500, 600, and even in 700 mM NaCl. (Rubio-Casal et al., 2002; Song et al., 2005) have stated that seeds of other specise could germinate when the environment becomes favorable; this kind of reaction has been linked to the need to take advantage of the periods with suitable conditions for establishment (Neo and Zedler 2000). Seeds that have very good germination, for example *Hordeum vulgare* and *Triticum aestivum*, could have very good yield giving the consequences that those that germinated better, may produce better food plants. From point of view of agronomic perspective, the improvement of germination (e.g. by 7-13%) might be significant for farmers or horticulturists in improving yield and reducing the costs of production (Panagopoulos and Margaritis, 2010). This project has successfully identified appropriate local, salt tolerant populations of these species in rural farms of the Al-Qassim region. Importantly, this project has persuaded local farmers to allow collection of part of their fiercely guarded heritage, sometimes with difficulty. Bulk collections of seed have been made in a fashion that would be expected to represent the genetic diversity within these land races of cereal species. This will need to be confirmed, preferably by molecular genetic analyses, in future development and use of the germplasm bank. The preparation and deposition of voucher herbarium specimens for each type should provide an invaluable archive of the material and will

allow cross-referencing with the morphological and taxonomic characteristics of future collections of the same species.

The most important outcome of this work, is that the fully documented seed collections made have now been dried down to less than 8% moisture content and placed in long-term storage at -20 °C, and have therefore been secured for the benefit of future research and exploitation. The validity of the methodology and the utility of the germplasm-banking resources established in the Kingdom of Saudi Arabia have therefore been established. However, bulk of seeds also has been stored at Millennium Seedbank, Wakehurst Place (Royal Botanic Gardens, Kew).

Chapter 3. Efficacy of seed germination testing in response to temperature and salinity

3.1 Introduction

Seed germination and initial plant development are multifaceted processes reliant on interactions between soil temperature and soil moisture, together with photoperiod (Montieth 1981), among other factors. Temperature is among the most important factors for germination: germination rate increases with higher temperatures, until a threshold or optimal temperature is exceeded, beyond which germination rate is reduced (Montieth 1981). Thus different cultivars may have different base temperatures, below which no biological progress will occur (Montieth 1984); similarly they will vary in their optimal and ceiling temperatures. However, not only the amount of germination but also the speed of germination can be affected by temperature. Hence, the length of time taken for germination differs between plant biotypes or cultivars (Weaver and Thomas 1986; Eagles 1988) and variations in the responses to temperature over the base temperature occur between varieties (Mann et al., 1985). Experiments have established the base temperatures for a wide range of species by providing optimum moisture conditions and photoperiod (e.g. Bierhuizen and Wagenvoort 1974; Del Pozo et al., 1987; Roche et al., 1997a,b). As germination is initiated after an imbibed seed is subjected to temperatures over the base temperature (Montieth 1981), base and other cardinal temperatures have been frequently determined through the assessment of germination rates over a range of temperatures (Montieth 1981; Garcia-Huidobro et al., 1982; Del Pozo et al., 1987). Even when seeds are able to germinate, there may be variations in the vigour and health of the resulting seedlings as a result of environmental conditions during the germination process and this might be expected to be reflected in the dry mass of seedlings shortly after germination.

Surprisingly little is known about how other environmental factors may affect the basal, optimal and ceiling temperatures for germination, or the subsequent vigour of seedlings, but it has been shown that the sensitivity of seeds to salinity may interact with the ability to germinate at extreme temperatures (Warner et al., 2000). In the particular context of cereal land-races from the hot, arid areas of Saudi Arabia, it would be of considerable interest to know whether salinity interacts with temperature to influence agronomic outcomes.

The identification of crop biotypes that differ in their base temperature, time of germination and growth reaction to temperature species, may allow the development of crop varieties that grow quicker at extremes of temperature, increasing food production. Breeding for cultivars that have high germination rates at low temperatures has been effective for beans (Dickson 1971), tomatoes (Cannon et al., 1973; De Vos et al., 1982; Scott and Jones 1985), maize (Eagles 1988) and cotton (Marani and Dag 1962). Temperature reduction comparison experiments have been conducted for maize (Hodges et al., 1994, 1995) and soybean (Bramlage et al., 1979).

The objectives of the research described in this chapter were: (1) to establish a protocol to investigate germination responses to temperature generally and, in particular, determine basal, optimal and ceiling temperatures for germination for these economically important Saudi Arabian cereal land-races; (2) to make broad preliminary comparisons of the effects of temperature, salinity and their interaction across a wide range of species and varieties of some of them.

3.2 Methods

These experiments were conducted at the Millennium Seedbank, Wakehurst Place (Royal Botanic Gardens, Kew). One land-race of each of five cereal species from the Al-Qassim region of Saudi Arabia (see Chapter 2) were investigated: *Triticum aestivum*, *Triticum durum*, *Hordeum vulgare*, *Panicum miliaceum* and *Pennisetum glaucum*. In addition, one commercial variety of winter wheat, *Triticum aestivum* (Istabraq), was used for comparison. Germination was tested at a wide range of constant temperatures and salinities using two complementary approaches; a thermogradient plate with a high resolution for temperature but limited capacity for species/treatment combinations, and a series of incubators with greater capacity but offering a more limited number of temperature treatments.

3.2.1 Thermogradient plate experiment

This experiment focused on wheat and barley species: *Triticum aestivum*, *T. aestivum* (Istabraq), *Triticum durum* and *Hordeum vulgare.* Twenty seeds were sown onto the surface of germination paper in Petri dishes (50 mm). Petri dishes were watered with one of four salinity treatments, 0 mM, 250 mM, 500 nM and 1000 mM of sodium

chloride. Three replicate petri dishes per species and salinity treatment were subjected to one of 13 constant temperatures across the thermogradient plate (Grant Instruments, Cambridge, UK, testing 1191672 GRD1) ranging from approximately 6 to 33 °C with a 12 h photoperiod (white light with photon flux density of 50 W m^{-2}). The temperature of a Petri dish at each position was measured using temperature probes and a Grant temperature logger set to record at each position on the gradient every 10 minutes for the duration of study (Figure 3.1). These measurements were used to define the precise treatment temperatures. Germination was scored every two hours from 08:00 to 19:00 daily only to see if there is any new seeds will germinate for a period of at least three weeks until no further germination was observed. Germination was defined as radicle emergence of 2 mm daily. As a measure of vigour, two germinated seeds were collected randomly from each Petri dish three days after germination and the seedlings separated into two roots and shoots, before drying and weighing to obtain dry mass.

3.2.2 Incubator experiments

All five Saudi Arabian cereals (*Triticum aestivum*, *Triticum durum*, *Hordeum vulgare*, *Panicum miliaceum* and *Pennisetum glaucum*) were examined. Incubators were set at five constant temperature regimes (10, 15, 20, 25, 30, 35 °C). The choice of temperature range was based on the climatic information recorded in the study area, which were taken from Meteorological Administration in Al-Qassim (see Chapter 4). Seeds were placed on 9 cm filter papers (Whatman No. 1) and 7 ml of one of four salinity treatments, distilled water (0 mM NaCl), 250 mM, 500 mM and 1000 mM of NaCl, added per dish, which was replaced every two days. For each species three replicate Petri dishes each containing 20 seeds was used in each salinity and temperature combination. Germinated seeds (with emerged radicles) were counted in every dish every two hours from 08:00 to 19:00, and any germinated seeds immediately removed after three days. This was continued for a period of 17 days. As previously described, seedlings were sampled, dried and weighed.

3.2.3 Analysis

Germination percentage was transformed to arcsin and then analysed using linear models that included temperature, salinity, species and the interaction between temperature and salinity, and a polynomial term that allowed for non-linear relationships (R Core Team 2012).

The time taken for the viable seeds to germinate was represented by the time to 50% germination (t_{50}), which was derived from the cumulative germination curves over time. Basal, optimal and ceiling temperatures were derived from relationships of $^{1}/_{t_{50}}$ against temperature, using the Kew Millennium Seedbank standard technique of fitting intersecting linear regressions; the data points were then divided into sub- and supra-optimal ranges and linear regressions fitted to the ascending and descending ranges.

Let x = temperature and y = $^{1}/_{t_{50}}$

For the sub-optimal range: $y = a + bx$

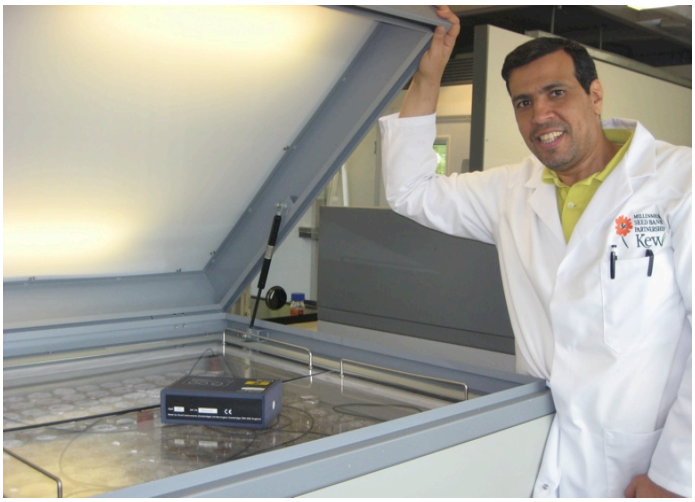
When y = 0, x = basal temperature $= -a/b$

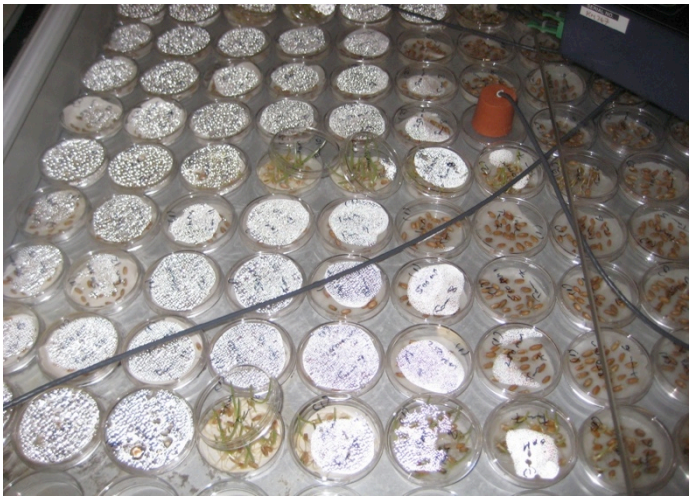
For the supra-optimal range: $y = c + dx$

When y = 0, x = ceiling temperature $= -c/d$

At the intersection temperature: $a + bx = c + dx$

Therefore optimal temperature $= (a - c) / (d - b)$





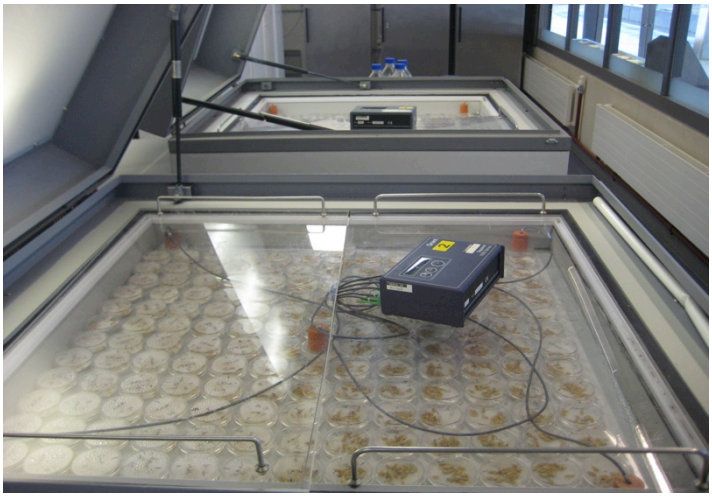


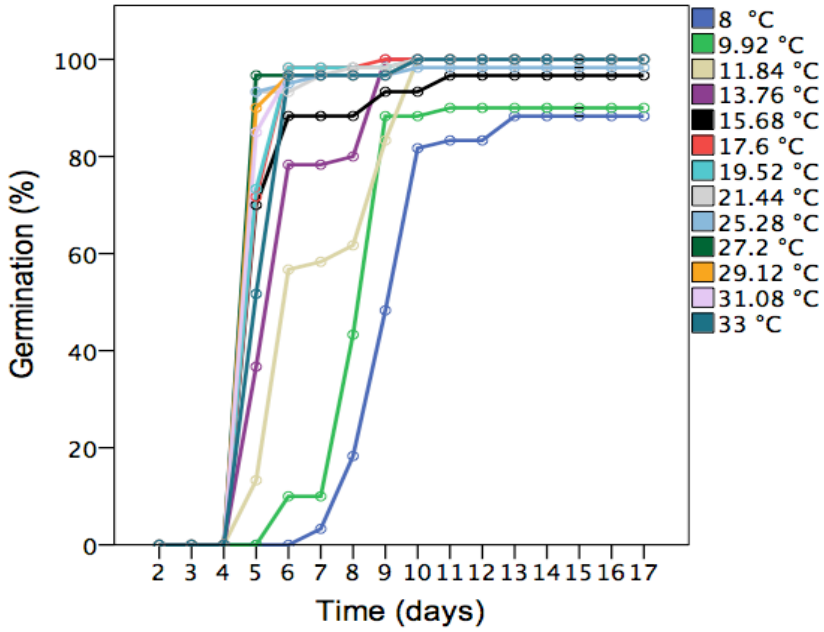
Figure 3.2. Three photographs showing the thermogradient plate, temperature logger and arrangement of the Petri dishes containing seeds.

3.3 Results

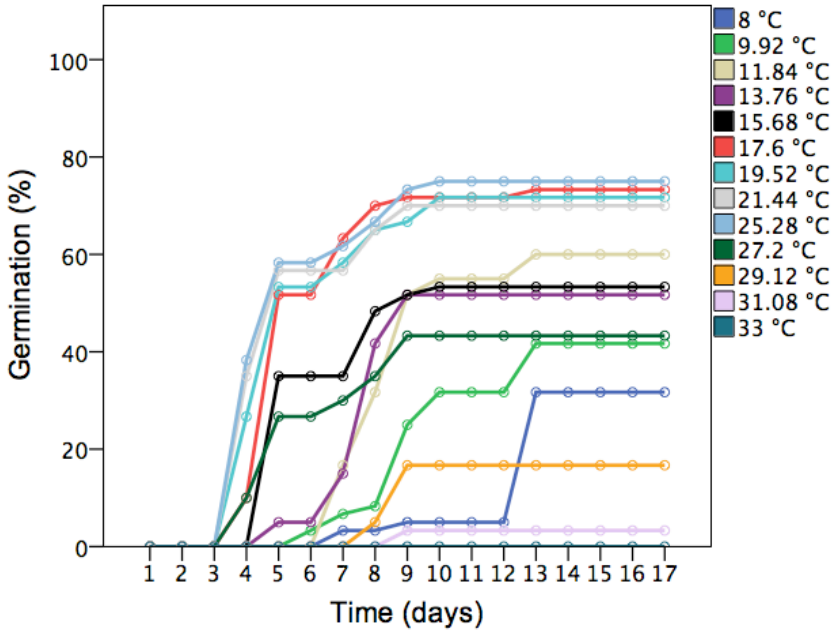
3.3.1 Thermogradient plate germination experiment

The cumulative germination curves at all the different combinations of temperature and salinity are shown for *Triticum aestivum*, *T. aestivum* (Istabraq), *Triticum durum* and *Hordeum vulgare* in Figs 3.2, 3.3, 3.4 and 3.5 respectively. In all cases these followed the inverse sigmoidal pattern expected. No germination occurred in any species/variety at 1000 mM Na Cl.

a)



b)



c)

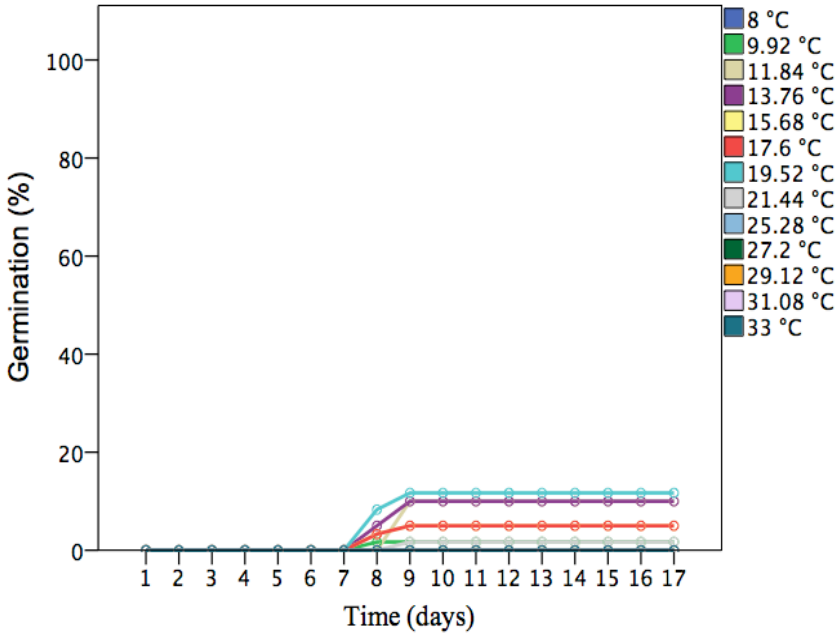
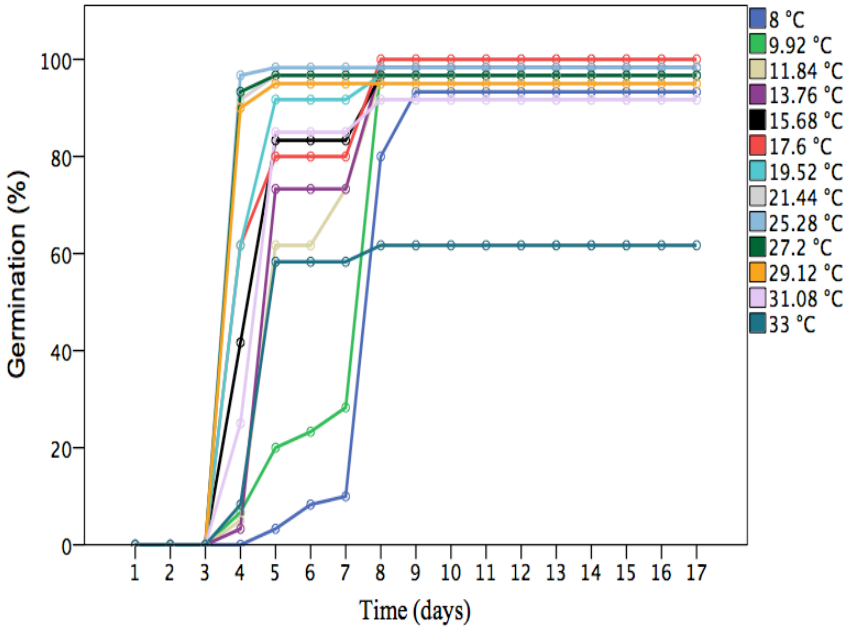


Figure 3.2. Time course of cumulative germination for *Triticum aestivum* (Qassim) at (a) 0 mM, (b) 250 mM, and (c) 500 mM NaCl on the thermogradient plate.

a)



b)

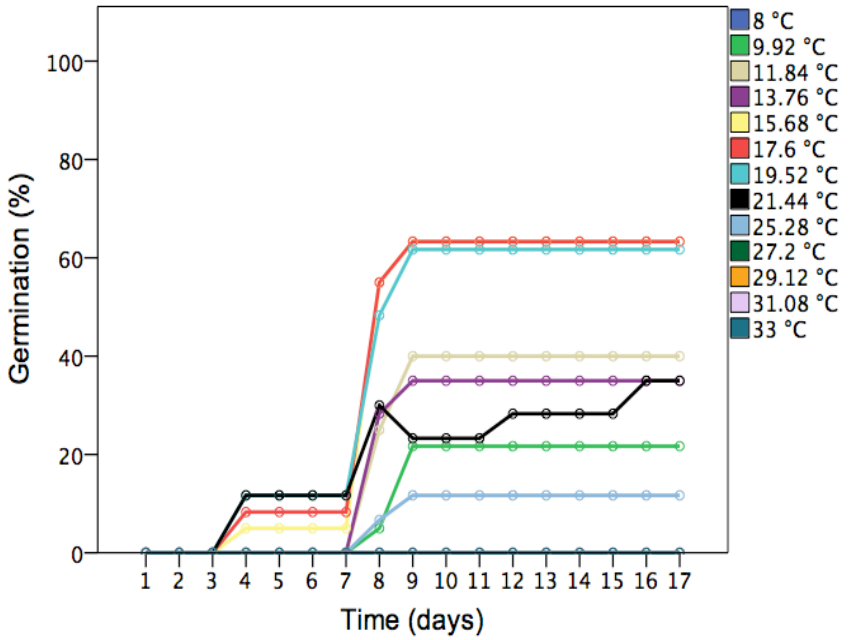
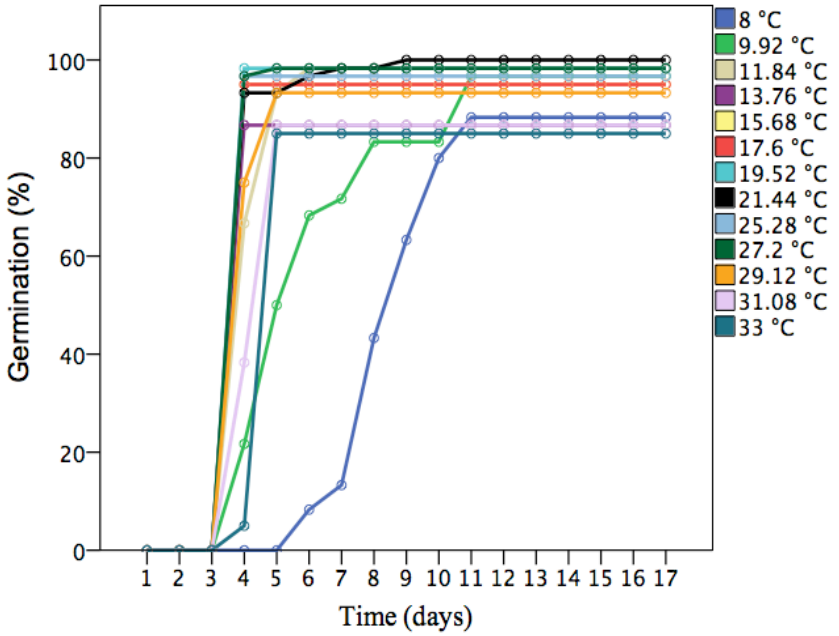


Figure 3.3. Time course of cumulative germination for *Triticum aestivum* (Istabraq) at (a) 0 mM, (b) 250 mM NaCl on the thermogradient plate.

a)



b)

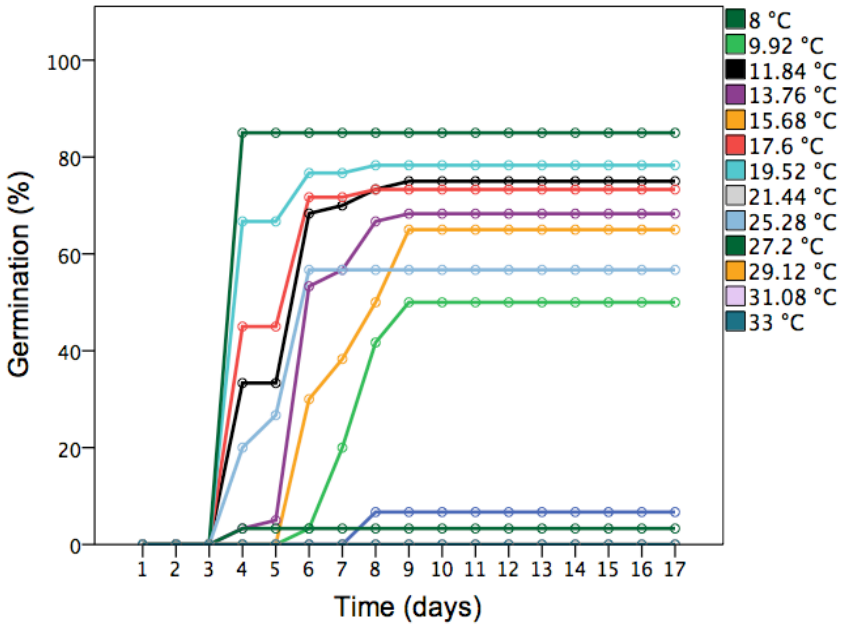
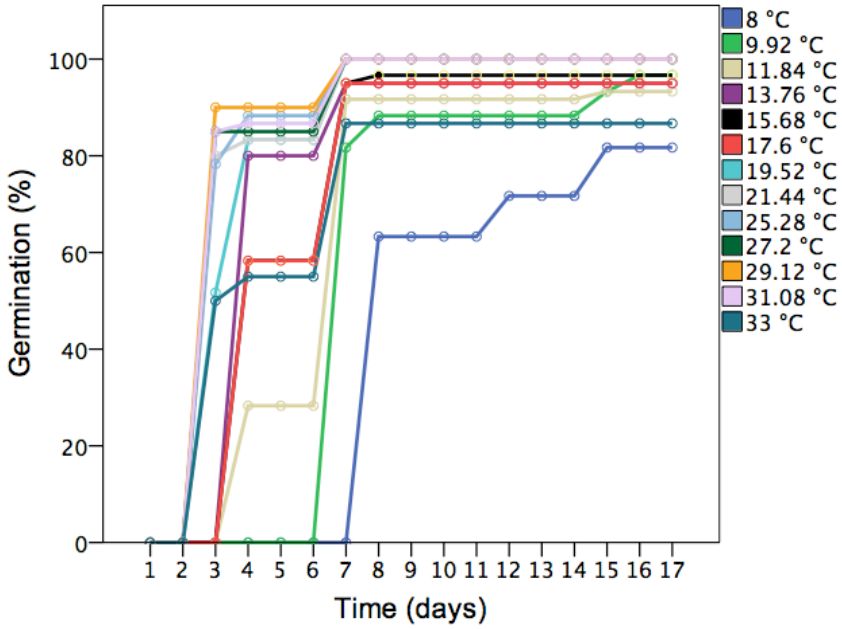
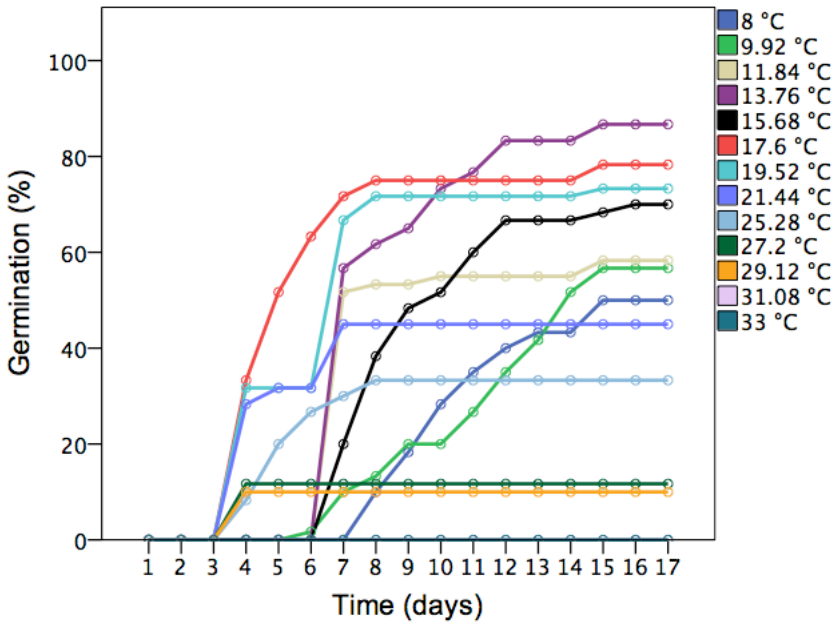


Figure 3.4. Time course of cumulative germination for *Triticum durum* at (a) 0 mM, (b) 250 mM NaCl on the thermogradient plate.

a)


Germination (%)
0
20
40
60
80
100
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Time (days)
8 °C
9.92 °C
11.84 °C
13.76 °C
15.68 °C
17.6 °C
19.52 °C
21.44 °C
25.28 °C
27.2 °C
29.12 °C
31.08 °C
33 °C

b)


Germination (%)
0
20
40
60
80
100
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Time (days)
8 °C
9.92 °C
11.84 °C
13.76 °C
15.68 °C
17.6 °C
19.52 °C
21.44 °C
25.28 °C
27.2 °C
29.12 °C
31.08 °C
33 °C

c)

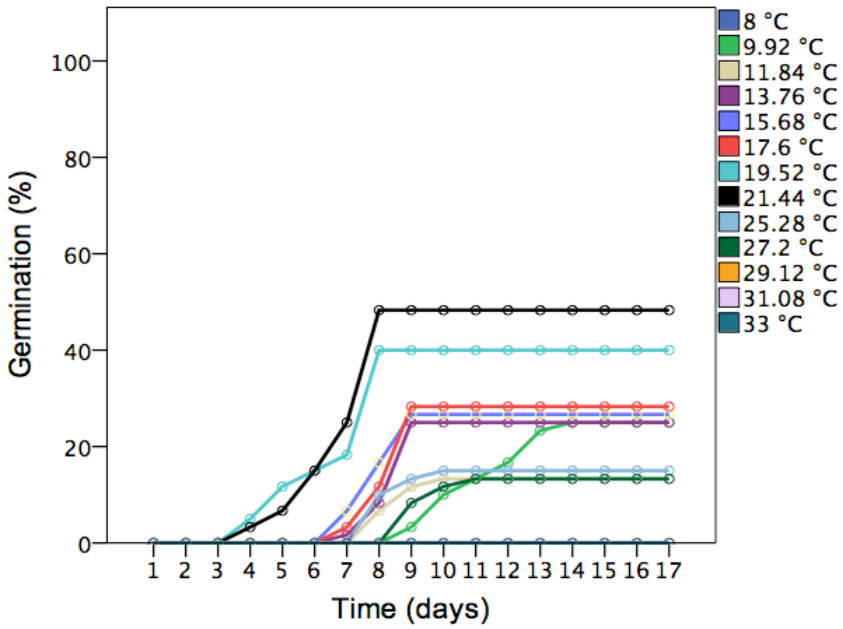
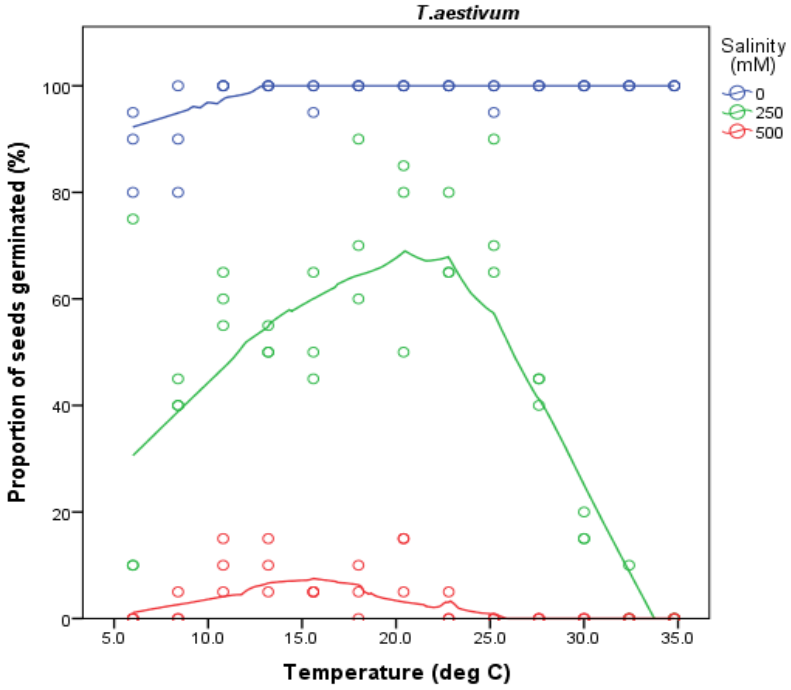


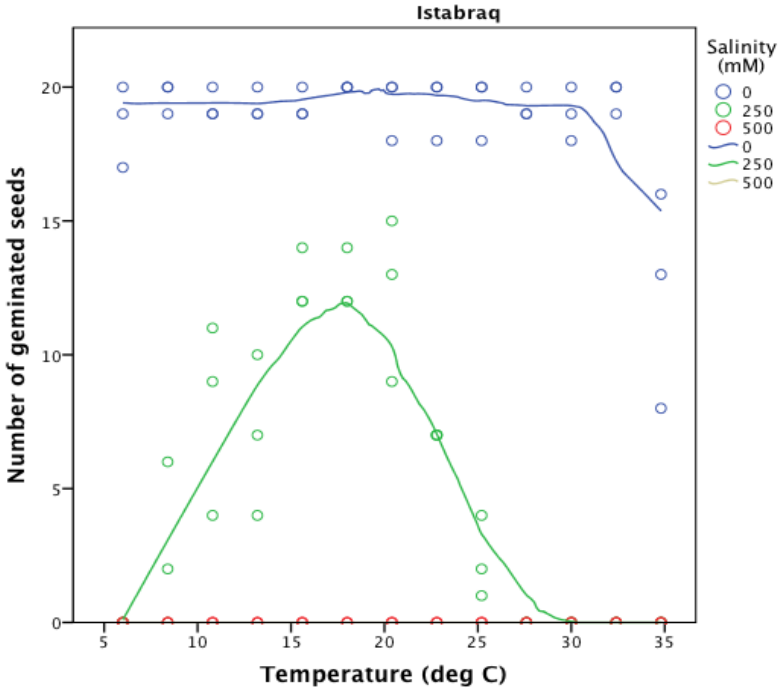
Figure 3.5. Time course of cumulative germination for *Hordeum vulgare* at (a) 0 mM, (b) 250 mM, and (c) 500 mM NaCl at (a) 0 (b) 250 (c) 500 (mM) NaCl on the thermogradient plate.

The final germination of these species in response to temperature and salinity is shown in Fig. 3.6.

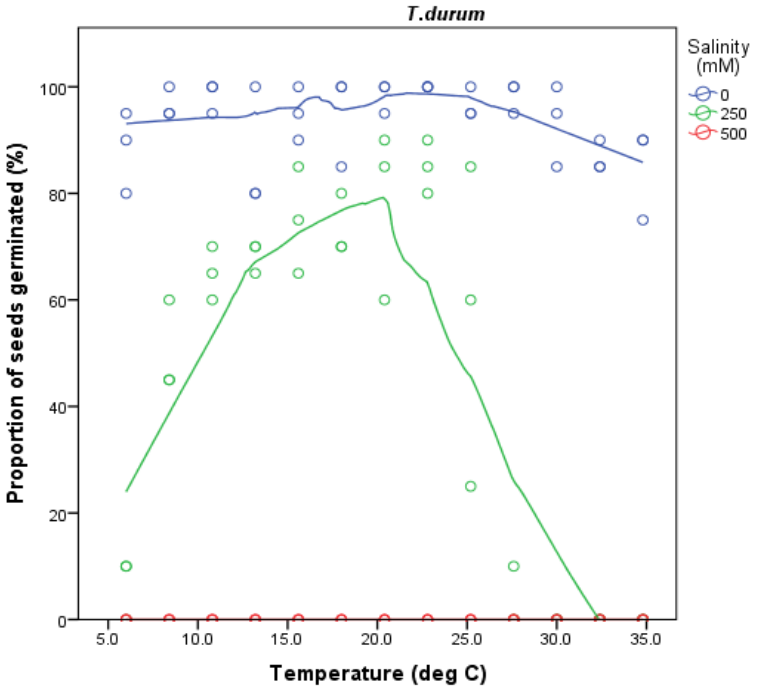
a)



b)



c)



d)

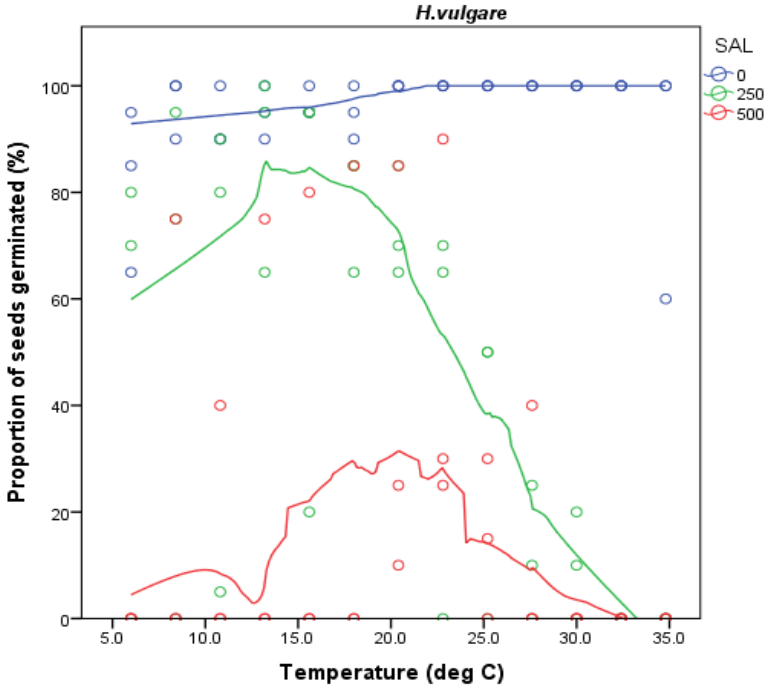


Figure 3.6. Response of final germination to temperature in (a) *Triticum aestivum* (Quassim), (b) *T. aestivum* (Istabraq), (c) *T. durum,* and (d) *H. vulgare* at salinities of 0, 250 and 500 mM NaCl on the thermogradient plate. Locally smoothed non-parametric regressions (LOESS) on temperature are shown.

In the absence of salinity, final germination was high and not much affected by temperature in any of the land-races or varieties of these species. At a salinity of 250 mM NaCl, the final germination was decreased and a temperature optimum of c. 18 – 23 °C became more evident in all species. There is a suggestion that the Istabraq variety of *Triticum aestivum* had a lower temperature optimum than the Qassim land race, and also than the Saudi land-races of the other two species. At 500 mM, germination was relatively low in *Triticum aestivum* (Qassim) and only slightly higher in *Hordeum vulgare*; there was no germination at all in *Triticum aestivum* (Istabraq) or *T. durum* at this salinity. General linear models showed that the effects of salinity, temperature and their interaction on germination were highly significant in all land-races or varieties of these species; in addition when all the land-races/varieties were considered together, they were also highly significantly different (Table 3.1).

Table 3.1. Summary of general linear models of the effects of salinity and temperature on arcsin-transformed germination percentage in the four species individually and all four combined, in the Thermogradient plate experiment. A polynomial term was included to allow for non-linear relationships.

Species	Effect	F	P
All combined	Species	13.68	<0.001
	Salinity	1238.80	<0.001
	Temperature	95.78	<0.001
	Temperature x salinity	44.03	<0.001
T. aestivum (Qassim)	Salinity	990.24	<0.001
	Temperature	51.28	<0.001
	Temperature x salinity	35.03	<0.001
T. aestivum (Istabraq)	Salinity	990.24	<0.001
	Temperature	35.23	<0.001
	Temperature x salinity	15.44	<0.001
T. durum	Salinity	619.85	<0.001
	Temperature	50.40	<0.001
	Temperature x salinity	38.43	<0.001
H. vulgare	Salinity	112.37	<0.001
	Temperature	18.03	<0.001
	Temperature x salinity	6.9	<0.001

Data for speed of germination, expressed as the reciprocal of the time to 50% germination $1/(t_{50})$, for this experiment are given in Table 3.2. These are generally more sensitive to temperature than final germination and can be used to derive the basal cardinal (basal, optimal and ceiling) temperatures (Table 3.3).

Table 3.2. Speed of germination, expressed as $^1/t_{50}$ at different temperatures for the four plant species at three concentrations of NaCl on the thermogradient plate.

Variety	Salinity NaCl (mM)	Temperature (°C)												
		8.0	9.9	11.8	13.8	15.7	17.6	19.5	21.4	25.3	27.2	29.1	31.1	33.0
T. aestivum (Qassim)	0	0.13	0.14	0.22	0.25	0.29	0.29	0.29	0.33	0.33	0.33	0.33	0.33	0.03
	250	0.11	0.09	0.10	0.09	0.09	0.20	0.20	0.22	0.25	0.07	0.08	0.07	0.00
	500	0.00	0.08	0.07	0.08	0.08	0.08	0.08	0.08	0.00	0.00	0.00	0.00	0.00
T. aestivum (Istabraq)	0	0.13	0.14	0.22	0.22	0.25	0.25	0.29	0.29	0.33	0.33	0.33	0.25	0.22
	250	0.00	0.08	0.08	0.08	0.13	0.13	0.13	0.08	0.08	0.00	0.00	0.00	0.00
	500	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T. durum	0	0.11	0.20	0.25	0.25	0.50	0.50	0.50	0.25	0.25	0.25	0.25	0.20	0.20
	250	0.00	0.11	0.13	0.17	0.17	0.17	0.25	0.25	0.17	0.33	0.00	0.00	0.00
	500	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
H. vulgare	0	0.13	0.14	0.14	0.25	0.25	0.25	0.33	0.33	0.33	0.33	0.33	0.33	0.03
	250	0.06	0.14	0.14	0.14	0.11	0.20	0.17	0.14	0.10	0.11	0.13	0.10	0.00
	500	0.00	0.14	0.15	0.14	0.13	0.07	0.11	0.13	0.13	0.13	0.00	0.00	0.00

Table 3.3. Estimates of basal (T_{min}), optimal (T_{opt}) and ceiling (T_{max}) temperatures for germination of four species/varieties at three salinities on the thermogradient plate, derived from pairs of linear regressions of $^1/t_{50}$ on temperature. The four regression parameters are described in the methods; non-significant regressions are not presented.

Species	Concentration of NaCl (mM)	Regression parameters				T_{min} (°C)	T_{opt} (°C)	T_{max} (°C)
		a	b	c	d			
T. aestivum (Qassim)	0	-0.0725	0.0237	0.6751	-0.0150	3.1	19.3	45.0
	250	-0.0125	0.0103	0.9444	-0.0347	1.2	21.3	27.2
	500	-0.0107	0.0051	0.5486	-0.0217	2.1	20.9	25.3
T. aestivum (Istabraq)	0	0.0769	0.0106	0.9651	-0.0235	-7.3	26.0	41.1
	250	-0.0444	0.0113			3.9		
	500							
T. durum	0	-0.1077	0.0344			3.1		
	250	-0.0377	0.0136	0.7821	-0.0252	2.8	21.1	31.0
	500							
H. vulgare	0	-0.0072	0.0157	0.6402	-0.0131	0.5	22.5	48.9
	250	-0.0138	0.0126	0.3375	-0.0087	1.1	16.5	38.8
	500	0.0469	0.0047	0.4137	-0.0137	-10.0	19.9	30.2

This technique depends to an extent on the goodness of fit of the pairs of regression lines. Because of the temperature range used, there were generally more points and therefore better fits in the sub-optimal ranges than in the supra-optinal ranges. Hence it

was possible to produce estimates of basal temperature for more land-race/salinity combinations than estimates of optimal and ceiling temperatures. The most complete data were for the Qassim race of *T. aestivum*, which showed evidence of a narrowing of its temperature range with increasing salinity. There was little evidence of systematic effects in the other races/species.

The dry masses of seedlings three days after germination in this experiment are given in Tables 3.4 and 3.5 for the 0 and 250 mM NaCl treatments, respectively. In the absence of salt, there were marked variations in seedling mass, reflecting better vigour in seedlings that had germinated at intermediate temperatures (20-21 °C) than at the extremes (Fig. 3.4). Seedlings that had germinated in 250 mM NaCl were all very much smaller indicating greatly reduced vigour as a result of salt stress; however the greatest mass for each race was seen at the slightly lower temperature of 19.5 °C (Fig. 3.5).

Table 3.4. The dry mass of seedlings of four plant races/species (mg) at an NaCl concentration of 0 mM three days after germination on the thermogradient plate.

Temperature °C	*T. aestivum*		*T. aestivum* Istabraq		*T. durum*		*H. vulgare*	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
8.0	18.1	22.3	2.0	19.4	12.3	22.3	20.0	22.4
9.9	23.2	25.6	11.5	26.7	32.3	24.3	12.4	35.7
11.8	20.4	23.2	13.2	37.6	15.3	24.0	19.8	44.4
13.8	18.6	31.0	11.5	34.0	13.5	37.9	22.6	43.8
15.7	37.2	34.5	20.2	27.8	16.2	37.9	41.6	54.7
17.6	46.4	33.3	25.7	31.5	35.4	41.3	48.4	63.1
19.5	51.3	22.2	33.2	20.1	40.8	30.2	57.3	56.3
21.4	53.2	15.7	31.6	23.9	42.2	15.5	66.2	36.4
25.3	18.1	15.8	19.2	5.0	15.1	17.5	23.1	23.9
27.2	16.0	9.1	5.6	7.5	14.7	10.9	16.0	22.6
29.1	25.4	7.5	3.2	4.3	14.3	11.8	11.4	20.1
31.1	19.4	14.7	2.0	3.5	13.7	18.1	13.4	18.4
33.0	24.8	7.3	2.2	0.7	9.9	3.0	9.5	4.0

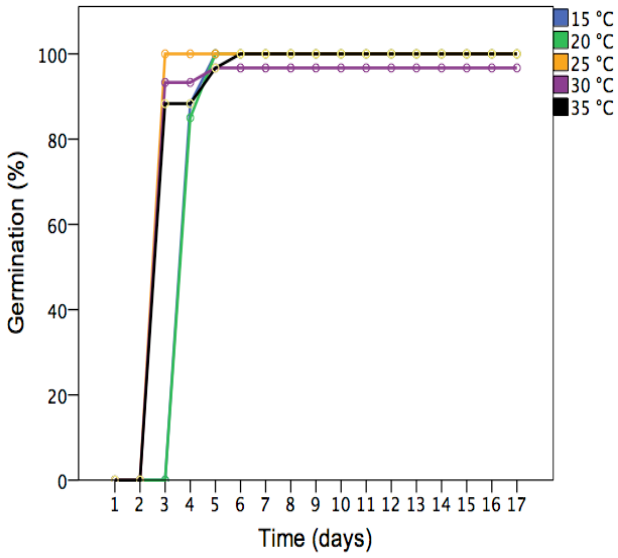
Table 3.5. The dry mass of seedlings of four plant races/species (mg) at an NaCl concentration of 250 mM three days after germination on the thermogradient plate.

Temperature ^{o}C	*T. aestivum*		*T. aestivum* Istabraq		*T. durum*		*H. vulgare*	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
8.0	8.0	0.0	0.0	0.0	0.0	0.0	0.0	8.0
9.9	9.9	0.0	0.0	0.0	0.0	0.0	0.0	10.4
11.8	11.8	0.0	0.0	0.0	18.8	0.0	17.9	14.7
13.8	13.8	5.7	20.8	3.7	32.1	3.7	34.2	11.6
15.7	15.7	5.9	33.9	3.8	10.9	5.0	8.1	17.8
17.6	17.6	3.8	14.0	4.8	43.3	1.8	34.6	32.1
19.5	19.5	8.7	36.2	5.9	13.7	5.2	14.2	39.6
21.4	21.4	3.6	14.7	4.6	11.3	3.6	7.0	38.8
25.3	25.3	2.0	10.6	3.0	5.6	1.9	5.2	18.1
27.2	27.2	2.0	8.9	2.8	9.7	2.0	4.7	15.1
29.1	29.1	1.1	8.4	1.5	0.7	0.7	0.3	4.9
31.1	31.1	0.0	0.0	0.0	0.0	0.0	0.0	8.2
33.0	33.0	0.0	0.0	0.0	0.0	0.0	0.0	10.1

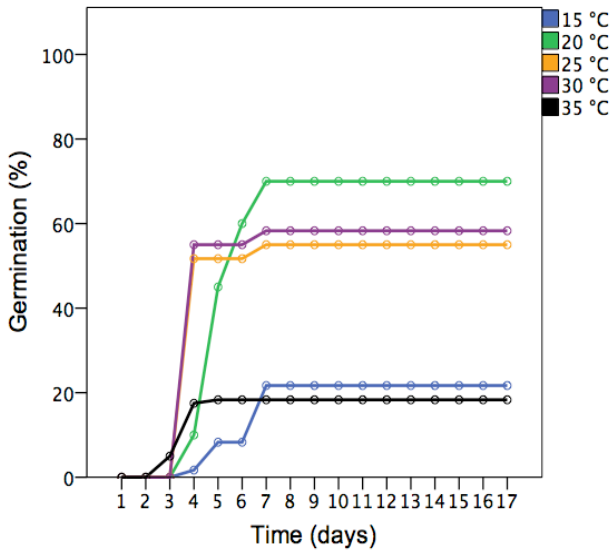
3.3.2 Incubator experiment

The cumulative germination curves at all the different combinations of temperature and salinity are shown for *Triticum aestivum*, *T. durum*, *Hordeum vulgare*, *Panicum miliaceum* and *Pennisetum glaucum* in Figs 3.7, 3.8, 3.9, 3.10 and 3.11 respectively. In all cases these followed the inverse sigmoidal pattern expected. No germination occurred in any species at 1000 mM Na Cl.

a)



b)



c)

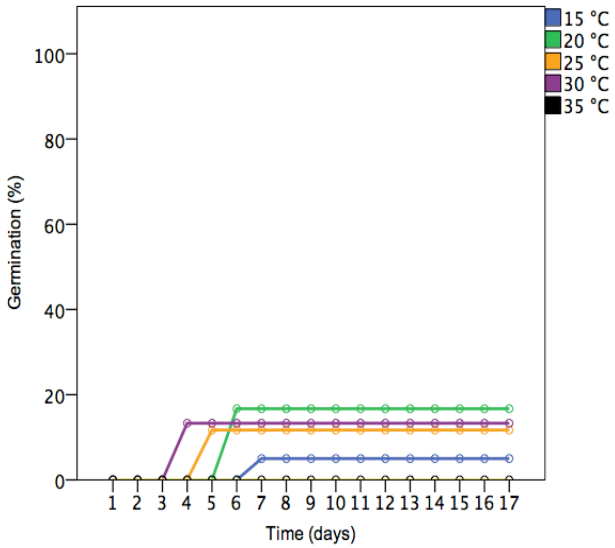
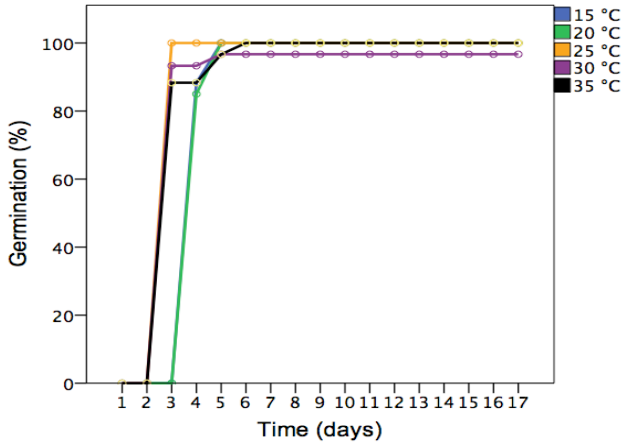
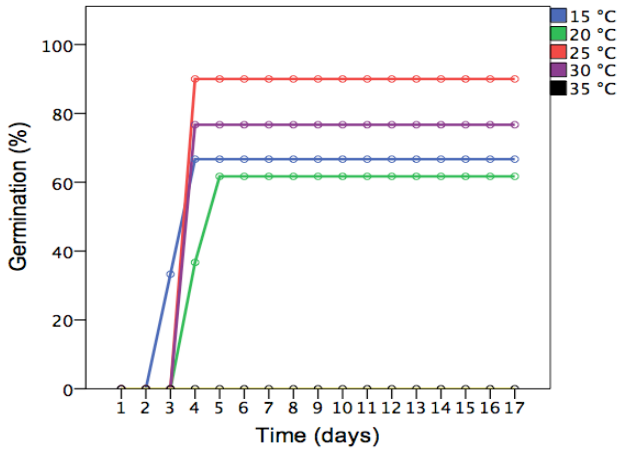


Figure 3.7. Time course of cumulative germination for *Triticum aestivum* (Qassim) at (a) 0 mM, (b) 250 mM, and (c) 500 mM NaCl in the incubator experiment.

a)



b)



c)

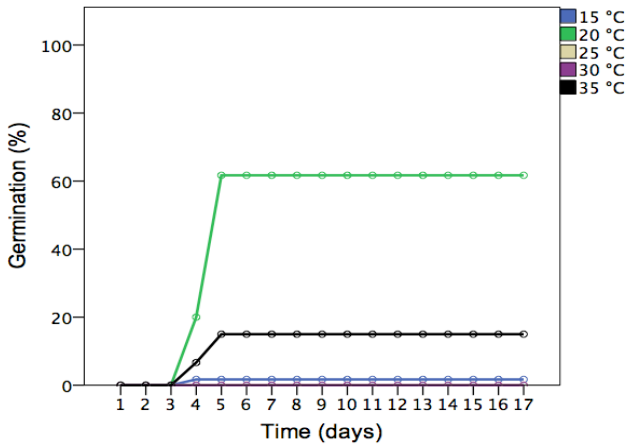
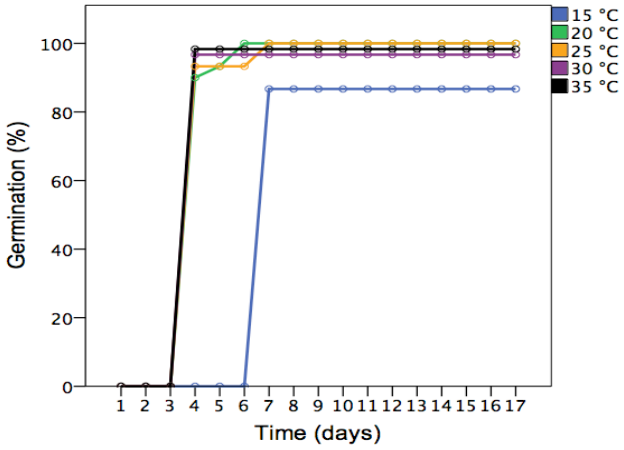
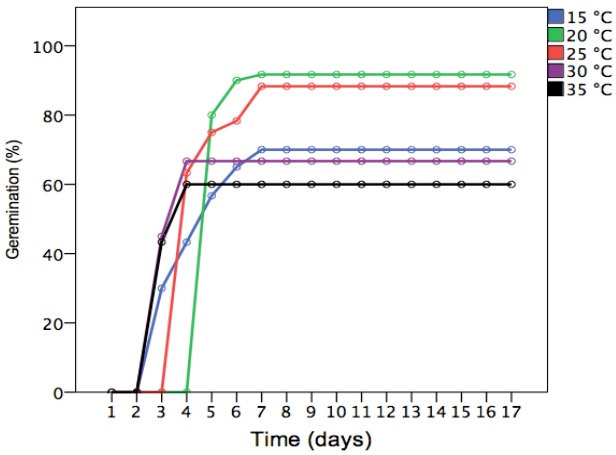


Figure 3.8. Time course of cumulative germination for *Triticum durum* at (a) 0 mM, (b) 250 mM, and (c) 500 mM NaCl in the incubator experiment.

a)



b)



c)

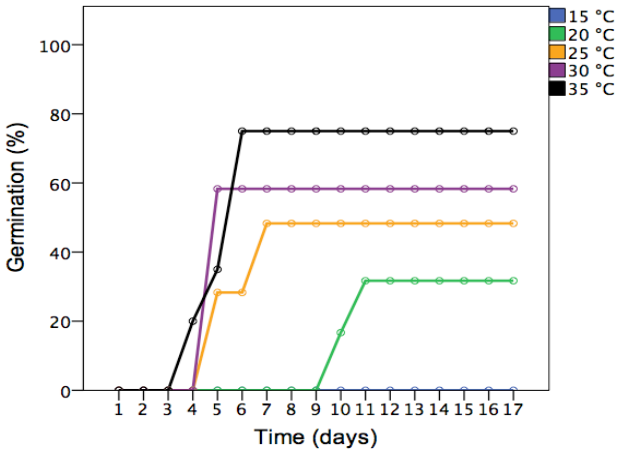
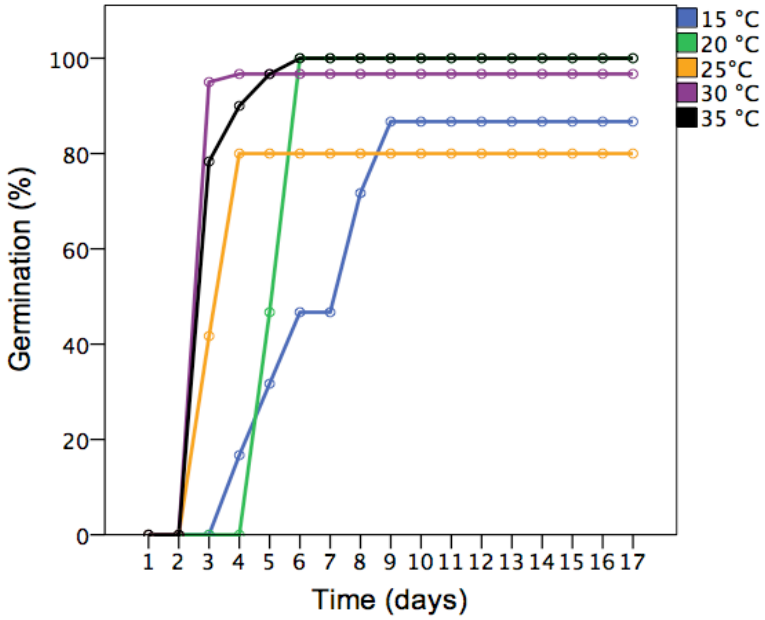


Figure 3.9. Time course of cumulative germination for *Hordeum vulgare* at (a) 0 mM, (b) 250 mM, and (c) 500 mM NaCl in the incubator experiment.

a)



b)

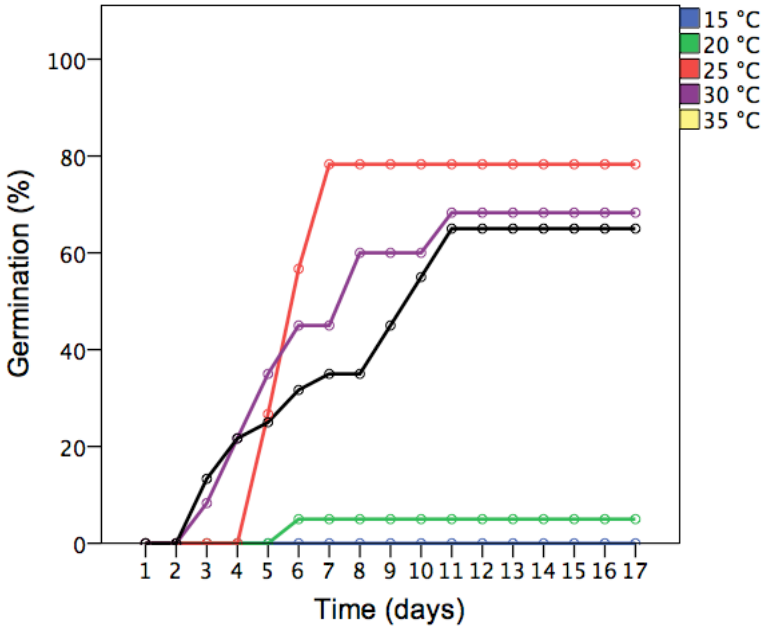
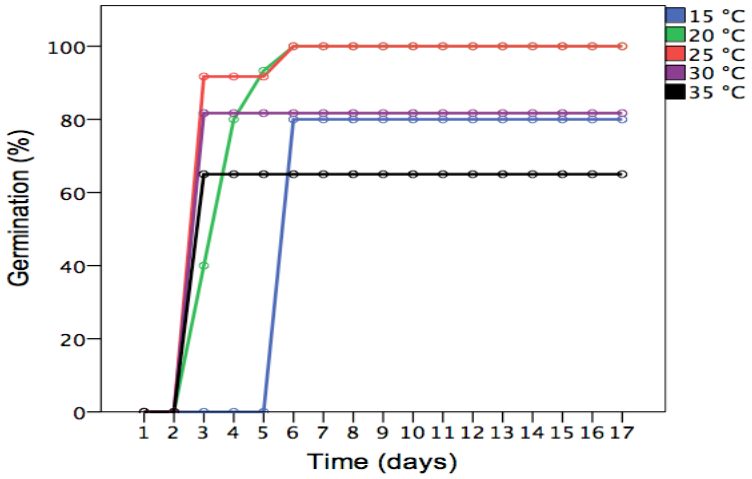
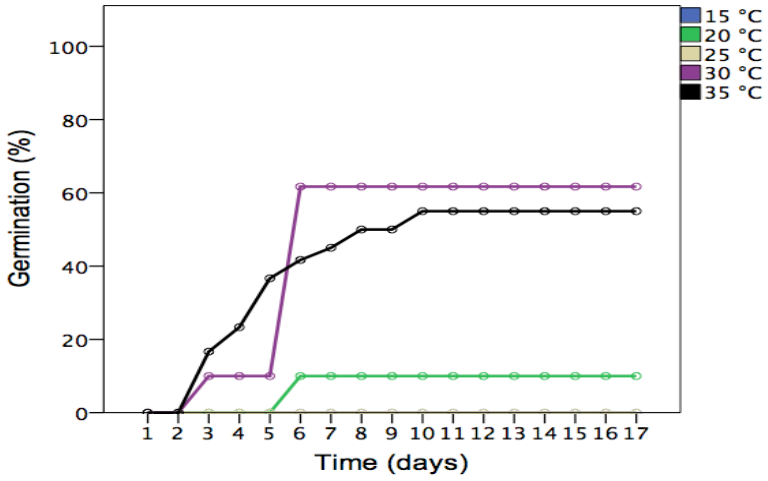


Figure 3.10. Time course of cumulative germination for *Panicum miliaceum* at (a) 0 mM, (b) 250 mM NaCl in the incubator experiment.

a)



b)



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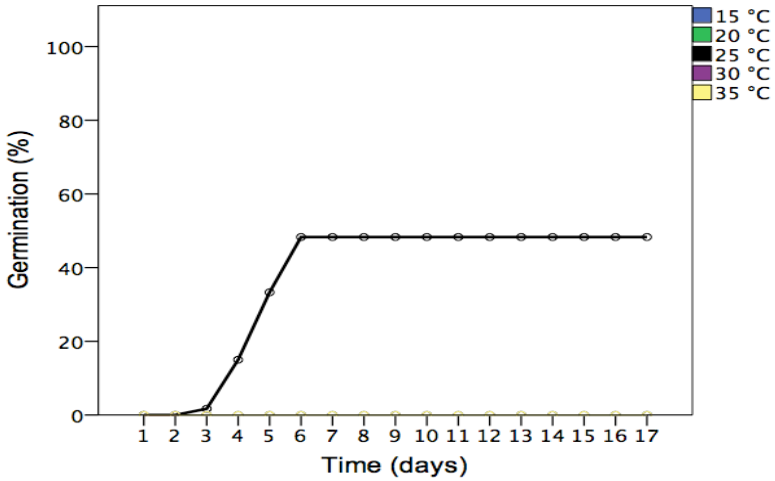
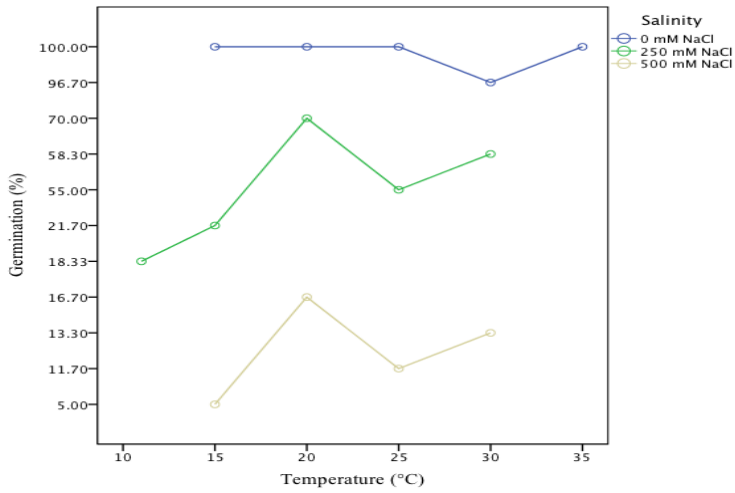


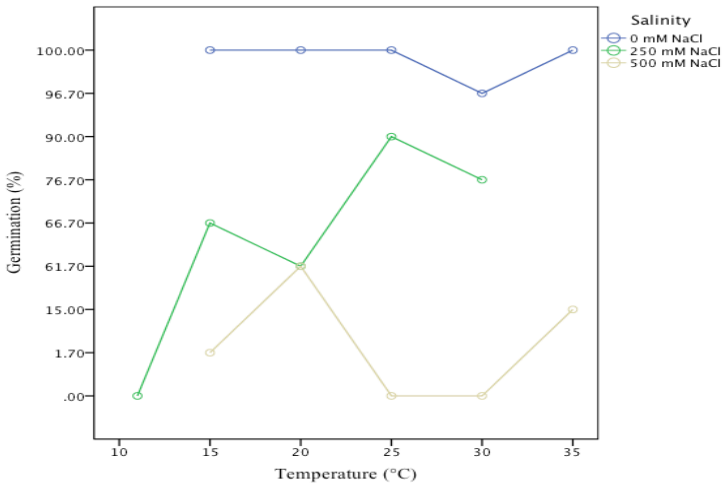
Figure 3.11. Time course of cumulative germination for *Pennisetum glaucum* at (a) 0 mM, (b) 250 mM, and (c) 500 mM NaCl in the incubator experiment.

The temperature response of final germination to salinity in the five species is summarized in Fig. 3.12. As in the thermogradient plate experiment, final germination was not very sensitive to temperature in the absence of salinity. *Triticum aestivum. T, durum* and *Panicum miliaceum* showed no obvious temperature optimum, whereas *Hordeum vulgare* showed somewhat reduced germination at low temperature, and *Pennisetum glaucum* displayed rather lower germination at both extremes. At 250 mM NaCl, however, final germination was distinctly lower in all species, and especially so at lower temperatures. These effects were further magnified at 500 mM NaCl, where germination was generally poor and rather erratic. *H. vulgare* showed a consistent trend towards higher germination with increasing temperature.

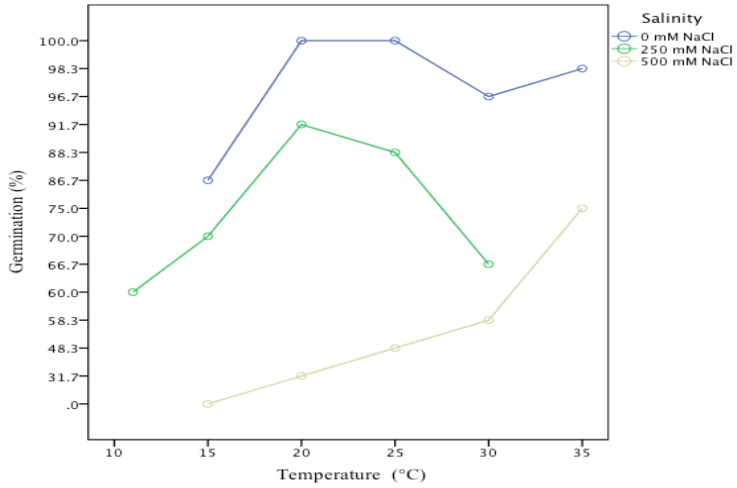
a)



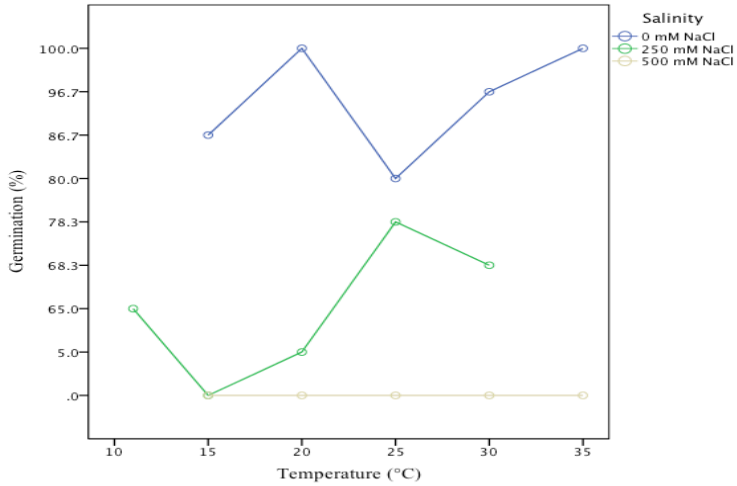
b)



c)



d)



e)

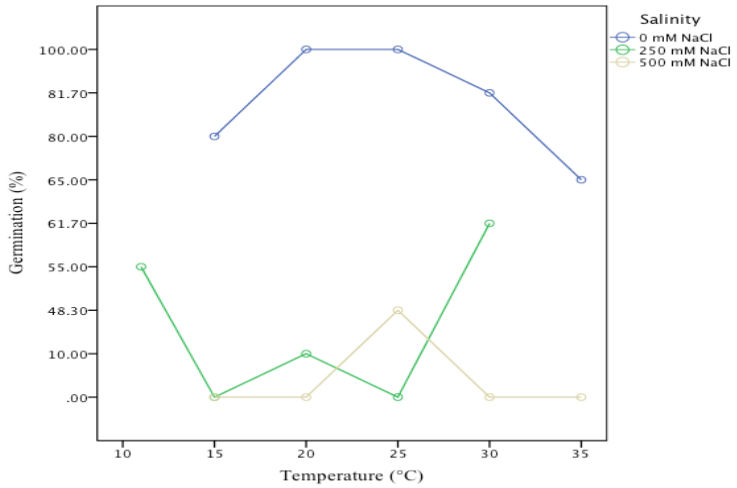


Figure 3.12. Response of final germination to temperature in (a) *Triticum aestivum* (Quassim), (b) *T. durum,* (c) *H. vulgare* (d) *P. miliaceum* and (e) *P. glaucum* at salinities of 0, 250 and 500 mM NaCl) in the incubator experiment.

General linear modelling showed that the effects of salinity and temperature on germination were highly significant in all of these species; the interaction between temperature and salinity was also highly significant in all species except *Triticum durum*. When all species were considered together, they were highly significantly different, and the overall temperature and salinity effects were also highly significant, but their interaction was not significant (Table 3.6).

Table 3.6. Summary of general linear models of the effects of salinity and temperature on arcsin-transformed germination percentage in the four species individually and all four combined, in the incubator experiment. A polynomial term was included to allow for non-linear relationships.

Species	Treatment	F	P
All combined	Species	15.96	<0.001
	Salinity	285.48	<0.001
	Temperature	12.53	<0.001
	Temperature x salinity	2.65	0.345
T. aestivum (Qassim)	Salinity	362.30	<0.001
	Temperature	17.06	<0.001
	Temperature x salinity	35.03	<0.001
T. durum	Salinity	73.54	<0.001
	Temperature	8.25	<0.001
	Temperature x salinity	4.32	0.006
H. vulgare	Salinity	122.92	<0.001
	Temperature	20.39	<0.001
	Temperature x salinity	22.44	<0.001
Panicum miliaceum	Salinity	150.18	<0.001
	Temperature	12.07	<0.001
	Temperature x salinity	9.72	<0.001
Pennisetum glaucum	Salinity	112.36	<0.001
	Temperature	6.68	<0.001
	Temperature x salinity	11.43	<0.001

Data for speed of germination, expressed as the reciprocal of the time to 50% germination $1(/t_{50})$, for this experiment are given in Table 3.7. Germination was generally rapid under these conditions. The trends are very similar in this case to final germination with not much variation over the temperature range 15 – 35 °C. However, the speed of germination was markedly reduced on average by increasing salinity in

every species, and this effect tended to be most pronounced at the lower temperatures (15-20 °C). *T. aestivum* showed a pronounce narrowing of its germination-speed temperature response with increasing salinity.

Table 3.7. Speed of germination, expressed as $^1/t_{50}$ at different temperatures for the four plant species at three concentrations of NaCl in the incubator experiment.

Species	NaCl concentration (mM)	Temperature (°C)				
		15	20	25	30	35
T. aestivum	0	0.33	0.33	0.33	0.25	0.33
	250	0.25	0.20	0.17	0.25	0.25
	500	0.00	0.17	0.25	0.25	0.00
T. durum	0	0.33	0.25	0.33	0.33	0.33
	250	0.25	0.20	0.25	0.25	0.00
	500	0.17	0.20	0.00	0.00	0.25
H. vulgare	0	0.14	0.25	0.25	0.25	0.25
	250	0.20	0.20	0.25	0.25	0.25
	500	0.10	0.17	0.14	0.20	0.17
P. miliaceum	0	0.13	0.20	0.25	0.33	0.33
	250	0.00	0.10	0.17	0.14	0.11
	500	0.00	0.00	0.00	0.00	0.00
P. glaucum	0	0.17	0.25	0.33	0.33	0.33
	250	0.00	0.17	0.00	0.17	0.13
	500	0.00	0.00	0.20	0.00	0.00

The dry masses of seedlings three days after germination in this experiment are given in Tables 3.8, 3.9 and 3.10 for the 0, 250 and 500 mM NaCl treatments, respectively. In the absence of salt, the greatest vigour of seedlings appeared to be at 15-20 °C, although results for *T. aestivum* and *H. vulgare* were more erratic (Fig. 3.8). Seedlings that had germinated in 250 mM or 500 NaCl showed similar trends with temperature but were progressively smaller with increasingly salinity indicating greatly reduced vigour as a result of salt stress.

Table 3.8. The dry mass of seedlings of four plant races/species (mg) at an NaCl concentration of 0 mM three days after germination in the incubator experiment.

Temperature °C	*T. aestivum*		*T. durum*		*H. vulgare*		*P. glaucum*		*P. miliaceum*	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
10	21.2	6.7	7.8	23.5	20.1	26.5	3.9	24.8	1.9	22.4
15	26.3	8.8	27.3	38.8	43.9	21.0	8.4	34.7	2.1	29.7
20	22.6	6.6	18.4	29.1	17.7	38.1	4.0	27.5	1.6	26.9
25	17.9	6.6	10.5	35.3	11.5	46.6	4.5	29.1	3.1	25.1
30	38.2	13.2	15.2	4.9	40.4	37.4	1.5	0.6	0.7	0.3
35	-	-	-	-	-	-	-	-	-	-

Table 3.9. The dry mass of seedlings of four plant races/species (mg) at an NaCl concentration of 250 mM three days after germination in the incubator experiment.

Temperature °C	*T. aestivum*		*T. durum*		*H. vulgare*		*P. glaucum*		*P. miliaceum*	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
10	13.8	4.2	7.6	12.7	7.2	22.8	4.0	23.6	2.0	4.5
15	11.1	3.8	8.2	15.5	5.3	24.3	2.7	30.4	3.0	19.0
20	8.5	2.6	17.8	21.8	31.6	14.7	3.2	33.5	3.3	30.8
25	5.3	1.6	5.6	20.5	9.6	27.5	7.5	15.9	2.5	20.3
30	4.3	1.5	3.6	27.4	19.0	16.3	4.9	1.8	1.9	11.0
35	-	-	-	-	-	-	-	-	-	-

Table 3.10. The dry mass of seedlings of four plant races/species (mg) at an NaCl concentration of 500 mM three days after germination in the incubator experiment.

Temperature °C	*T. aestivum*		*T. durum*		*H. vulgare*		*P. glaucum*		*P. miliaceum*	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
10	3.6	1.1	4.5	16.1	3.2	13.3	-	-	-	-
15	5.0	1.5	4.0	11.6	2.6	15.9	-	-	-	-
20	6.8	2.0	5.5	20.4	6.9	26.6	-	-	-	-
25	2.5	0.7	2.9	20.3	7.6	15.1	-	-	-	-
30	1.8	0.6	2.2	15.0	5.4	8.2	-	-	-	-
35	-	-	-	-	-	-	-	-	-	-

3.5 Discussion

The fine resolution of temperature provided by the thermogradient plate allowed both an evaluation of the method for examining basal, optimal and ceiling temperatures, using a limited range of the Saudi-Arabian land-races, and also preliminary information on how their temperature responses to germination are affected by salinity. The complementary incubator experiment, with fewer temperature treatments, embraced a wider range of species and was more effective in providing further information on the effects of salinity on their germination. Both approaches yielded useful information on the effects of salinity on the subsequent vigour of germinated seedlings.

It was striking that when no salinity treatment was applied, temperature had little effect on the total percentage germination of *Triticum aestivum, T. durum* or *Hordeum vulgare* in either experiment; effects on *Panicum miliaceum* and *Pennisetum glaucum*, investigated, only in the incubator experiment, were also relatively small. On the other hand, the speed of germination (t_{50}) was much more sensitive to temperature, and this was reflected in the cardinal temperatures discussed later. Increasing salinity both decreased the total germination generally and decreased it especially towards the extremes of temperature, generally revealing a more obvious optimum temperature and a narrowing of the temperature range over which germination could occur. This is consistent with the findings for a range of salt-tolerant species found in the vegetation of coastal shingle by Walmsley and Davy (1997). The Saudi land-race of *T. aestivum* was clearly much more tolerant of salinity than the cultivar Istabraq, former having low but appreciable germination at 500 mM NaCl and the latter showing no germination at all above 250 mM NaCl. This indicates an evolved adaptation in the Saudi land-race, as Istabraq is a conventional winter variety that has not been bred for salt tolerance and which performs well in the cool climate of the UK (Fenwick 2012). The effects of salinity on germination are discussed more fully in succeeding chapters of this thesis.

The Kew Millenium Seedbank regression techniques (Newton et al., 2009) for determining cardinal temperatures showed considerable efficacy for use with Saudi material. The probably genetically heterogeneous land–races showed more variability than might be expected for highly bred cultivars and certain of the regressions proved inadequate as predictors. Surprisingly, however, the commercial variety of *T. aestivum* Istabraq performed least reliably in this experiment; results for this cultivar and the

negative basal temperature for *Hordeum vulgare* should probably be disregarded. Otherwise the basal temperatures of c. 1-3 °C were consistent with a previous estimate of 2.6 °C for wheat (Angus et al., 1981) and the fact that these landraces are 'winter' forms. Basal temperature can be very different between species, for instance as high as 9.8 °C in the C4 species maize (Angus et al., 1981). Similarly the optimal temperatures and ceiling temperatures were within the ranges that might be expected from previous work (e.g. Garcia-Huidobro et al., 1982a; Covell et al., 1986; Benech-Arnold et al., 1990a). Consequently there is every reason to expect that methodology employed here would work well with other cereal land-races. There was no clear evidence that salinity affected basal or optimum temperature, but an interesting indication that it reduced the ceiling temperature, in two of the species. As has been mentioned previously, a narrowing of the temperature range for germination with increasing salinity has been reported for species of coastal shingle vegetation by Walmsley and Davy, 1997.

Measurements of dry mass shortly after germination, of those seedlings that were able to germinate, have also proved to respond to temperature and salinity and, although the results were a little erratic for some species, this parameter should represent a good indicator of their effects on seedling vigour. As expected, seedling dry masses tended to be greatly reduced towards the more extreme temperatures and by increasing salinity, in both the thermogradient and incubator experiments. The trends mostly followed those seen in total germination and speed of germination, but seedlings of Saudi-Arabian *T. aestivum* performed better at high temperatures under saline conditions on the thermogradient plate than might have been expected. The reasons for this are not clear and this is an approach that would benefit from further research, as it has been exploited rather little previously.

Non-dormant seeds respond to continually varying temperature conditions in the field but their temperature responses can be characterized from these constant temperature germination experiments if the concept of thermal time is employed. Germination below the optimal temperature is generally modelled as a linear response to accumulated day-degrees above the basal or threshold temperature (Garcia-Huidobro et al., 1982b; Benech-Arnold et al., 1990a). This underlines the importance of determining basal temperatures in experiments such as those described here. They represent a succinct distillation of experimental data that allows generalizations and comparisons,

across populations and between environmental conditions (Garcia- Huidobro et al., 1982a; Covell et al., 1986; Benech-Arnold et al., 1990a; Probert 1992). These resulting models can also help to define future experimental strategies for acquiring the data needed to assess the differences in thermal responses between populations and between seed lots (Covell et al., 1986; Ellis et al., 1986; Benech-Arnold et al., 1990a; Fidanza et al., 1996; Holshouser et al., 1996).

Chapter 4. Ecophysiology of germination in *Triticum aestivum* from contrasting environments in Saudi Arabia

4.1 Introduction

Five percent of the dry zones in the world are located in the Kingdom of Saudi Arabia (Hajrah 1979), but there is little information on the salinity of the soils in the Kingdom. Most of Saudi Arabia consists of arid or semi-arid land. Soil salinity is a common problem in arid and semi-arid regions, where poor irrigation water always holds a considerable volume of salts. Twenty-five percent of the irrigated land is affected with salts in arid and semi-arid areas, making the effects of salinity more visible (Azevedo et al., 2004). Certain climatic parameters, for example high temperature, low rainfall and wind, can aggravate the salinity problem by increasing evaporation rates, which in turn increase the soil's salt concentration. Soil salinity is a major factor of soil degradation and considered to be one of the most limiting factors for plant growth. There is also little information on the relationship between the distribution of plants and their environment. Soil salinity plays a key role in the composition of vegetation, particularly in coastal areas (Al-Oudat and Qadir 2011).

It is possible to apportion the Kingdom of Arabia into five regions for the characteristics of soil: (1) the Najd region (2) region of the Arabian Shield (3) Eastern Region (4) area of the Red Sea coast and (5) of the Empty Quarter (Abd El Rahman 1986). The Empty Quarter area is characterized by soil find according to medium varying depth and contains a high percentage of calcium carbonate, and near the hydrogen ion concentration (pH = 8) with low, cation exchange capacity. However, Al-Qassim comes from the Najd region and Al-Bahah from area of the Red Sea coast (Figure 4.1) The Arabian Shield area of soil is usually deep medium-soft and neutral for the hydrogen ion. In the eastern region, characterized by the Arabian Gulf Coast, soil salinity is high with many soluble salts, particularly sodium chloride. Coastal soils are characterized by low altitude surface. Further from the coast the land is covered by wind deposited sand dunes. The soil of the Red Sea coast is characterized highly saline and the surrounding areas of the coast are vulnerable to the effects of the tsunami and by infiltration of sea water. The Empty Quarter covered with loose soil and deep sand (Abd El Rahman 1986).

Salt is a common environmental feature of the deserts of the Middle East (Zohary 1973) and only a few halophytic plant species can tolerate such environments, including *Halopeplis perfoliata* and *Limonium axillare* (Mahmoud et al., 1982; Mahmoud et al., 1983b), *Avicennia marina* and *Hammada elegans* (Al-Huqail 2008; Mahmoud 1985; Al-Homaid et al., 1990) and *Suaeda aegyptiaca*. However, the distribution of plants in relation to the environment is generally poorly described in Saudi Arabia.

This study aims to compare in detail the environments of land-races *Triticum aestivum* in the contrasting Al-Bahah and Al-Qassim regions of Saudi Arabia, and relate these to the germination characteristics of the two races. This involves characterization of the two climates as well as characterization of the physical and chemical characteristics of soil collected from the root zones of the field sites from which seed was collected. Soil conditions characterized include (1) soil texture, (2) electrical conductivity (EC), (3) pH and (4) ecologically significant ions, Na^{+}, Cl^{-}, Ca^{2+}, K +, Mg^{2+}, PO_4^{3-}, SO_4^{2-}. *Triticum aestivum* was selected because of the importance of this crop in these regions. It is very common in both regions of the Kingdom of Saudi Arabia, which share more or less the same agricultural practices of cultivation. Soil samples were collected from the root zone of this plant to understand the soil structure and the availability of the nutrient to this crop plant in these regions.

It is hypothesized that the germination response of *T. aestivum* seeds collected from the two regions will differ under differing environmental stresses (temperature and salinity) and that this will be related to the climatic and soil conditions from where they originated.

Figure 4.1. Map shows Al-Qassim and Al-Bahah and there location in the map of Saudi Arabia.

4.2 Details of the Weather Station in Al-Qassim

The weather station at Unaizah City records provided the majority of the climate data used in this study for both regions. However, all data for Al-Bahah came from the weather station in Al-Bahah city and sent to Unaizah weather station, Al-Bahah city is at latitude 20.0000° N, longitude 41.4500° E, with a site elevation of 2,155 m. The Ministry of Water and Agriculture (Hydrology Section) provided daily and monthly time series of maximum and minimum temperature, total daily rainfall and potential evaporation. Climate data were obtained for both regions for a 30-year period, 1971-2000 (Table 4.1). All data series were prepared for analysis by careful reading through these records sheets and checking visually for errors and break points in the series. The data were used to investigate the differences in climate between the two regions, Al-Qassim and Al-Bahah.

Table 4.1. The meteorological data of Unaizah weather station used in this study.

Parameters	Period	Period
Max temperature (^{o} C)	1971-2000	Daily
Min temperature (^{o} C)	1971-2000	Daily
Rainfall (mm)	1971-2000	Daily
Evaporation (mm)	1971-2000	Monthly

Figure 4.2. Weather station a, Unaizah City, at latitude 26^{o} 04’ N, longitude 43^{o} 56’ E, elevation 724m.

4.3.1 Study sites

Al-Bahah

Al-Bahah highland is considered one of the richest and most variable floristic regions of the Asir Mountains, located in the south-west of Saudi Arabia. This highland is a part of the Arabian Shield, comprising Precambrian crystalline rocks. It spreads for a distance of 70 km in the north-south direction (19°50'-20°18' N, 41°38'-42°10' E) with rocky topography and elevation above sea level ranging between 1700 m eastwards and 2400 m westwards. The soils in the area vary substantially, being shallow and coarse-textured in elevated and sloping sites, but deep and of sedimentary texture in valley (lowland) sites.

The climate in Al-Bahah Province is greatly influenced by its varying topography. It is generally moderate in summer and cold in winter with average temperatures ranging between 7 °C in January to 35 °C in July, and relative humidity ranging between 24% in July to 54% in December. At Bahah city, the rainfall ranges from 85 mm in January to 10 mm in September, with a distinct peak in evaporation in April and May (Fig. 4.3).

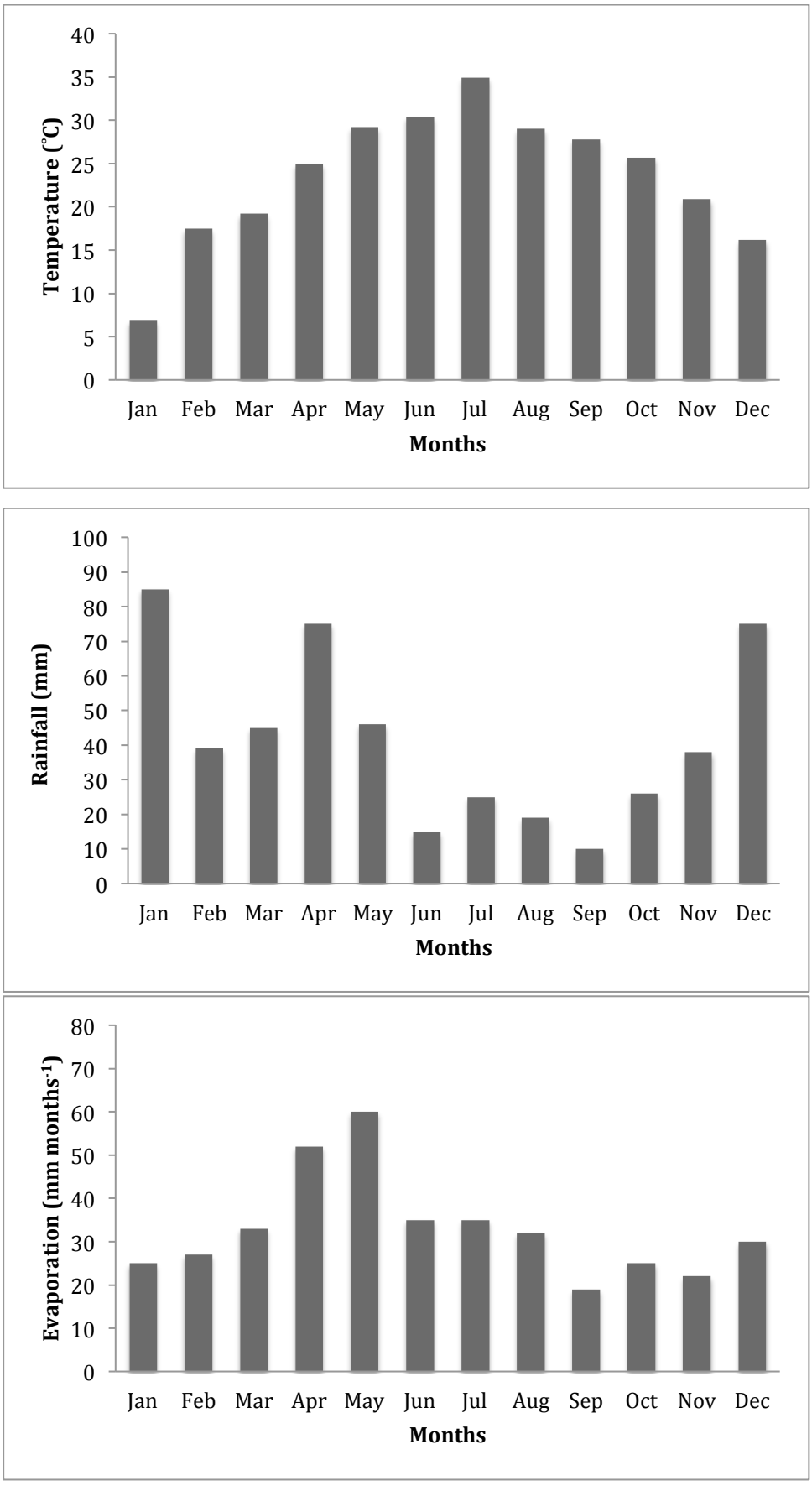


Figure 4.3. Mean monthly temperature (°C) rainfall and evaporation (mm) in Al-Bahah, Saudi Arabia, between 1971 and 2000.

Al-Qassim

Al-Qassim area is situated between latitudes 24° 30’ and 27° 15’ N, and longitudes 41° 50’ and 44° 50’ E, in the centre of Saudi Arabia. It is an arid zone, where the annual potential evaporation exceeds the rainfall. The climate at Al-Qassim is characteristically continental with long, hot and dry summers, and short, cool winters. The average monthly temperature, rainfall, and evaporation, for the 30-year period 1971 to 2000 are plotted in Figure 4.3.

Rainfall is substantially lower in Al-Qassim compared to Al-Bahah, with less than 2 mm of rain in June, July, August and September (Fig. 4.4). Monthly rainfall has a high degree of variability through the year; rainfall is highly seasonal and variable from year to year, because rainfall events are infrequent and of irregular occurrence.

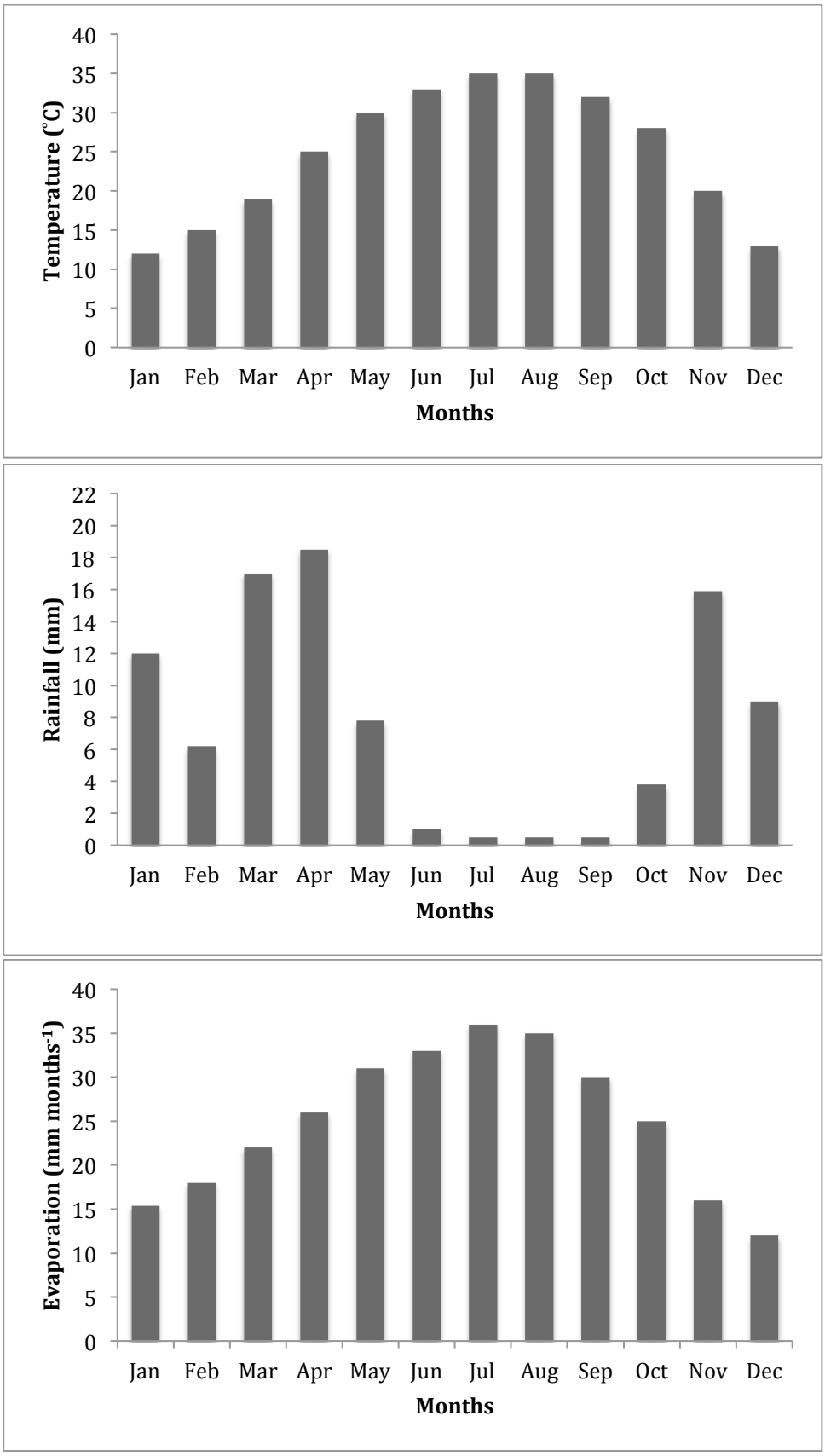


Figure 4.4. Mean monthly temperature (˚C) rainfall and evaporation (mm months^{-1}), in Al-Qassim, Saudi Arabia, between 1971 and 2000.

4.3.2 Collection of soil samples

In July 2013, one soil sample for each species (500 – 1500 g) was collected from three locations within the same field at each of four depths, 0-25 cm, 25-50 cm, 50-75 cm, and 75-100 cm, in areas where *Triticum aestivum* dominated. Samples were placed in sealed plastic bags and transported in plastic bags to the Organic Farming Research Centre, Onaizah, where they were air-dried for about a week. Samples were then sieved to <2 mm in preparation for physical and chemical analysis.

4.3.3 Soil texture hydrometer method

Three replicate sub-samples of 50 g of soil were each blended with 20 ml of a solution of sodium hexametaphosphate (50 g of sodium hexametaphosphate + 25 g sodium hydroxide in a litre of distilled water) and then suspended in a litre of water in a cylinder. A Bouyoucos hydrometer was inserted after 4 min to measure the mass of clay + silt in the soil, and again after two hours to measure the mass of clay alone. The masses of sand and silt were obtained by subtraction. The percentage contents of sand, silt and clay were calculated and used to assign a textural class on the soil texture triangle, using the USDA classification scheme (Bouyoucos 1962).

4.3.4 Chemical analysis of soils

Three replicate sub-sample of 1 g soil was mixed with 100 ml distilled water and left for 2 h. The mixuture was then filtered with 0.45 μm filter paper. One ml of extract was diluted with distilled water to 10 ml and a 20 μl sample was injected into an ion chromatograph and analyzed for the anions Cl^-, SO_4^{2-}, NO_3^-, HCO_3^- and PO_4^{3-}. The same procedure was used for analyzing the cations Na^+, $K^{+,}$ Ca^{2+} and Mg^{2+}. Carbonate was analyzed by titration with H_2SO_4 (0.02 M), using methyl orange and phenolphthalein as indicators. Total nitrogen was determined by the Kjeldahl method using two steps: sulphuric-salicylic acid digestion, followed by steam distillation with mossy zinc into boric acid, and titration with sulphuric acid (Bremner 1965).

A sub-sample of 25 g of soil was mixed with 25 ml distilled water, using the method of (Rhoads 1982), in order to make a paste to test the soil pH and electrical conductivity (EC). The pH of the soil was measured using a pH-meter D8120 (Weilheim pH40), following the method in (Mclean 1982), and placing a glass electrode in to the paste. Similarly, electrical conductivity (EC) was measured using an electronic meter and electrode (Wissenschaftlich-Technische Werkstatten LF530). Using the same soil paste, concentrations of sodium and potassium were measured by spectral analysis using the a (M7D) Flame photometer (Knudsen et al., 1982). The carbonate in the paste was measured by titration with sulphuric acid, using the indicators phenolphthalein and orange bromide (Richards 1954). Phosphorus was determined spectrophotometrically using a Spectronic 20 D (Watanabe and Olsen 1965). Total nitrogen in the soil paste was determined by the Kjeldahl method using two steps, as described above.

4.3.5 Germination experiments

The germination methods in this Chapter follow those in Chapter 3. Seeds from a population of *Triticum aestivum* from Al-Qassim and a population from Al-Bahah, were collected between 15 December 2012 and 7 January 2013 (Table 4.2). For each population, approximately 2-3 kg of seed in total was collected randomly from at least 50 plants. Seeds were transported in paper bags to the laboratory, cleaned of impurities and particulates and left to dry in the lab rooms, at a temperature 22 °C. The seed were brought to U.K. and have been stored in the dry room at the Millennium Seedbank, Wakehurst Place (Royal Botanic Gardens, Kew).

For each population, three replicates of 20 seeds were sown onto the surface of germination paper in Petri dishes (50 mm) and watered with one of three salinity treatments, 0 mM, 250 mM and 500 mM of sodium chloride. Petri dishes were placed on a thermogradient plate (Grant Instruments, Cambridge, UK), set at 13 constant temperatures ranging from approximately 6 - 35 °C with a 12 h photoperiod (white light with photon flux density of 50 Wm^{-2}). The temperature of each Petri dish was measured using temperature probes set to record every 10 minutes for the duration of study (Figure 3.1). Germination was scored every two hours during the day from 08:00 to 18:00 for a period of at least three weeks until no further germination was observed.

Germination was defined as radicle emergence of 2 mm. Two seedlings were selected from each Petri dish in thermogradient experiment three days after germination, and each seedling separated into two parts, roots and leaves. The plant material was weighed and then dried at 45 °C for 24 hours and the dry masses measured.

The analysis used in this part of the study is a linear model including temperature, salinity, site and the interaction between temperature and salinity and a polynomial term that allows for non-linear relationships with (R Core Team 2012).

Table 4.2. The plants scientific and local Arabic name, collector, GPS, from different location from Al-Qassim, Al-Bahah reigons, collected between15 December to 7 January 2013

Family	Scientific name	Arabic Local Name	Collector	G.P.S.
Poaceae	*Triticum aestivum*	Meyeh	Sami Albarih	N 26,134,89 E 043,96864
	Triticum aestivum	Haap		N 20,108233 E 41,285070

4.4 Results

4.4.1 Physical and chemical characteristics of soils from Al-Bahah

Soil textures in the topsoil of the three sites in Al-Bahah were mainly loamy sand types, with sand content ranging between 36-45% and silt between 39-48% and clay between 14-19% (Table 4.3). The results indicate that EC ranges between 2.8 and 4.6 mmhos/cm. There was no consistent change in soil texture or conductivity with depth. The pH and chemical composition at different depths are reported in Fig. 4.4. The difference in electrical conductivity between physical and chemical analysis is due to the titration.

Table 4.3. Mean (SD) composition and electrical conductivity (EC) in soil collected from Al-Bahah, Saudi Arabia, at differing depths.

Depth (cm)	Sand (%)	Silt (%)	Clay (%)	Texture	EC (mmhos cm^{1})
0-25	43 (10)	41 (10)	18 (2)	Loamy Sand	3.03 (2)
25-50	38 (11)	48 (8)	14 (4)		3.4 (2)
50-75	45 (12)	39 (12)	16 (0)		2.8 (3)
75-100	36 (12)	45 (10)	19 (4)		4.6 (3)

Table 4.4. Chemical analyses of soil samples collected from locations where *Triticum aestivum* was found in the Al-Bahah region of Saudi Arabia.

Depth (cm)	pH	Conductivity (mmhos cm^{1}) EC	Anion (mM $100g^{-1}$)			Cation (mM $100g^{-1}$)				HCO^{3} (%)	N (%)	CO^{3} (%)	Depth (cm)
			SO_4^{2-}	PO_4^{3-}	Cl^{-}	K^{+}	Mg^{2+}	Ca^{2+}	Na^{+}				
0-25	8.46	1.1	3.55	3.6	1.6	2.64	4.33	5.5	2.16	1.09	2.7	0.63	0-25
25-50	7.81	1.55	3.02	1.77	8	3.97	4.3	3.2	3.31	0.89	1.24	0.23	25-50
50-75	7.16	0.44	2.33	1.23	8	2.12	3.8	1.5	3.31	0.21	0.77	0.01	50-75
75-100	7.34	6	1.79	1.07	6.79	1.78	2.78	2.02	1.79	0.02	0.65	Nil	75-100

4.4.2 Physical and chemical characteristics of soils from Al-Qassim

Soil textures in the topsoil of the site in Al-Qassim were also loamy sand types. However, the sand content at Al-Qassim was greater than that in Al-Bahah (69-85% compared to 36-46%). The proportion of silt range was also greater, 54-63%, but the proportion of clay was lower (Table 4.5). The EC ranged between 5.8 and 6.43 mmhos cm^{-1}, higher than that found at Al-Bahah. The pH and chemical composition at different depths are reported in Fig. 4.6.

Table 4.5. Mean (SD) composition and electrical conductivity (EC) in soil collected from Al-Qassim, Saudi Arabia, at differing depths.

Depth (cm)	Sand (%)	Silt (%)	Clay (%)	Texture	EC (mmhos cm^{-1})
0-25	68 (27)	54 (42)	12 (7)	Loamy Sand	5.93 (0)
25-50	85 (5)	58 (47)	11 (1)		5.80 (1)
50-75	70 (29)	63 (40)	5 (3)		6.43 (1)
75-100	69 (33)	63 (43)	8 (4)		6.27 (0)

Table 4.6. Chemical analyses of soil samples collected from locations where *Triticum aestivum* was found in the Al-Qassim region of Saudi Arabia.

Depth (cm)	pH	Conductivity (mmhos cm^{1}) EC	Anion (mM $100g^{-1}$)			Cation (mM $100g^{-1}$)				HCO^3 (%)	N (%)	CO^3 (%)	Depth (cm)
			SO_4^{2-}	PO_4^{3-}	Cl^-	K^+	Mg^{2+}	Ca^{2+}	Na^+				
0-25	8.98	12.50	2.51	NIL	4.77	0.43	0.97	3.1	2.71	0.77	0.23	0.22	0-25
25-50	9.33	9.11	2.04	NIL	3.57	0.19	0.05	3.78	1.28	0.75	0.18	0.27	25-50
50-75	7.16	4.44	1.13	NIL	2.13	2.12	0.08	2.51	1.31	0.01	0.32	0.01	50-75
75-100	7.34	6	1.19	NIL	1.11	1.78	NIL	2.21	1.07	Nil	0.12	Nil	75-100

4.4.3 Germination response to salinity and temperature

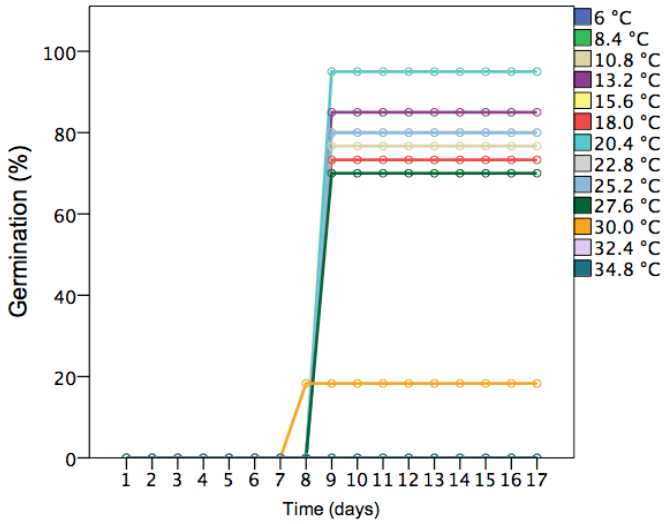
The overall germination of seeds from Al-Bahah and Al-Qassim is compared in Table 4.7. The time-course of germination at the 13 temperatures and 3 salinities for Al-Baha and Al-Qassim is shown in Figs 4.5 and 4.6, respectively.

Table 4.7. The percentage of seed germinating of *Triticum aestivum* from Al–Bahah and Al-Qassim.

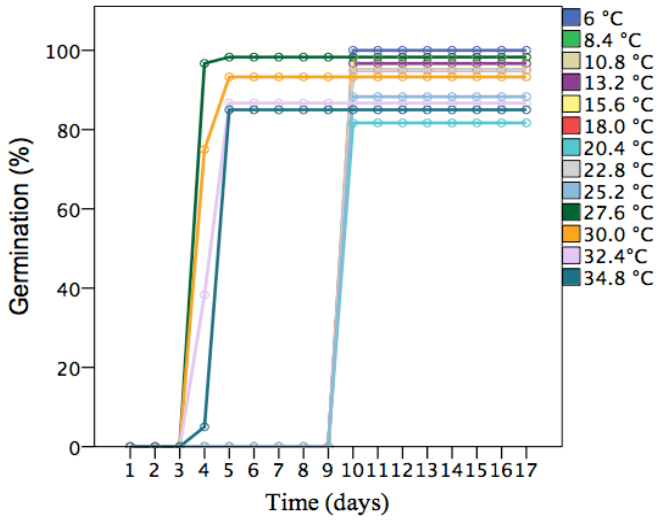
Descriptive Statistics

SITE		N	Minimum	Maximum	Mean	Std. Deviation
Al-Qassim	GERM	39	0.0	100.0	65.6	39.1
	Valid N (listwise)	39				
Al-Bahah	GERM	39	0.0	100.0	56. 7	39.9
	Valid N (listwise)	39				

a)



b)



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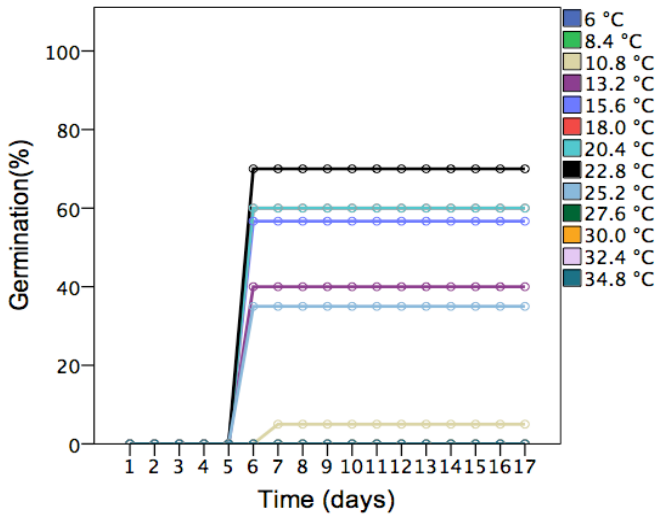
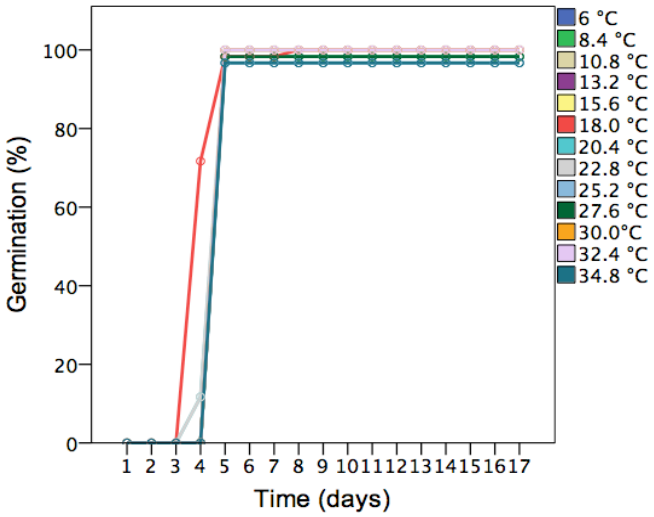
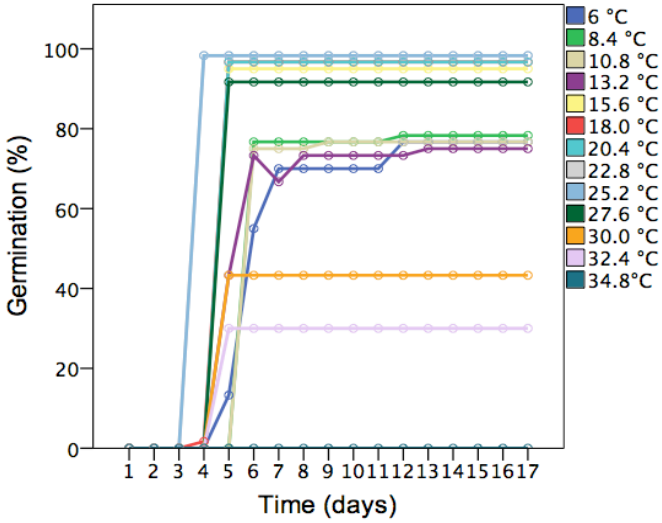


Figure 4.5. Mean proportion of *Triticum aestivum* seeds from Al-Bahah germinating under range of temperature (6-35°C) at, (a) 0 (b) 250 (c) 500 (mM) NaCl in the thermogradient table.

a)



b)



c)

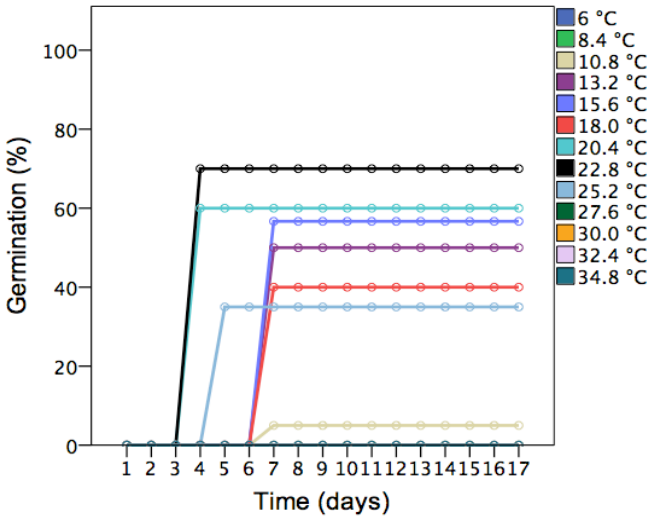


Figure 4.6. Mean proportion of *Triticum aestivum* seeds from Al-Qassim germinating under range of temperature (6-35 °C) at, (a) 0 (b) 250 (c) 500 (mM) NaCl in the thermogradient table.

The origins of the seeds were a significant determinant of the proportion germinating (F=16.75, P<0.001), with more seeds from Al-Qassim germinating that from Al-Bahah (mean±SD, Al-Qassim 66±39%, Al-Bahah 57±40%). Temperature and salinity were both significant determinates of germination (temperature F=52.36, p<0.001; F=207.48 P<0.001) and, importantly, there was also a significant interaction between salinity and temperature (F=14.07, P<0.001). Taking both land races together, in the 0 mM treatment, there was no relationship between temperature and germination, with the majority of seeds germinating at all temperatures (Fig. 4.7). At 250 mM, germination tended to be constant at the lower temperatures, but dropped off quickly above 27 °C. However, at 500 mM, there was a distinctive humped relationship between temperature and germination.

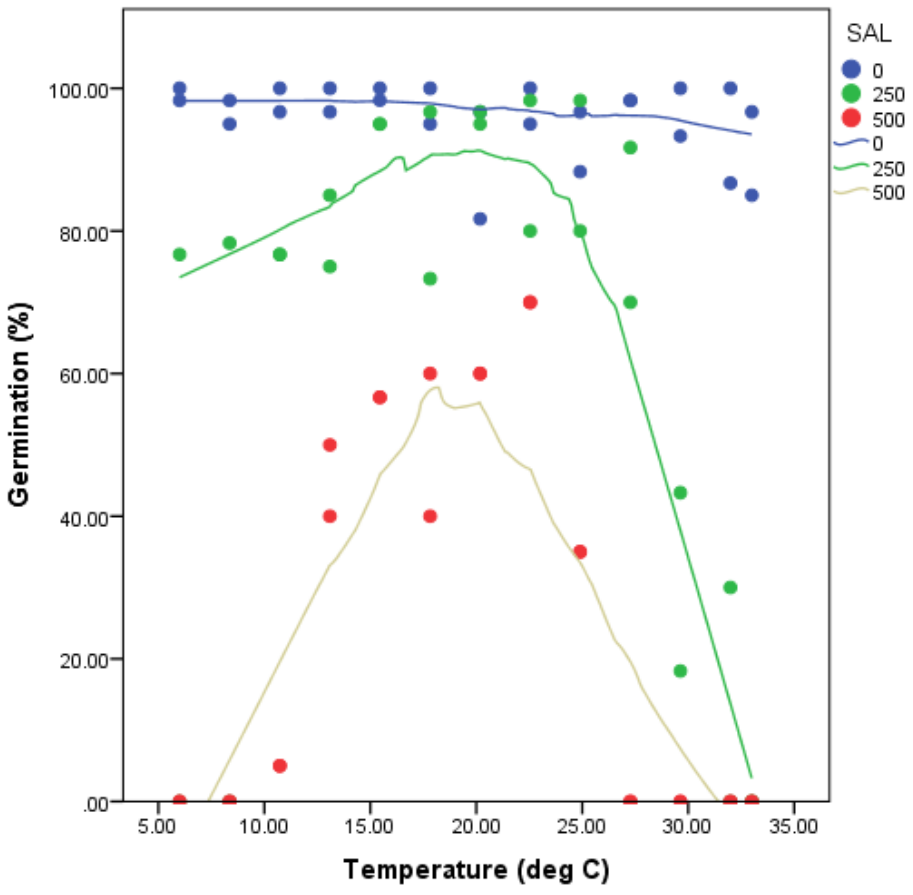
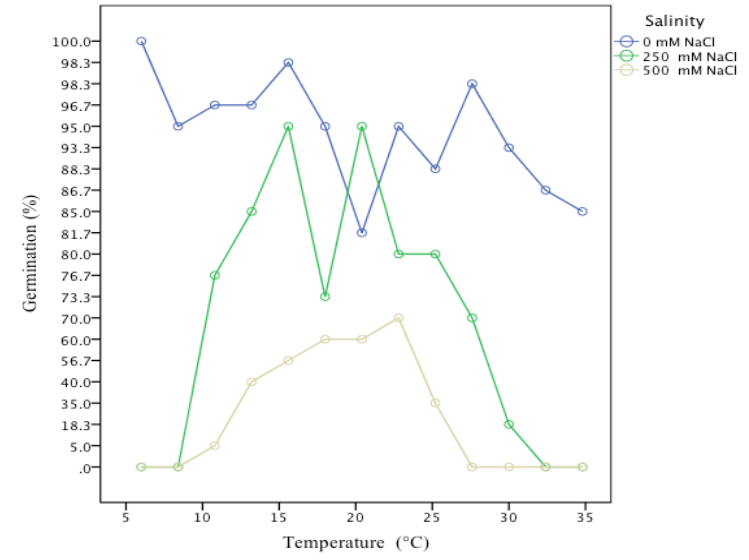


Figure 4.7. Mean proportion of *Triticum aestivum* seeds germinating under range of salinity (0 mM, 250 mM and 500 mM NaCl) and temperature conditions (thermogradient table). Loess lines are fitted.

a)



b)

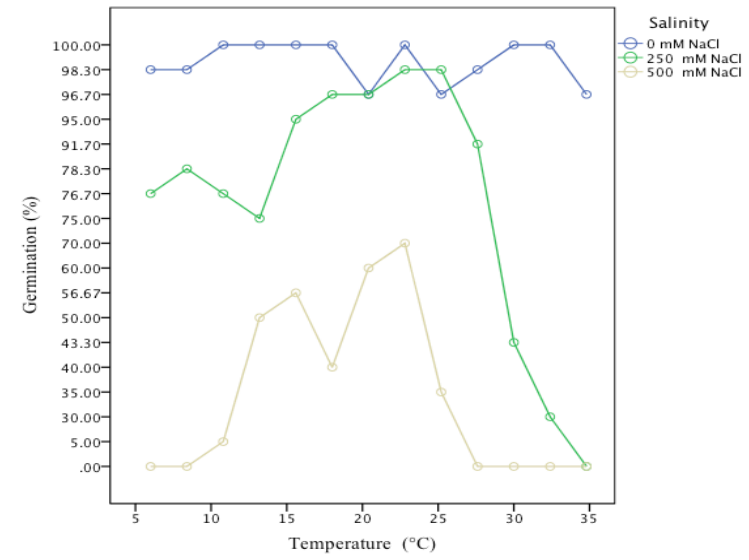


Figure 4.8. Response of final germination to temperature in (a) *Triticum aestivum* (Al-Bahah) (b) *Triticum aestivum* (Al-Qassim) at salinities of 0, 250 and 500 mM NaCl thermogradient plate experiment.

The speed of germination (t_{50}) of both populations of *T. aestivum* were reduced by high concentrations of sodium chloride (Tables 4.9 and 4.10) and by the lowest and highest temperatures (Figs 4.5 and 4.6).

Table 4.7. Time (days) to achieve 50% germination (t_{50}) of *T. aestivum* from Al-Bahah on the thermogradient plate.

NaCl Concentration	Temperature °C												
	6	8.4	10.8	13.2	15.6	18	20.4	22.8	25.2	27.6	30	32.4	34.8
0 mM	9	5	4	4	4	2	2	4	4	4	4	5	5
250 mM	0	9	8	6	6	6	4	4	6	3	0	0	0
500 mM	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 4.8. Time (days) to achieve 50% germination (t_{50}) of *T. aestivum* from Al-Qassim on the thermogradient plate.

NaCl Concentration	Temperature °C												
	6	8.4	10.8	13.2	15.6	18	20.4	22.8	25.2	27.6	30	32.4	34.8
0 mM	4.5	4.5	5	5	5	5	5	5	5	5	5	4.5	4
250 mM	6	6	6	6	6	4	4	4	6	2	2	0	0
500 mM l	0	0	7	7	3	2	2	2	2	0	0	0	0

Seedling masses for both land-races 3 days after germination on the thermogradient plate are given in Tables 4.9 and 4.10 for salinities of 0 and 250 mM NaCl, respectively. Seedling masses peaked at 18-23 °C in the absence of salinity and were greatly decreased by salinity in both land-races. The largest mean mass in Al-Qassim seedlings was approximately double that of the Al-Baha seedlings.

Table 4.9. Mean seedling dry mass (mg) three days after germination and standard deviation (SE) of *T. aestivum* germinated in 0 mM NaCl on a thermogradient plate.

	T. aestivum Al-Bahah		*T. aestivum* Al-Qassim	
Temperature °C	Mean	SE	Mean	SE
6.0	4.0	0.0	18.2	4.4
8.4	13.3	0.7	22.5	6.0
10.8	17.8	1.4	21.0	2.2
13.2	11.9	1.4	18.9	6.4
15.6	20.9	4.9	27.5	3.7
18.0	23.9	2.9	47.4	5.9
20.4	29.9	1.7	51.0	3.5
22.8	31.6	5.9	64.2	18.9
25.2	21.3	1.0	18.1	4.5
27.6	6.9	0.7	17.6	4.3
30.0	3.9	0.0	27.3	3.5
32.4	3.1	0.6	20.1	0.1
34.8	3.3	0.5	26.1	2.7

Table 4.10. Mean seedling dry mass (mg) three days after germination and standard deviation (SE) of *T. aestivum* germinated in 250 mM NaCl on a thermogradient plate.

	T. aestivum Al-Bahah		*T. aestivum* Al-Qassim	
Temperature °C	Mean	SE	Mean	SE
6.0	0.0	0.0	0.0	0.0
8.4	0.0	0.0	0.0	0.0
10.8	0.0	0.0	0.0	0.0
13.2	3.3	1.7	0.0	0.0
15.6	5.1	0.5	7.5	0.6
18.0	4.2	0.2	4.9	0.7
20.4	7.9	1.1	7.6	0.1
22.8	4.7	0.9	2.8	0.4
25.2	4.0	0.3	3.1	0.6
27.6	3.7	0.2	3.2	0.6
30	2.7	7.3	2.8	0.4
32.4	0.0	0.0	0.0	0.0
34.8	0.0	0.0	0.0	0.0

4.5 Discussion

Comparison of the long-term mean data for the two sites confirms that the two land-races experience distinctly contrasting climates. Although both sites have similarly hot summers, there is virtually no rainfall at Al-Qassim from May to October (and little in October itself), whereas low to moderate rainfall is maintained throughout the summer at Al-Baha. The winters, when most growth occurs, have similar rainfall and mostly mild temperatures. This has implications for the timing of germination and harvest. Sowing of these land-races in Saudi Arabia can be from mid-September to early January (see Chapter 6). Clearly, irrigation would be needed if sowing was before November at Al-Qasim and by then temperature would be lower than at Al-Baha; however, as irrigation can be used, selection may not necessarily be expected to have favoured germination at the lower temperatures. Given the constraints of drought at the end of the growing season, early establishment at Al-Qassim might be advantageous.

The soil analyses confirmed the extreme nature of the environments that these land-races of cereal were growing in. Both were saline, and salinization is typical of arid and semi-arid environments, where annual evapotranspiration is greater than annual precipitation, and is exacerbated by irrigation with groundwater containing salts (Abd El Rahman 1986). The high pH values, and sulphate and chloride concentrations were consistent with salinization, Presumably because of the summer drought and substantially greater annual water deficit, Al-Qassim has average higher soil salinities, when expressed as conductivity readings. The soil environments are markedly different, with much sandier, less water retentive substrates at Al-Qassim, which might exacerbate the irrigation requirement and salinization, or potentially limit germination in its absence. Al-Qassim was also nutrient deficient, in terms of concentration of total nitrogen, phosphate and potassium, notwithstanding the use of manures (See Chapter 6). AI-Jaloud et al. (1996) and Hussain et al. (1996) in their field experiments on wheat cultivars found that the yield of wheat and its nitrogen use efficiency were greatly affected by nitrogen application and treatment with effluent irrigation: wheat grain yield ranged between 5.20-6.87 Mg ha^{-1} for well-watered crops.

The results of the germination experiment revealed that wheat land-races collected from two different locations indeed reacted differently to salinity stress, with a highly significant interaction between land-race and salinity in the GLM: most strikingly, seeds from Al-Baha did not germinate at all at 500 mM NaCl. whereas those from Al-Qassim, which had the higher soil salinity, showed up to 70% germination at optimal temperatures (c. 23 °C). Even at 250 mM NaCl, Al-Qassim seeds consistently germinated faster (lower values of t_{50}) and had a broader range of temperature tolerance than those from Al-Baha. Other studies have also found that different wheat cultivars have different responses to salinity stress, resulting in differing grain yields (Richards et al., 1987; Slavich et al., 1990; Albarih 2010). In a large-scale study of 103 wheat genotypes from across Europe, Asia and the Middle East, genotypes from locations in Pakistan were among the most and least salt tolerant (El-Hendawy et al., 2005).

The results of this experiment strongly support an important finding also seen in the work described in chapters 3 and 5: a progressive narrowing of the range of temperature at which germination can occur as salinity was increased; as elsewhere this narrowing was evident at the higher extreme temperatures at moderate salinity and then also at the lower extreme temperatures at higher salinity. This is discussed in more detail elsewhere.

Chapter 5. Effects of seed ageing on the germination response to salinity and electrolyte leakage in wheat land-races

5.1 Introduction

The advancement of technology and mechanization of agricultural production has improved the prospects for long-term seed storage. During storage, physical and physicochemical transformations occur in seeds as a result of ageing (Silva et al., 2005; Sisman 2005). The deterioration in quality that accrues with ageing is usually manifested primarily as a decrease in germination percentage; however aged seeds that do germinate may also produce weak seedlings. Throughout the ageing process, seeds lose their vigour, ability to germinate and eventually become significantly less viable (Maity et al., 2000). The decline in seed quality can begin before harvest, depending on field weather conditions and harvesting time but is most important during seed storage. The deterioration is greater if seeds are stored at high temperatures and/or high relative humidity. For example, one of the most sensitive seeds, Cottonseed, shows substantial deterioration after just one year of storage (Powell et al., 2000). The susceptibility of seeds to ageing depends on their ability of seed to resist degradation through various defence mechanisms, which may vary in different plant species (Gupta and Aneja 2004; Sisman and Delibas 2004; Mohammadi et al., 2011). Thus seeds of different plant species in similar storage environments may lose viability to higher or lower degrees and seed storage has obvious consequences for seed viability.

The main external conditions influencing seed damage during storage are the relative humidity of the air, temperature and oxygen concentration. The opportunity to control these factors creates the foundation for longer seed storage. However, seeds with low viability are the first to die. Seeds that contain large amounts of lipids tend to have limited longevity because of their particular chemical composition (Voelker and Kinney 2001). The moisture content of seed is the most important factor of longevity under storage as the chemical potential of water in the system determines the activity of all chemical reactions (Basra 1984). Mature seed collected from the field may have moisture content as high as 14%, too high for long-term storage in most species. The seed of the majority of agricultural species can be stored for several years if the moisture content is maintained at 5-8%. However, the seed moisture content depends on

air relative humidity; high air humidity will increase seed moisture content, which could lead to rapid seed deterioration, especially at over 12% moisture content. Similarly, the chemical reactions leading to deterioration in quality will tend to proceed faster at higher storage temperatures, Oxygen is required for the ageing repair mechanisms but, on the other hand, oxidative damage is more likely with storage at higher oxygen concentrations.

One possible consequence of deteriorating seed quality is a disproportionately reduced ability to germinate under conditions of stress. As has been investigated in previous chapters, of particular interest for land-races in arid environments is the ability to germinate under high salinity. Little is known about this, but Walmsley and (Walmsley and Davy 1997) found that storage of seeds of coastal-shingle species for 7 years at low humidity and temperature resulted subsequently in lower germination rates at both high salinities and temperatures.

Practically, it is valuable to be able to assess the effects of seed ageing on viability quickly prior to sowing and germination (Perry 1972). Measurements of electrical conductivity (EC) during artificial ageing treatments potentially are an effective way to measure seed vigour, as has been demonstrated for of pea and soybean (Association of Official Seed Analysts, 2002). Although such investigations are generally dependable for these two species, application of this approach to other species remains less well tested; its effectiveness in defining seed vigour of additional species correspondingly needs further investigation (Marcos-Filho 1998). The EC method depends on the fact that seeds, after soaking in water, release ions, sugars and other metabolites from the beginning of the soaking phase, due to changes in the integrity of the cell membranes. As seeds deteriorate, the repair mechanisms become ineffective, or the membranes are severely damaged (Bewley and Black 1985), therefore enabling leakage of higher electrolyte volumes. EC experimental results can be highly reproducible because certain conditions are known to affect the results, for example seed size (Tao 1978; Deswal and Sheoran 1993), water soaking temperature (Murphy and Noland 1982), soaking time (Loeffler et al., 1988; Schmidt and Tracy 1989), preliminary seed moisture content (Tao 1978; Loeffler et al., 1988), and physical injury to the seeds (Tao 1978; Duke and Kakefuda 1981). Thus all of these influences can be controlled to decrease their effects.

Other factors, requiring investigation, cannot be so easily controlled, for example the effect of genotype (Short and Lacy, 1976; Panobianco and Vieira 1996), or developmental stage at harvest (Styer and Cantliffe, 1983; Powell 1986) and the storage environment (Ferguson 1988; Vieira et al., 2001). Reports on soybean seeds have shown the results of the EC examination could be affected by storage temperature, particularly low temperatures, for example 10 °C (Ferguson 1988; Vieira et al., 2001). EC test results have been successful in predicting seed germination and stand establishment under a wide range of field conditions, as reported for soybean seeds (Colete et al., 2004; Vieira et al., 2004), showing the importance of vigour testing for this species (Vieira et al., 1999a,b). Increasing conductivity in seed leachates has been found to correlate with reduction in germination and seed vigour in several crop species (Ghosh 1981 and Rudrapal 1979). Ultimately, the outcomes of EC experiments are determined by the integrity of seed membrane systems, and thus can be used for the evaluation of potential seed vigour, since they detect seed deterioration in the early stages of its progression.

Such vigour tests can be used to predict the essential emergence performance of seed lots more quickly and accurately than traditional germination tests. Two validated tests, now in the International Seed Testing Association Rules (ISTA 2006), are the electrical conductivity (EC) of seed soak water (Matthews and Powell 2006) and accelerated ageing tests (TeKrony and Egli 1977). Accelerated ageing, generally achieved by subjecting seeds to a combination of high temperature and humidity, allows the effects of natural ageing to be uncovered on a relatively short time scale.

The aims of this part of the study were two-fold. The first was to subject seeds from two land races *Triticum aestivum* from different climatic zones represented by the Al-Qassim and the Al-Bahah region to accelerated ageing and investigate their subsequent ability to germinate under saline conditions. The hypothesis was that aged seeds of poorer quality would be less salt tolerant. The second aim was to investigate the practical value of EC measurements of electrolyte leakage in predicting relative seedling emergence for these land-races of *Triticum aestivum* under different salinity conditions, where average germination lay in the commercially conventional range. A supplementary aim was to measure electrolyte leakage from different parts of the seed and establish how it was affected by the different salinity treatments.

5.2 Methods

5.2.1 Experimental materials

Seeds of land-races of *Triticum aestivum* from climatic zones represented by the Al-Qassim region (Arabic local name Meyeh) and the Al-Bahah region (Arabic local name, Haab) were collected from the farms in Saudi Arabia in 15 December to 7 January 2013 and deposited in the drying room at the Millennium Seedbank, Wakehurst Place (Royal Botanic Gardens, Kew), where part of this study was conducted. Details of the collection sites and their environments are presented in Chapter 4.

5.2.2 Controlled ageing procedure

This method aims to generate a single seed survival curve, using a carefully controlled ageing environment. Seeds were withdrawn from dry storage and first rehydrated by equilibration with a closed atmosphere at 47% RH for 14 d, to minimise the change in water content when samples were transferred to accelerated ageing conditions. Seeds were placed in open dishes on a grid over lithium chloride (385 gL^{-1} LiCl) solution at 20 °C in sealed electrical enclosure boxes (Fig. 5.1; Ensto, UK Ltd).

For controlled ageing, the seeds were transferred to another sealed electrical enclosure box at 60% RH (in equilibrium with 300 gL^{-1} LiCl; Fig.5.2) which was placed in a fan-assisted oven at 45 °C. Ageing times that would result in 75, 50, 25 and 0% germination were estimated from the predicted seed moisture content as 0, 5, 14, 23 and 30 d using the Kew website dedicated calculator (http://www.kew.org/data/sid.) Three replicate seed samples were withdrawn on each of these days and transferred 15% RH at 15 °C to stop the ageing process. Five replicates of 3 seeds were also taken to check that their moisture content was consistent. The aged seeds were sealed in vials and transferred to the University of East Anglia for germination tests.

5.2.3 Germination tests

Each of the three replicate samples of 100 seeds of each land-race for each ageing period were allocated randomly to 4 Petri dishes (25 seeds per dish) and each was subjected to a different salinity treatment: 0, 250, 500 and 1000 mM NaCl. Seeds were placed on 9 cm filter papers (Whatman No. 1) and 7 ml of distilled water or the

appropriate concentration of sodium chloride solution per dish was added. Dishes were placed in a temperature-controlled incubator at 25 °C. Solutions were topped up every four days, according to need. Germinated seeds (with emerged radicles) were counted in every dish every twenty-four hours, and the germinated seeds immediately removed. The solutions were replaced completely every four days. Seeds that had not germinated after 17 d were washed with distilled water thoroughly several times, and then transferred to new Petri dishes, and incubated for a further 5 days to make sure that no further germination would occur. A 'cut test' performed at the end of each germination test, to confirm that any non-germinated seeds were dead and not otherwise unviable (empty or infested). Incompetent seeds were excluded from the calculation of germination percentage.

A cumulative germination curve was used to calculate the rate of germination (t_{50}; the period of time needed to achieve 50% germination) by dropping a vertical line from 50% germination to intersect with the time axis. Statistical analysis was by ANCOVA with salinity as the main effect and ageing time as the co-variate (SPSS version 21).

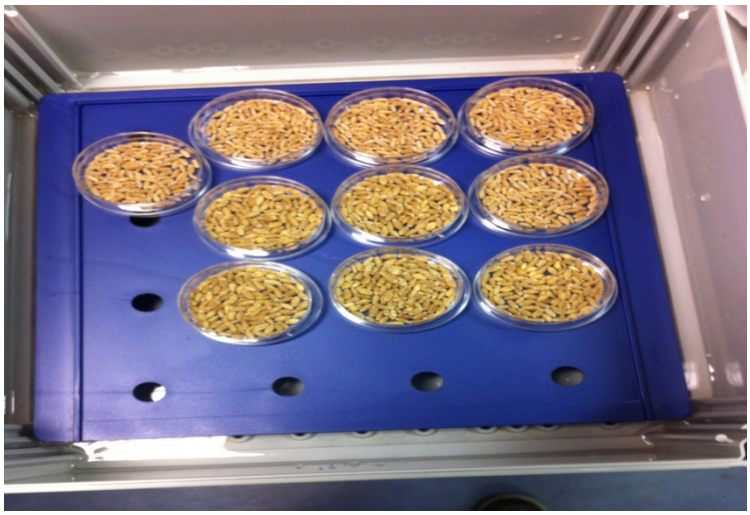


Figure 5.1.The rehydration box with seed samples inside.

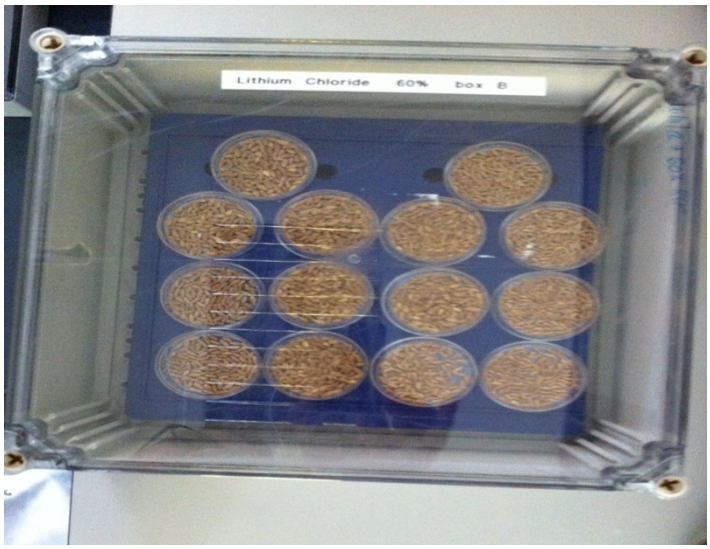


Figure 5.2. The ageing-treatment box with seeds sealed inside.

5.2.4 Electrical conductivity testing

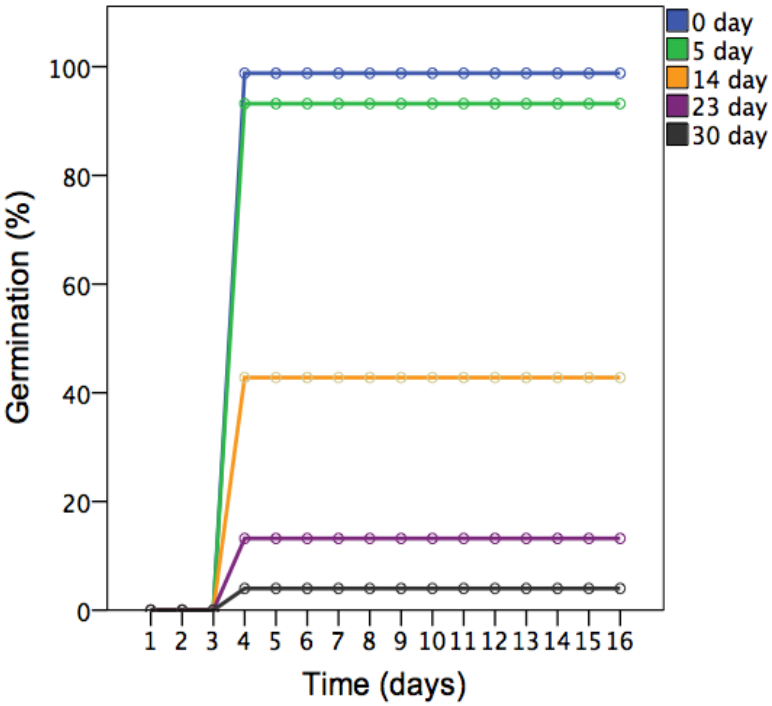
Electrical conductivity measures the leakage of electrolytes during imbibition. Seeds of the two land-races were drawn from seed lots two days before the beginning of the controlled ageing experiment. Three replicates of 25 seeds for each salinity were soaked on the surface of germination paper in a Petri dish after adding 5 ml distilled water and then left for 4 h in an incubator at 25 °C to imbibe. Then seeds were transferred to another Petri dish and treated with 7 ml of a solution of 0, 250, 500 or 1000 mM NaCl, before being incubated for 5 h at 25 °C. Then sub-samples of five seeds were chosen randomly from each Petri dish and washed with distilled water to remove any surface salt, and then different batches were used to examine three different components: whole seeds, seed coats, and embryos. Five seeds were kept as a reserve in case there were problems during the separation of the seeds into parts. Each component was placed separately in a glass vial to which was added 12 ml distilled water, and the electrical conductivity meter (Oakton T-100 Conductivity Meter Resolution 0.01, 0.1, 1 Accuracy ± 2% of reading) was used to measure electrical leakage every 2 h from 8:00 am - 6:00 pm until there was no further change.

5.3 Results

5.3.1 Germination response to salinity in aged seed

No germination at all occurred in either of the 500 and 1000 mM NaCl treatments. Cumulative germination curves at 0 and 250 NaCl mM salinities for the seeds from Al-Qassim and and Al-Bahah are shown in Figures 5.3 and 5.4 respectively. In the absence of salinity and with no ageing treatment germination was high (95-97%) and showed no notable differences between the two land races. However, as predicted controlled ageing greatly reduced germination in both races, with only 8% for *Triticum aestivum* from Al-Qassim and 14.8% for that from Al-Bahah after 30 days of ageing at 250 mM NaCl concentration.

a)



b)

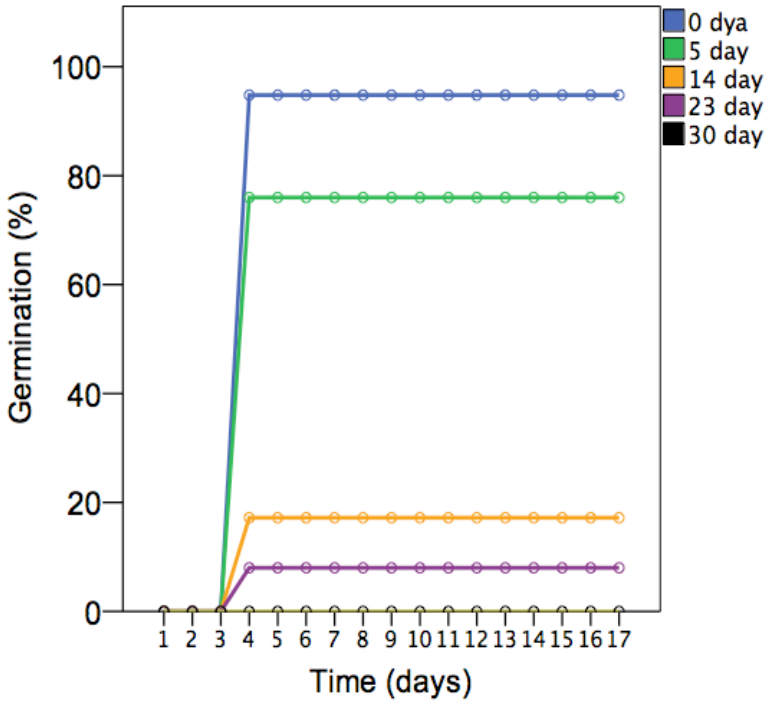
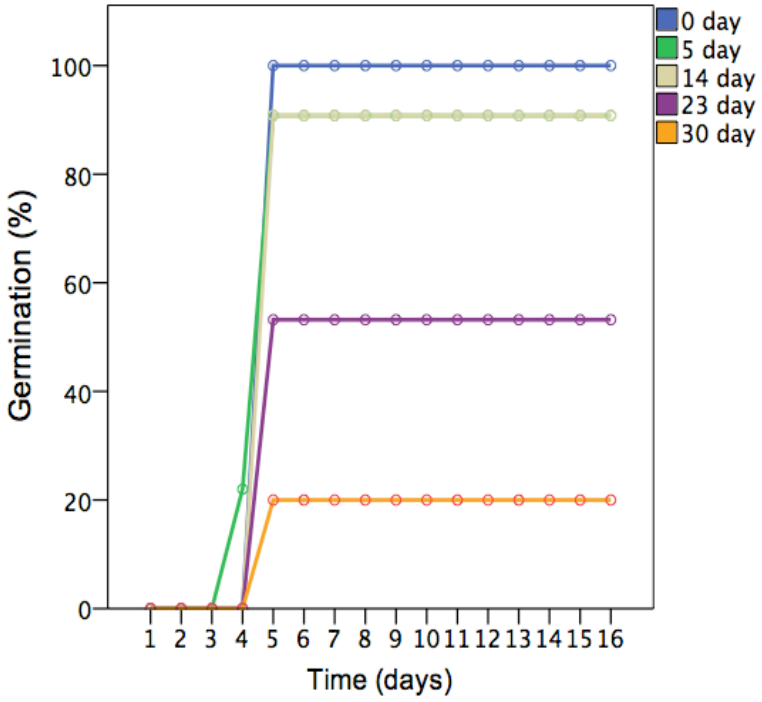


Figure 5.3. Cumulative germination of *Triticum aestivum* from Al-Qassim region, after different ageing times, treated with (a) 0 and (b) 250 mM NaCl.

a)



b)

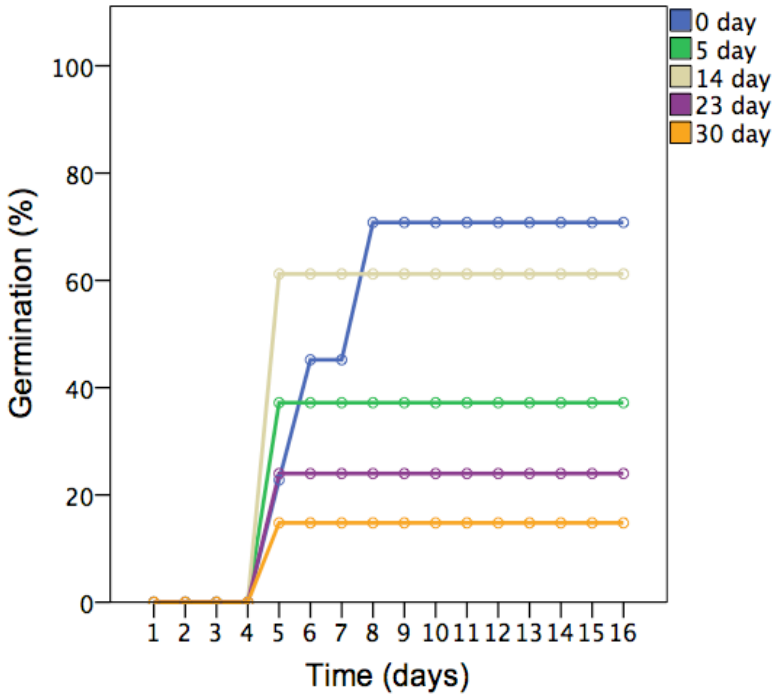


Figure 5.4. Cumulative germination of species *Triticum aestivum* from Al-Bahah region, after different ageing times, treated with (a) 0 and (b) 250 mM NaCl.

The germination survival curves in the 0 and 250 mM NaCl treatments (Figures 5.5 and 5.6) followed the inverted sigmoidal expected. At both both races at all ageing times, germination was reduced by the higher salinity. One-way ANCOVA, with salinity as a fixed factor and ageing as covariate, yielded highly significant models (Table 5.1). The effect of ageing was highly significant for both races; the effect of salinity and the salinity*ageing interaction were both highly significant for the Al-Baha race, whereas for the Al Qassim race salinity was marginally significant and the interaction was not significant. A two-way ANCOVA, allowing for comparison of the two races, confirmed the significance of salinity and ageing main effects but showed that the effect of race and the race*salinity interaction were significantly; however, race*ageing interaction was highly significant, indicating the two races responded differently to the ageing treatment at both salinities.

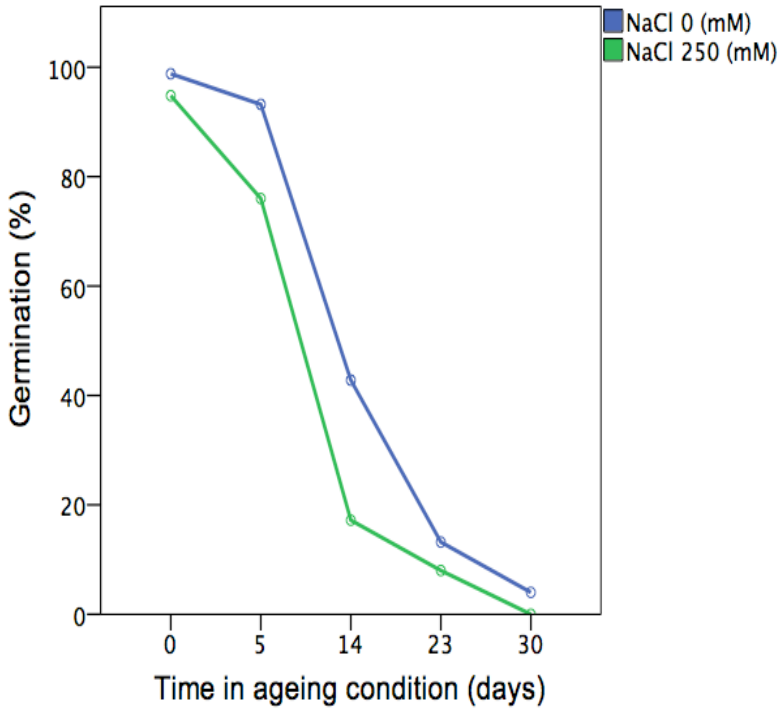


Figure 5.5. Germination survival curve in response to controlled ageing in the Al-Qassim race of *Triticum aestivum* when subsequently germinated at salinities of 0 or 250 mM NaCl and 25 °C.

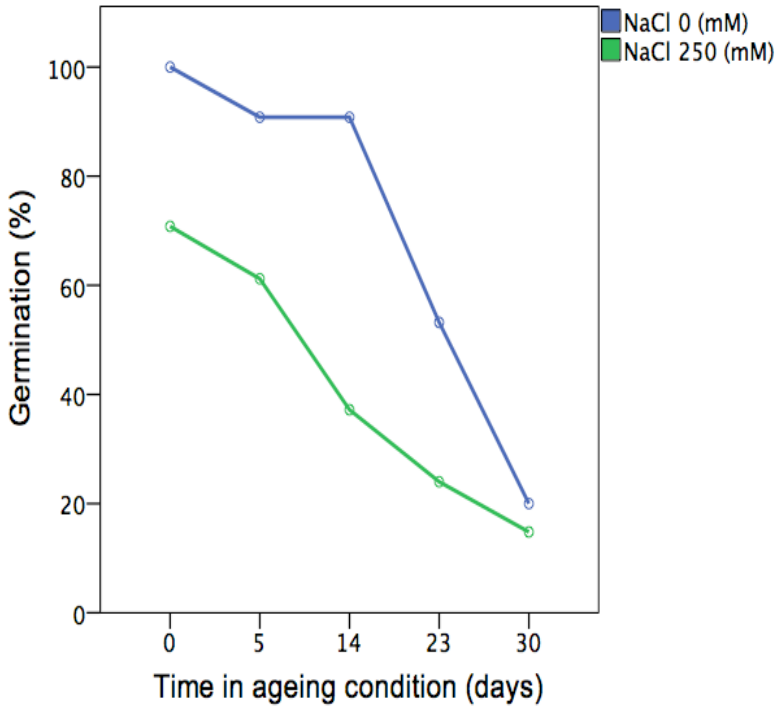


Figure 5.6. Figure 5.5. Germination survival curve in response to controlled ageing in the Al-Bahah race of *Triticum aestivum* when subsequently germinated at salinities of 0 or 250 mM NaCl and 25 °C.

5.1. One-way ANCOVA, with salinity as a fixed factor and ageing as covariate, for arc-sin percentage germination of seeds of the land-race of *T. aestivum* from (a) Al-Qassim region and (b) Al-Bahah region

(a)

T. aestivum Al-Qassim	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	8.351[a]	3	2.784	47.112	<0.01
Intercept	17.399	1	17.399	294.457	<0.01
Salinity	0.242	1	0.242	4.093	0.053
Ageing	8.048	1	8.048	136.209	<0.01
Salinity * Ageing	.057	1	0.057	0.967	0.335
Error	1.536	26	0.059		
Total	19.831	30			
Corrected Total	9.888	29			

a. R Squared = 0.845 (Adjusted R Squared = 0.827)

(b)

T. aestivum Al-Bahah	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5.208[a]	3	1.736	44.624	<0.01
Intercept	13.432	1	13.432	345.310	<0.01
Salinity	1.774	1	1.774	45.612	<0.01
Ageing	3.047	1	3.047	78.330	<0.01
Salinity * Ageing	0.458	1	0.458	11.784	0.002
Error	1.011	26	0.039		
Total	20.223	30			
Corrected Total	6.219	29			

a. R Squared = 0.837 (Adjusted R Squared = 0.819)

Table 5.2 Two-way ANCOVA, with salinity and race as a fixed factors and ageing as covariate, for arc-sin percentage germination of seeds of the two land-races of *T. aestivum*.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	66403.019[a]	7	9486.146	33.520	<0.001
Intercept	176875.129	1	176875.129	625.004	<0.001
Salinity	4214.650	1	4214.650	14.893	<0.001
Race	453.570	1	453.570	1.603	0.211
Ageing	52681.133	1	52681.133	186.154	<0.001
Salinity * Race	1004.262	1	1004.262	3.549	0.065
Race * Ageing	3723.492	1	3723.492	13.157	. <0.001
Salinity * Race * Ageing	631.995	2	315.997	1.117	0.335
Error	14715.914	52	282.998		
Total	234336.000	60			
Corrected Total	81118.933	59			

a. R Squared = 0.819 (Adjusted R Squared = 0.794)

The time taken to achieve 50% of the final germination (t_{50}) in all of the treatments in this experiment is shown in Table 5.3. In general, germination was rapid (with t_{50} of 1-7 d). There was a general trend for t_{50} to increase with ageing time in some treatments (e.g. the Al-Qassim race at 250 mM and the Al-Baha race at 0 mM NaCl); however t_{50} was rather erratic in other treatments and ANCOVA did not yield significant effects from either ageing time or salinity for either race (Table 5.4).

Table 5.3. Time to 50% germination ±SE in two land races from Al-Qassim and Al-Bahah regions after controlled ageing and subsequent germination at salinities of 0 or 250 mM NaCl and 25 °C. MC indicates the mean seed moisture content (%) during ageing.

Land races	Controlled ageing time (days)					MC (%)
	0	5	14	23	30	
Al-Qassim race:						
0 mM NaCl	1.23 ±0.5	1.38 ±1.7	3.8 ±9.1	5.38 ±3.5	1.38 ±1.7	10.37
250 mM NaCl	4.8 ±1.1	1.14 ±1.0	14.9 ±5.8	6.14 ±2.0	0.38 ±1.0	10.68
Al-Bahah race:						
0 mM NaCl	0.83 ±0.5	1.15 ±1.1	1.14 ±1.0	3.8 ±9.2	6.38 ±3.5	10.34
250 mM NaCl	1.15 ±1.1	1.14 ±1.0	5.9 ±5.8	7.14 ±2.0	------___	10.39

Table 5.4. One-way ANCOVA, with salinity as a fixed factor and ageing as covariate, for the time to 50% germination (t_{50}) of seeds of the land-race of *T. aestivum* from (a) Al-Qassim region and (b) Al-Bahah region.

(a)

T. aestivum Al-Qassim	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	23.396[a]	3	7.799	0.322	0.810
Intercept	58.069	1	58.069	2.399	0.172
Salinity	17.254	1	17.254	0.713	0.431
Ageing	0.059	1	0.059	0.002	0.962
Salinity * Ageing	3.201	1	3.201	0.132	0.729
Error	145.236	6	24.206		
Total	332.900	10			
Corrected Total	168.632	9			

a. R Squared = .139 (Adjusted R Squared = -.292)

(b)

T. aestivum Al-Bahah	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	21.430[a]	3	7.143	0.986	0.460
Intercept	5.444	1	5.444	0.751	0.419
Salinity	4.577	1	4.577	0.632	0.457
Ageing	16.155	1	16.155	2.230	0.186
Salinity * Ageing	4.862	1	4.862	0.671	0.444
Error	43.470	6	7.245		
Total	146.867	10			
Corrected Total	64.899	9			

a. R Squared = .330 (Adjusted R Squared = -.005)

5.3.2 Electrolyte leakage in response to salinity

Electrical conductivity measurements representing the leakage of electrolytes from whole seeds, seed coats and embryos after incubation at a range of salinities are shown in Figures 5.7 and 5.8 for the races from Al-Qassim and Al-Baha regions, respectively. The two races behaved very similarly. Whole seeds released increasing amounts of electrolytes after incubation with increasing salinities. However the difference between the 0 and 250 mm NaCl treatments was very small, and electrolyte leakage increased more after incubation at, 500 mM. Leakage doubled after incubation at 1000 mM NaCl. The trends were similar in isolated seed coats and embryos, but overall levels of conductivity were about an order of magnitude lower in both cases, presumably reflecting their relative masses. In particular there was very little evidence of an adverse effect on the embryos below 1000 mM NaCl.

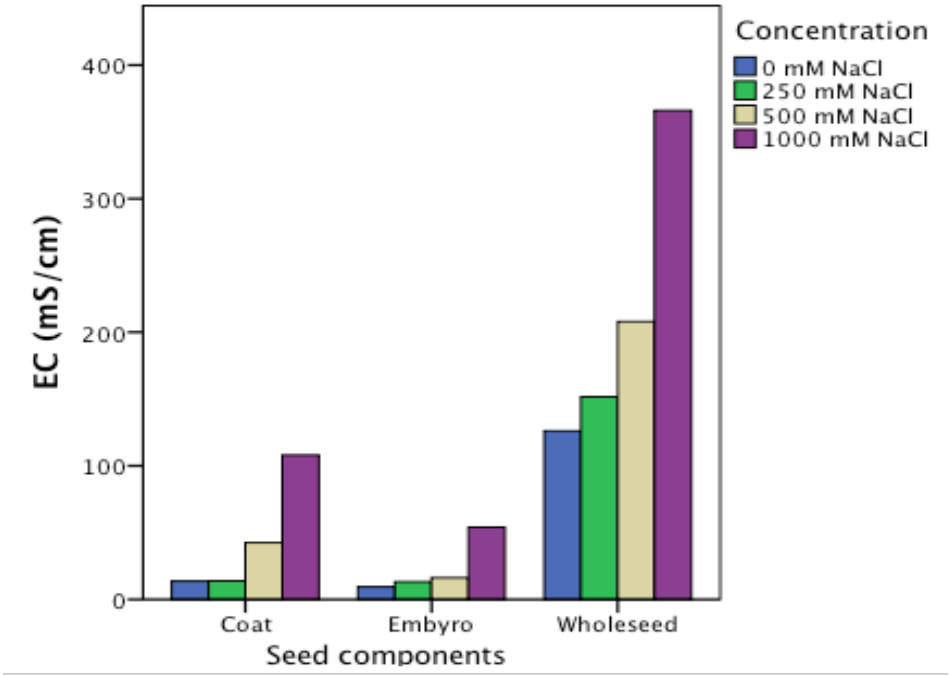


Figure 5.7 Electrical conductivity of leachates (representing electrolyte leakage) from seeds and isolated seed coats and embryos of *Triticum aestivum* from Al-Qassim region, after incubation at a range of salinity (0, 250, 500 and1000 mM NaCl).

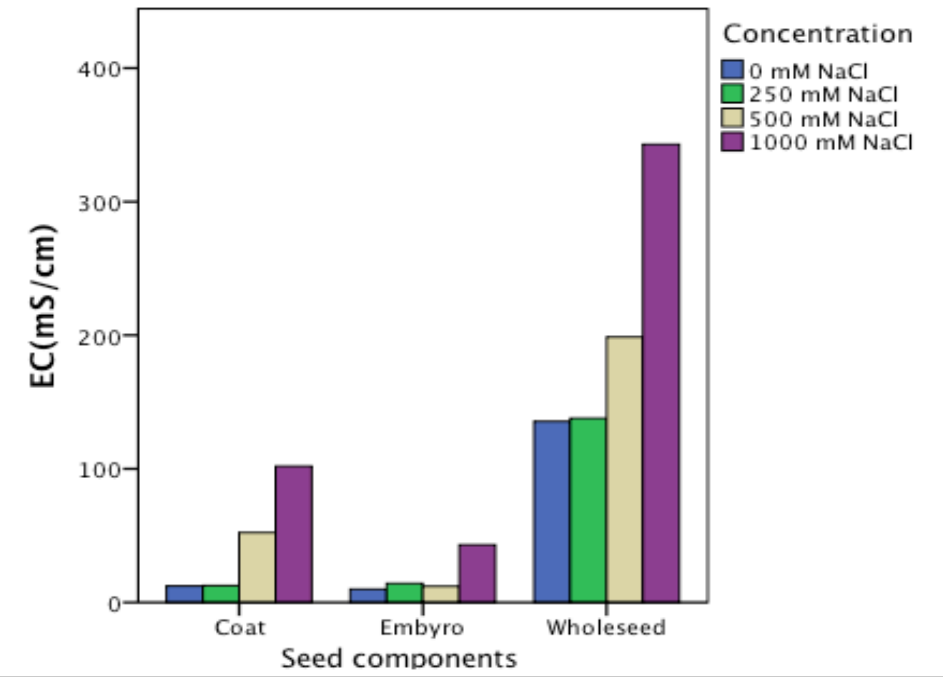


Figure 5.8 Electrical conductivity of leachates (representing electrolyte leakage) from seeds and isolated seed coats and embryos of *Triticum aestivum* from Al-Baha region, after incubation at a range of salinity (0, 250, 500 and 1000 mM NaCl).

5.4 Discussion

Germination of both races showed substantial salt tolerance, by the standards of wheat varieties and in comparison with halophytes (Woodell 1985; Pujol et al., 2000; Garcia-Huidobro et al., 1982b; Benech-Arnold et al., 1990a), as they germinated well at a salinity of 250 mM NaCl. However, the absence of any germination at 500 or 1000 mM NaCl suggests that they are not among the most salt-tolerant types investigated in this study. There was some suggestion that the Al-Qassim race was more tolerant of 250 mM NaCl than the Al-Bahah race (cf. Figures 5.5. and 5.6) but the race*salinity interaction in Two-way ANCOVA narrowly failed to achieve significance ($P = 0.065$).

The accelerated ageing treatment (60% RH at 45 °C) was extremely effective, as has been shown previously (Dearman et al., 1986; Bailly et al., 1998; Dell'Aquila and Tritto 1990; Walmsley and Davy 1997), with declining viability at both salinities during the 30 days of ageing. Interestingly, the Al-Bahah race was less severely affected by the ageing treatment than the Al-Qassim one ($P < 0.001$ for the race*ageing interaction in the Two-way ANCOVA), highlighting the genetic diversity of local land races (Hassan 1979). This might be an adaptation to the much higher summer rainfall (and therefore humidity) in Al-Bahah than in Al-Qassim (see Chapter 4), as there is likely to have been selection for good storage properties under local humidity conditions.

The key question was whether accelerated ageing affected salinity tolerance, as this has implications for seed storage conditions and time (Ellis et al., 1990; Navarro and Noyes 2010). Perhaps because it was only possible to test this at two salinities in the event, the answer is somewhat equivocal. On the one hand there was no evidence that the salt tolerance of germination was affected by ageing in the Al-Qassim race, whereas on the other, salt tolerance in the Al-Bahah race was retained significantly better during ageing ($P = 0.002$ for the salinity*ageing interaction in One-way ANCOVA). Again this emphasizes the genetic diversity among local land-races, and possible selection for good storage properties under the more humid conditions of Al-Bahah. Walmsley and Davy (1997) examined the germination response to salt of six species of coastal shingle vegetation after one and 7 years of storage over silica gel at -20 °C; they found a significant degradation of salt tolerance after 7-year's storage in four of them (*Crambe maritima, Eryngium maritimum, Glaucium flavum* and *Honckenya peploides*. In general

is has been suggested that seeds of poorer quality become more responsive than vigorous ones to a variety of stress factors (Perry 1973).

The main aim of the electrolyte leakage experiment was to determine the effects of salinity during imbibition on membrane integrity in seeds and their component parts, as a rapid indication of seed quality (Tao 1978; Loeffler et al., 1988). The conductivities measure clearly reflected the masses of the material involved: highest for whole seeds, lower for the seed coats and lowest for the embryos. However the individual component can be compared across salinity treatments. In both land-races and whatever the component examined, the results indicate very little loss of membrane integrity (seed quality) after imbibition in 250 mM NaCl (Vieira et al., 1999a, 1999b). This is consistent with the percentage germination results, discussed previously, which showed very little impairment at this salinity. In contrast, treatment with 500 or 1000 mM NaCl during imbibition resulted in much greater electrolyte leakage subsequently, which is also consistent with the failure of all seeds to germinate at these salinities. Interestingly, the damage suffered at 500 mM NaCl, although evident in whole seeds and their coats, was not observable in the embryos themselves. Consequently the electrolyte leakage tests for seed vigour, carried out in a few hours, have proved to be good indicators for the germination response to salinity in the full-scale experiment (Bewley and Black, 1985; Don et al., 1981; Hill et al., 1988).

Chapter 6. Effects of salinity on plant growth

6.1 Introduction

The main focus of this thesis has been on the crucial germination stage of the life cycle. However, evolutionary fitness and therefore agricultural productivity necessarily depend on the entire life cycle. The work described in this chapter represents a preliminary investigation of the next phase of life history - responses of vegetative growth in young plants to salinity. This is of particular interest because of the distinctive agronomic techniques that have been evolved by the artisanal farmers using local land-races of wheat in the semi-arid environments of Saudi Arabia (see section 6.2).

Growth reduction in both halophytes and glycophytes occurs because of total osmotic potential of soil water and/or toxic concentrations of soluble salts (Flowers et al., 1977; Greenway and Munns 1980). Negative effects are detected at the whole-plant level, for example as the death of plants or reduction in yield. Suppression of growth may be evident in every part of the plant, but plant tolerance to salinity differs extensively between plant species. Salt stress disturbs all of the main processes that underpin growth, including, photosynthesis, protein synthesis, nutrient uptake and lipid metabolism.

Salinity stress has many effects in checking of plant growth (Hernandez et al., 1995; Cherian et al., 1999). A rapid reaction to salt stress is typically a decrease of the rate leaf area expansion (Wang and Nil 2000). Salt stress similarly results in a significant reduction in the fresh and dry masses of leaves, stems, and roots (Hernandez et al., 1995; AliDinar et al., 1999; Chartzoulakis and Klapaki 2000). In halophytes such as *Rhizophora mucronata,* optimal growth may be achieved at a salinity equivalent to 50% sea-water but growth drops with further increase in salinity (Aziz and Khan 2001). Similarly, in *Salicornia rubra* optimal growth is evident at 200 mM NaCl and the growth deteriorates at higher salinity (Khan 2001). However, most plants (glycophytes) have little tolerance of salinity before growth is impaired. For example in *Raphanus sativus* (radish) whole plant dry mass is reduced at high salinity approximately 80% reduced which could lead to decrease in leaf area development and consequently

decrease the light capture. The small leaf area at high salinity is related to a reduction in specific leaf mechanisms that lead to increase tuber/shoot weight ratio and decrease plant dry weight at high salinity (Marcelis and VanHooijdonk 1999). Nevertheless a salt mangrove *Aegiceras corniculatum* could tolerate up to 250 mM NaCl and 300 mM was discovered to be toxic in this circumstance (Mishra and Das 2003). Salt accumulation was ccompanied by substantial decreases in shoot mass, plant height, number of leaves per plant, root length, and root surface area in tomato plants (Mohammad et al., 1998). Increased levels of NaCl resulted in a significant reduction, in root, shoot, and leaf growth biomass but an increase in root:shoot ratio in cotton (Meloni et al., 2001). (Khan et al., 1999) stated that when *Halopyrum mucronatum* (a perennial grass found on coastal dunes near Karachi, Pakistan) was treated in sand media with 0, 90, 180, and 360 mM NaCl, it was found that fresh and dry masses of roots and shoots reached an optimum at 90 mM NaCl, and further increase in salinity impeded growth, resulting eventually death at 360 mM NaCl (Parida et al., 2004a). (Kurban et al., 1999) reported that in *Alhagi pseudoalhagi* (a leguminous plant), overall plant mass reductions were low at 50 mM NaCl but nevertheless were severe higher at salinity (100 and 200 mM NaCl).

Clearly plant mass and other metrics, such as plant height and tiller or organ number, can provide valuable insights into the effects of salinity on growth. However, the more coherent and powerful trechniques of plant growth analysis, which allow the partitioning of growth into ecologically interpretable components (Evans 1970), have less commonly been applied to these problems (but see Redondo-Gomez et al. 2007).

The hypothesis in this part of the study was that the osmotic effects of salinity on water availability and/or the directly toxic effects of salt would inhibit the growth of the Saudi-Arabian land-races races of wheat. The objectives were (1) to compare the effects of salinity on them with those on known salt-sensitive and salt-tolerant varieties of wheat and (2) partition those growth effects using the methods of quantitative growth analysis to understand better their significance.

6.2. Traditional wheat growing in Al-Qassim region

6.2.1 Land-races

'Land-races' of wheat are genetically diverse mixtures traditionally planted by an area's farmers. They are cleaned and stored for future sowing on their farms by the local farmers. Land-races are generally more rugged and resistant to a variety of problems. Planting of a landrace, or possibly a mix of older varieties, can produce a crop which is reputed to keep out weeds better, be more saline-tolerant and less susceptible to disease, therefore giving a more consistent crop year-on-year under adverse and variable conditions**.**

6.2.2 Sowing time and cultivation practices

Wheat is separated into spring and winter types. Spring wheat grows relatively fast and is sown at the start of spring (usually March); winter wheat is sown at the beginning of winter, between October and the end of November. Winter wheat needs a period after initial growth with consistent temperatures under 7 °C in order for it to be vernalised and become reproductive. However these wheat varieties may be sown earlier with advantage for two different reasons, first to reduce the quantity of seeds needed and second to provide grazing. Seeds may be sown in the middle of September at about 50 kg ha^{-1} and the crop grown until the plants reach 25-30 cm in height; then irrigation is stop completely to allow grazing by animals for forty days, before intense irrigation is applied allow to further growth and the production of spikes at about 12-13 per plant. This produces a full-sized of seed and reduces the height of the plants to approximately 150 cm. On the other hand, a the later sowing at the beginning of January requires 120 kg seeds ha^{-1} and regular irrigation until the harvest time to produce 2-3 spikes per plant.

The sowing site can be a normal bed or a raised bed. A raised bed should mean the soil will warm up approximately a week earlier. The site should be as little over-shadowed as possible both for rain and sun. Some shelter from wind can however be an advantage. However, soil preparation is important before sowing. If the bed is at normal ground level, traditionally this would mean digging over with spade before start sowing, digging and breaking up with fork a week to two before sowing, if necessary re-plowing

immediately before sowing and then raking into a fairly good surface tilth. Treatment for a raised bed could be similar but probably requires less digging as there is less likelihood that soil has become compacted. Usually a wheat crop would be rotated in the next year being a bean or other nitrogen-fixing crop, followed by another crop, which might be alfalfa.

Both winter and spring wheat in an average year will be ready for harvesting in late July to August. If harvested in a traditional manner the crop will be cut and bundled into sheaves and then these stacked into stooks to dry in the open for three to four weeks. The crop will then be ready to be threshed and winnowed at the beginning of September. All this information has been derived from interviewing local farmers and, although they differ in detail from one another, these are the general practices.

6.3 Material and methods

6.3.1 Experimental design

Three bread-wheat genotypes including one salt-tolerant genotype (Karachi 65), and two salt-sensitive genotypes (W9940, TW161) were obtained from the John Innes Centre, Norwich Research Park, Colney, Norwich, Norfolk NR4 7UH. They were compared with the land-race Meyeh *Triticum aestivum* from the al-Qasssim region of Saudi Arabia.

The experiment was conducted in the glasshouse located at University of East Anglia, Norwich, Norfolk, on movable benches 95 cm high 2000 cm wide and 3000 cm long, with daily glasshouse temperature ranging from 25°C during the day and 15°C during the night artificial light are used to give more bright. It was a factorial experiment with 4 genotypes (races) x 4 salinities x 4 harvests x 10 replicate plants. Seeds were selected for uniform size and mass, and surface-sterilized with sodium hypochlorite (1%). Seeds were planted in pots (25 cm in diameter) containing perlite rooting medium size (Figures 6.4-6.9). From one week after sowing, pots were irrigated three times a week with a 20% Hoagland's solution (Hoagland and Arnon 1950). Control plants were irrigated twice a day with 50 ml of distilled water. In the first and second weeks salt-treatment plants were subjected to 50 ml of 100 mM NaCl; in the third week the two

higher salt treatments were increased to 200 and 300 mM NaCl respectively. Salt treatments were maintained for 55 days.

Ten plants were harvested randomly from each treatment, at weekly intervals between 24 and 45 days from sowing, and transported in paper bags directly to the laboratory. Fresh mass was recorded and leaf area determined by using a leaf area meter (LIC-COR Portable area meter Model LI-300). Then plants were partitioned into roots, stems and leaves and placed to dry in an oven at 60 °C for 42 hours, before dry masses were recorded.

6.3.2 Growth analysis

Increases in dry mass were used to derive relative growth rate (R) and this was partitioned into leaf area ratio (LAR) and unit leaf rate (ULR) (Evans 1972). This was accomplished using stepwise polynomial regressions of ln dry mass and ln leaf area on time with the software **HPcurves** for the Windows 95/98/XP platform (Hunt and Parsons 1974). After presentation with replicated measurements of two plant variables Y (mass) and Z (leaf area) at four or more harvests in time t, **HPcurves** fits first, second or third order polynomial exponential curves to the trends in ln$Y$ versus t and in ln$Z$ versus t. The choice of order of polynomial exponential was determined automatically by the program (at $P< 0.05$). The output from **HPcurves** contained tables of primary and derived growth-analytical data. These constituted observed and fitted values of ln$Y$ and ln$Z$, and values of dY/dt, dZ/dt, $(1/Y)(dY/dt)$ (R), $(1/Z)(dZ/dt)$ (relative leaf growth rate), Z/Y (LAR) and $(1/Z)(dY/dt)$ (ULR). 95% confidence intervals are provided for all estimates.

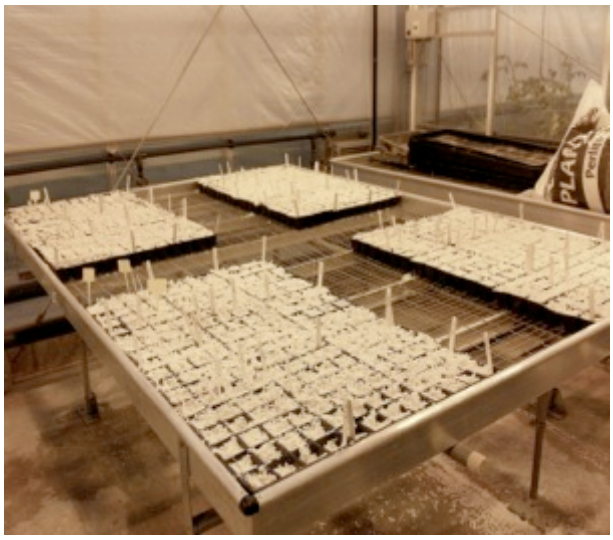


Figure 6.1. The experimental set-up in the UEA glasshouse

Figure 6.2. First emergence of seeds after five days of planting

Figure 6.3. First emergence of seeds after five days of planting

Figure 6.4. Emergences of seeds after two weeks of planting

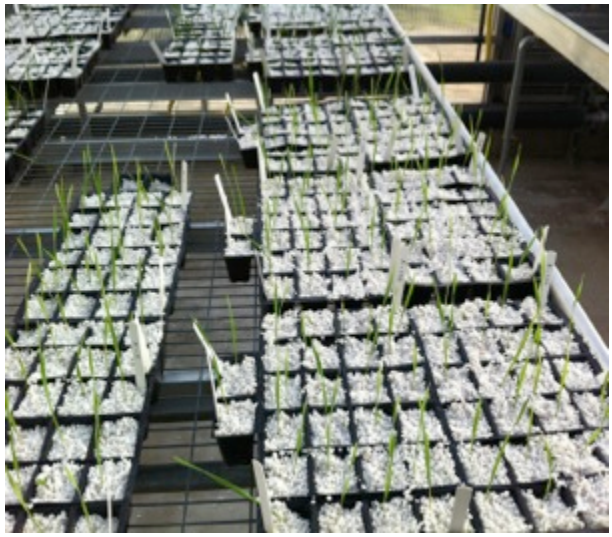


Figure 6.5. Emergences of seeds after three weeks of planting

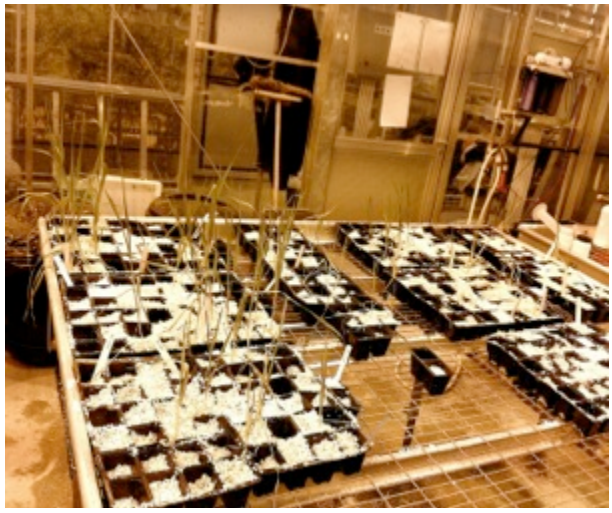
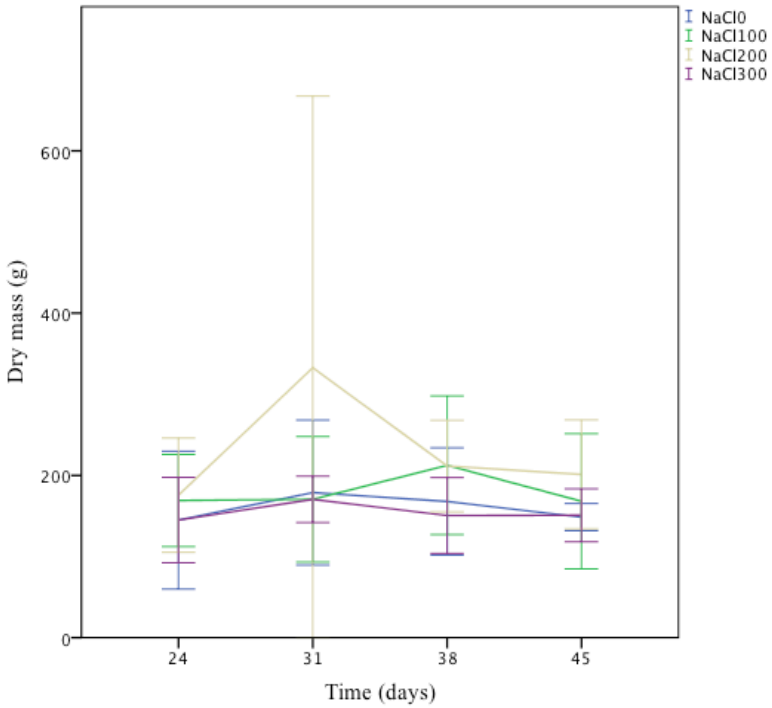


Figure 6.6. The plant near the end of the experiment

6.4 Results

The trends of total dry mass and total leaf area with time for *Triticum aestivum* Meyeh at the four salinities are shown in Figure 6.7. A growth analysis using polynomial exponential regression is presented in Figure 6.8. A linear regression was the best fit for ln dry mass for all treatments and for ln leaf area in all except 300mM NaCl, for which a cubic was fitted; the estimating equations are given in Table 6.1. Relative growth rates (R) in all treatments were constant throughout the experiment. R was not reduced relative to the control (0 mM NaCl) by treatment with 100 mM NaCl, but was progressively lower at 200 and 300 mM (Figure 6.8b). It is clear that the differences in R between salinity treatments could be attributed to differences in Unit Leaf rate rather than Leaf Area Ratio (compare Figure 6.8 c &d).

a)



b)

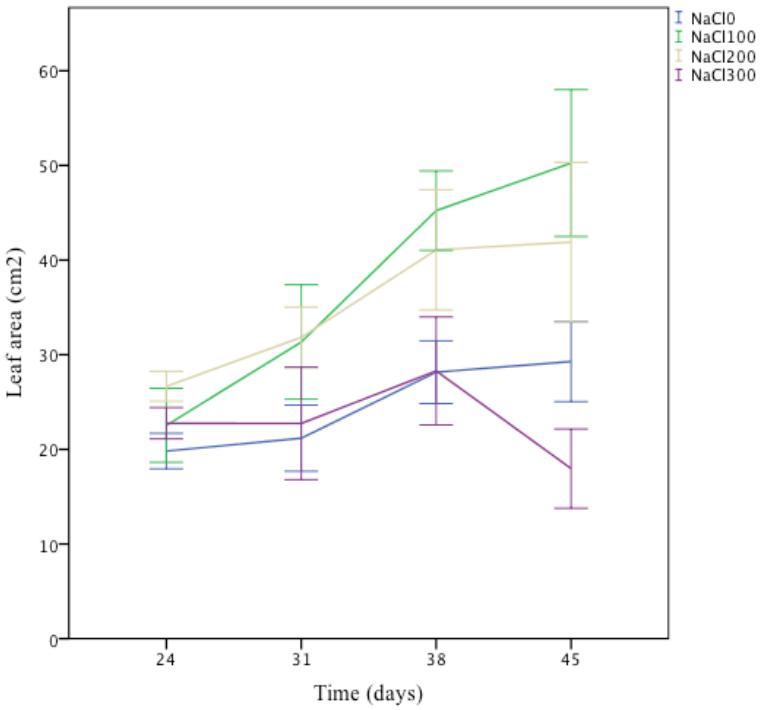
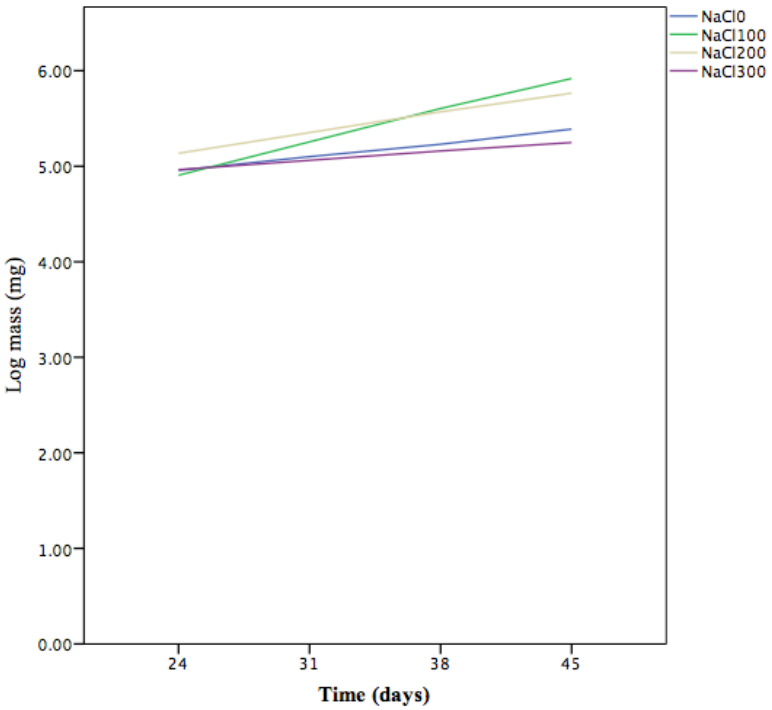
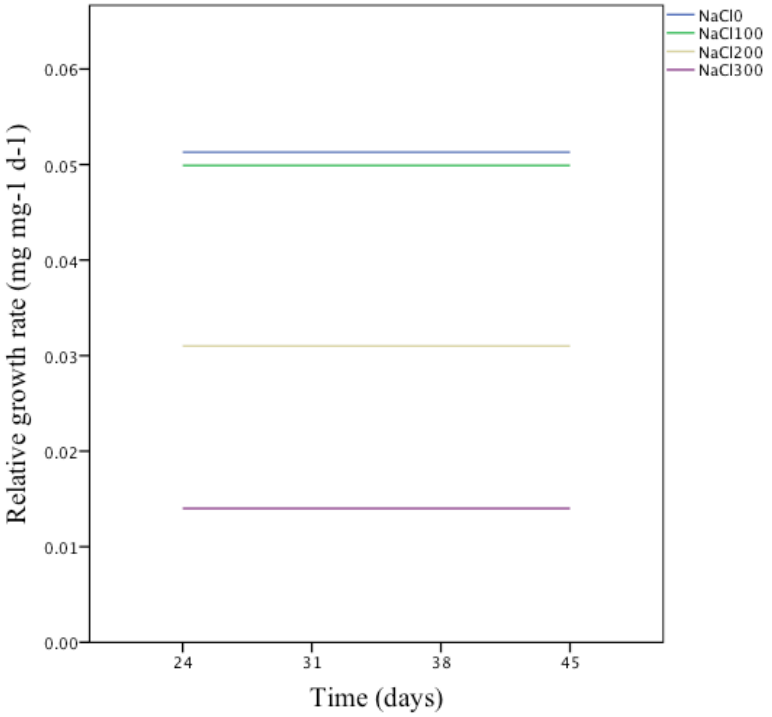


Fig. 6.7. Growth of *Triticum aestivum* Meyeh in response to treatment with 0, 100, 200 and 300 mM NaCl in a glasshouse experiment: (a) dry mass; (b) leaf area.

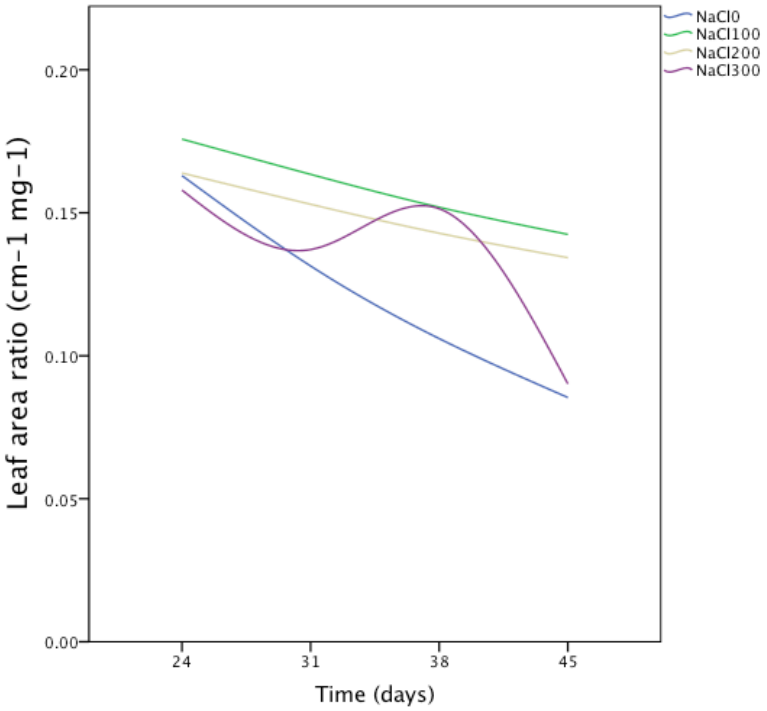
(a)


NaCl0
NaCl100
NaCl200
NaCl300
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Log mass (mg)
24
31
38
45
Time (days)

(b)


NaCl0
NaCl100
NaCl200
NaCl300
0.06
0.05
0.04
0.03
0.02
0.01
0.00
Relative growth rate (mg mg-1 d-1)
24
31
38
45
Time (days)

(c)



(d)

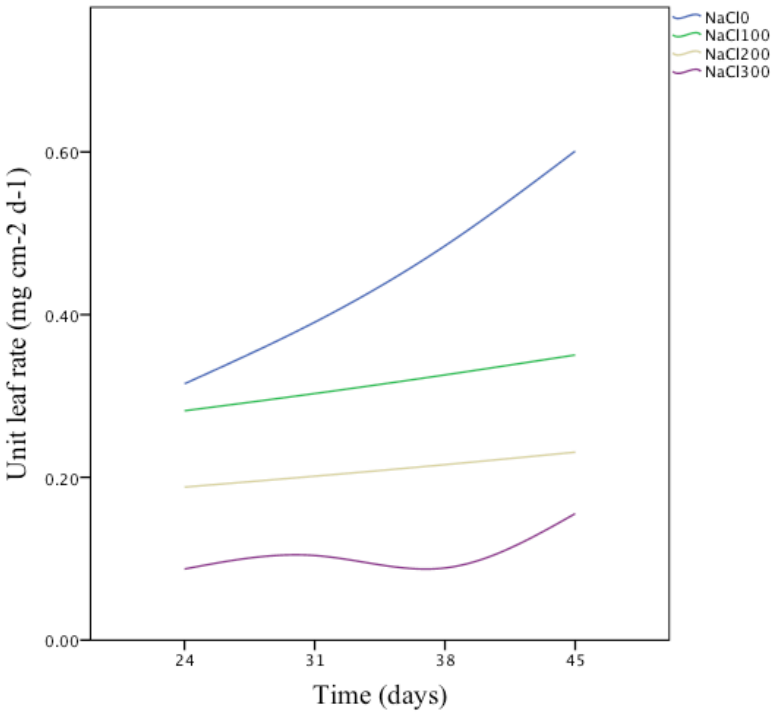
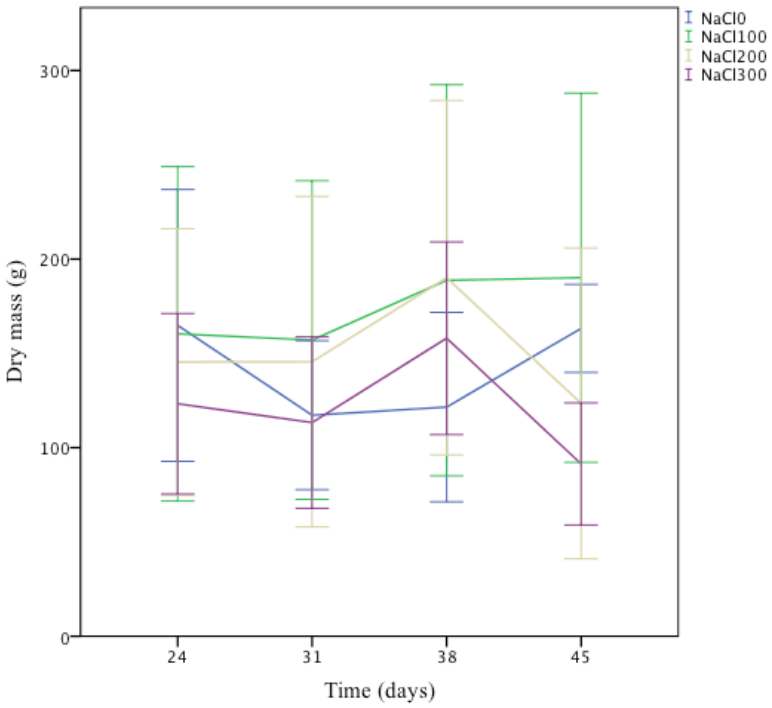


Figure 6.8. Growth analysis of *T. aestivum* Meyeh in response to treatment with 0, 100, 200 and 300 mM NaCl in a glasshouse experiment: (a) ln dry mass; (b) Relative growth rate; (c) Leaf area ratio; (d) Unit leaf rate. Estimating equations are presented in Table 6.1.

The trends of total dry mass and total leaf area with time for *Triticum aestivum* Karachi at the four salinities are shown in Figure 6.9. A growth analysis using polynomial exponential regression is presented in Figure 6.10. A linear regression was the best fit for ln dry mass for all treatments and for ln leaf area in all except 0mM NaCl, for which a cubic relationship was fitted; the estimating equations are given in Table 6.1. In all treatments R was constant throughout the experiment. Relative to the control (0 mM NaCl) R was reduced progressively by increasing salinity (Figure 6.10b). The differences in R between salinity treatments were mainly related to differences in Unit Leaf rate rather than Leaf Area Ratio (compare Figure 6.10 c &d).

a)



b)

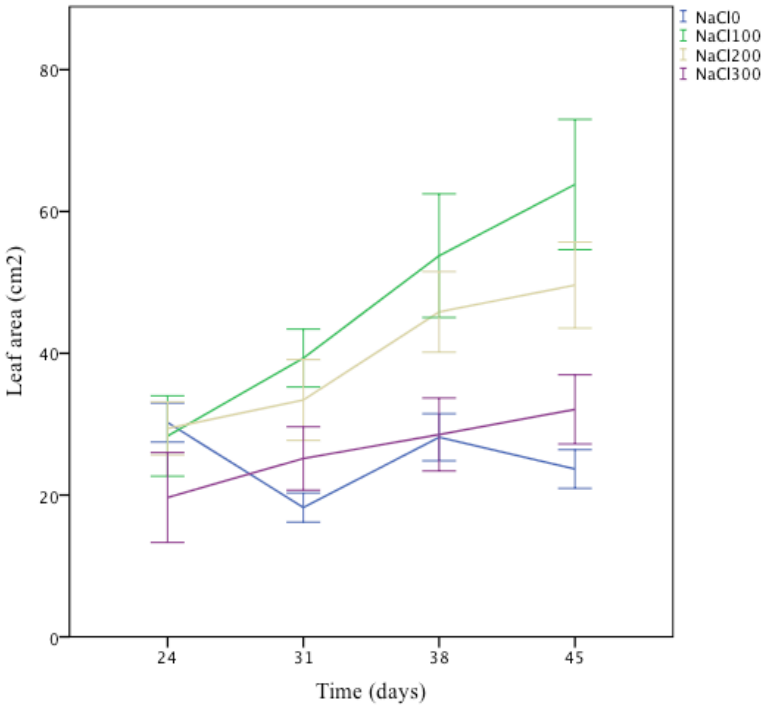
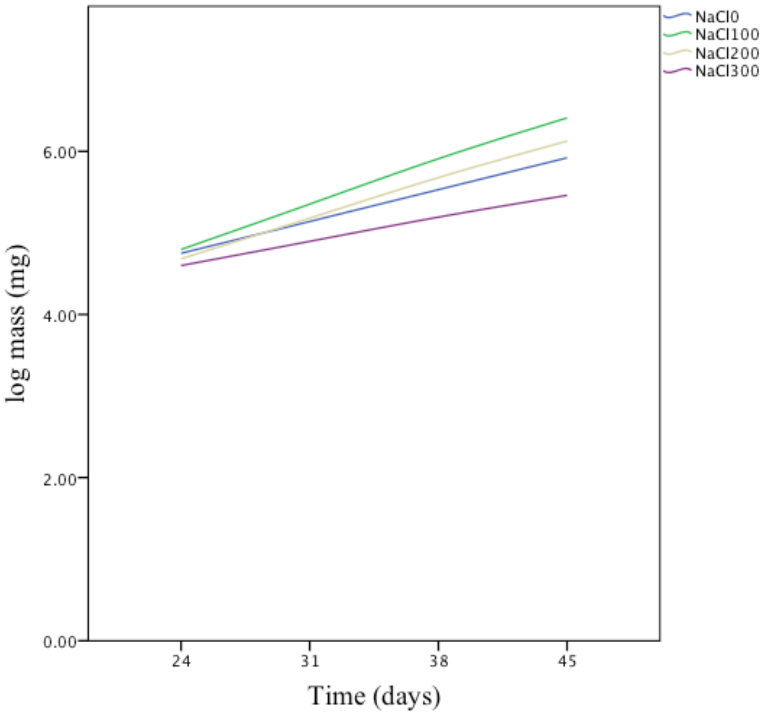
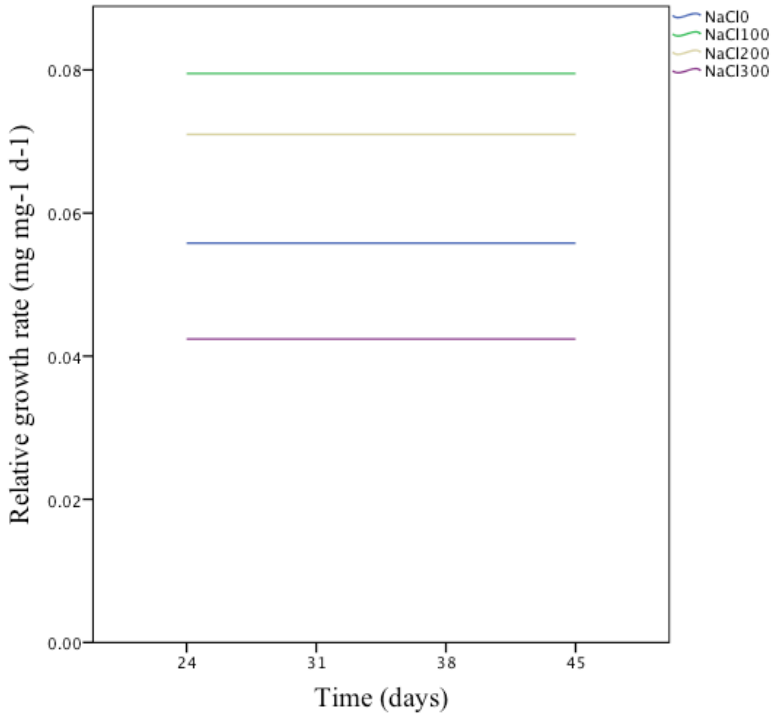


Fig. 6.9. Growth of *Triticum aestivum* Karachi in response to treatment with 0, 100, 200 and 300 mM NaCl in a glasshouse experiment: (a) dry mass; (b) leaf area.

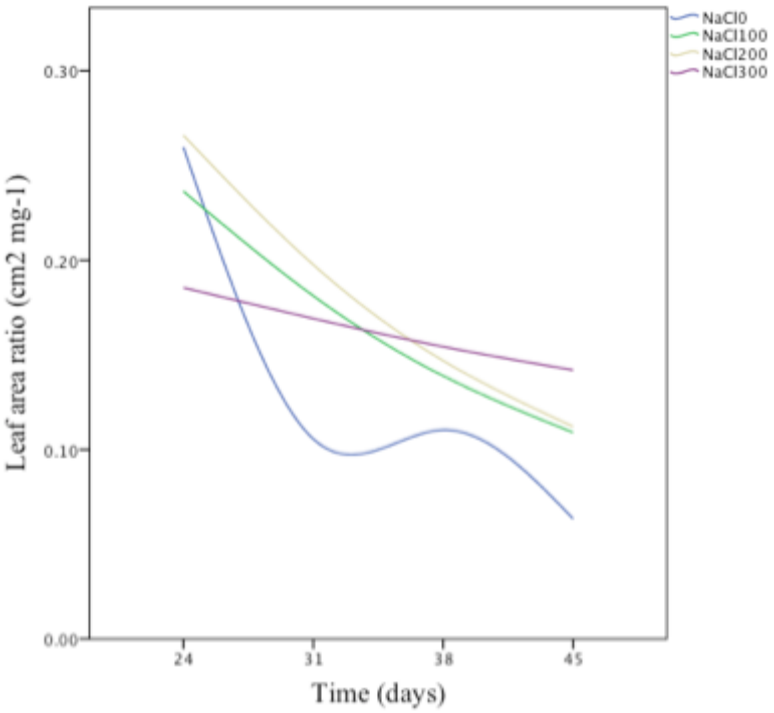
a)



b)


NaCl0
NaCl100
NaCl200
NaCl300
Relative growth rate (mg mg-1 d-1)
0.08
0.06
0.04
0.02
0.00
24
31
38
45
Time (days)

c)


NaCl0
NaCl100
NaCl200
NaCl300
Leaf area ratio (cm2 mg-1)
0.30
0.20
0.10
0.00
24
31
38
45
Time (days)

d)

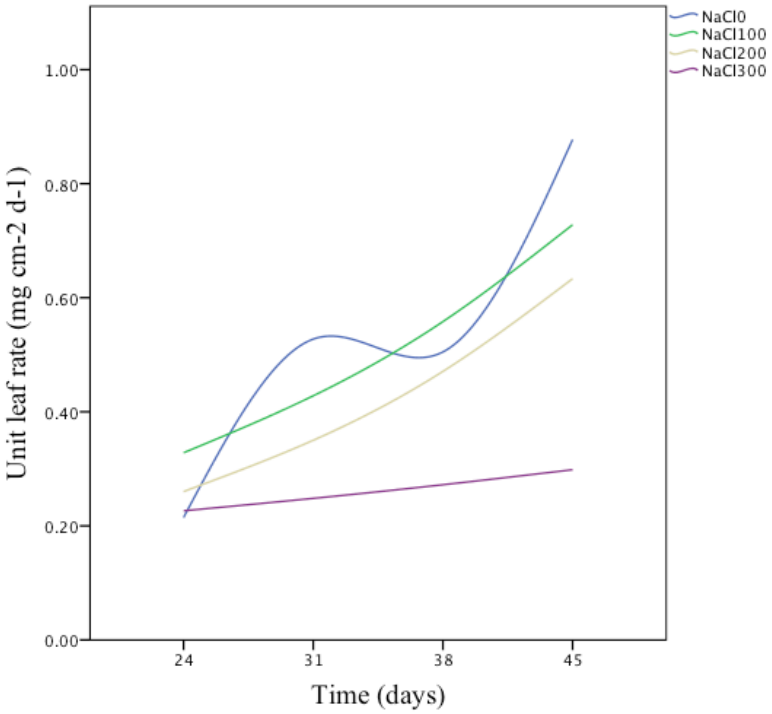
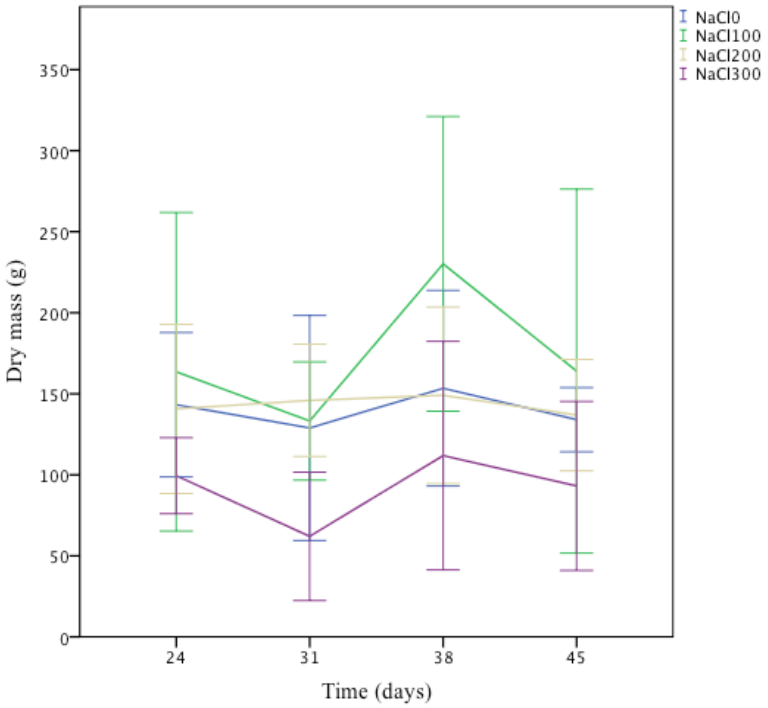


Figure 6.10. Growth analysis of *T. aestivum* Karachi in response to treatment with 0, 100, 200 and 300 mM NaCl in a glasshouse experiment: (a) ln dry mass; (b) Relative growth rate; (c) Leaf area ratio; (d) Unit leaf rate. Estimating equations are presented in Table 6.1.

The trends of total dry mass and total leaf area with time for *Triticum aestivum* TW161 at the four salinities are shown in Figure 6.11. A growth analysis using polynomial exponential regression is presented in Figure 6.12. A quadratic regression was the best fit for ln dry mass for three of the treatments but a linear fit was best for 200 mM NaCl; for ln leaf area, a quadratic fttted best at 0 and 200 mM, but a cubic was employed at 100 and 300 mM; the estimating equations are given in Table 6.1. At 200 mM R was constant but it declined throughout the experiment in the other treatments. Overall, there was little difference in average R, ULR of LAR in the four different treatments (Figure 6.12 b, c & d).

a)



b)

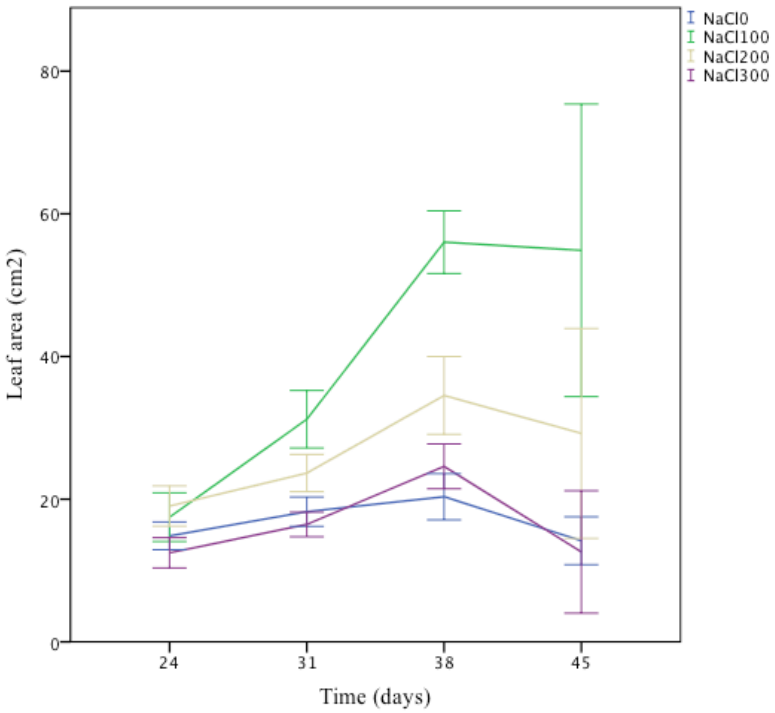
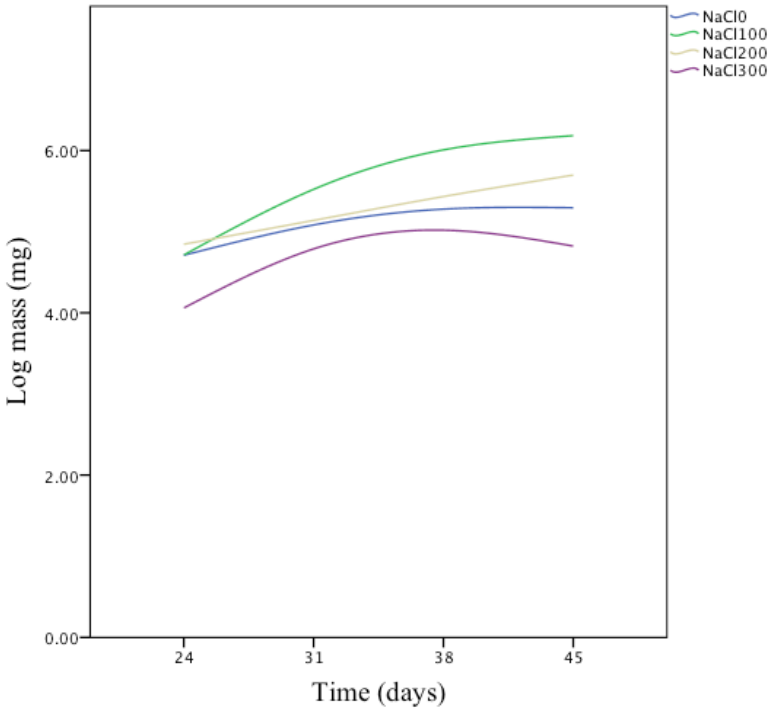
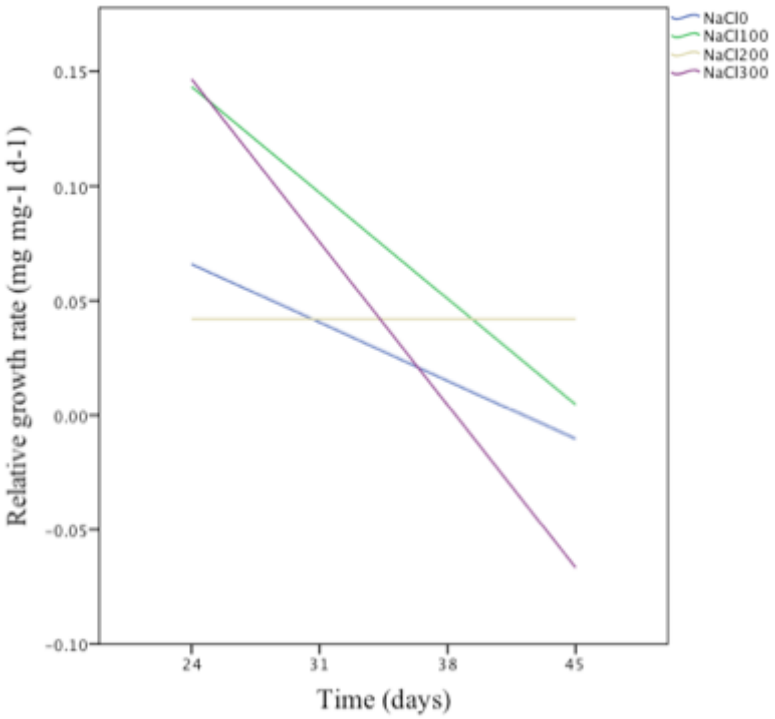


Fig. 6.11. Growth of *Triticum aestivum* TW161 in response to treatment with 0, 100, 200 and 300 mM NaCl in a glasshouse experiment: (a) dry mass; (b) leaf area.

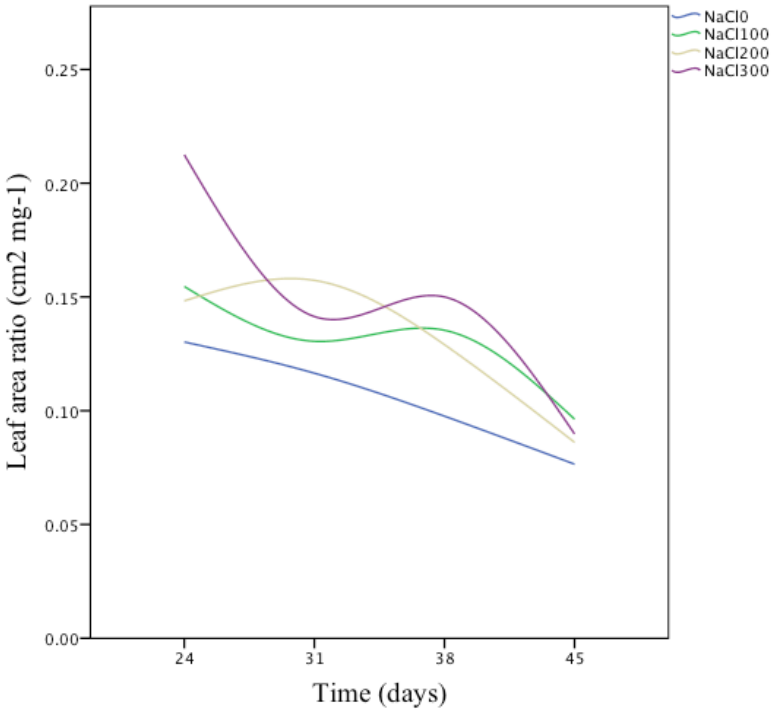
a)


NaCl0
NaCl100
NaCl200
NaCl300
Log mass (mg)
6.00
4.00
2.00
0.00
24
31
38
45
Time (days)

b)


NaCl0
NaCl100
NaCl200
NaCl300
Relative growth rate (mg mg-1 d-1)
0.15
0.10
0.05
0.00
-0.05
-0.10
24
31
38
45
Time (days)

c)



d)

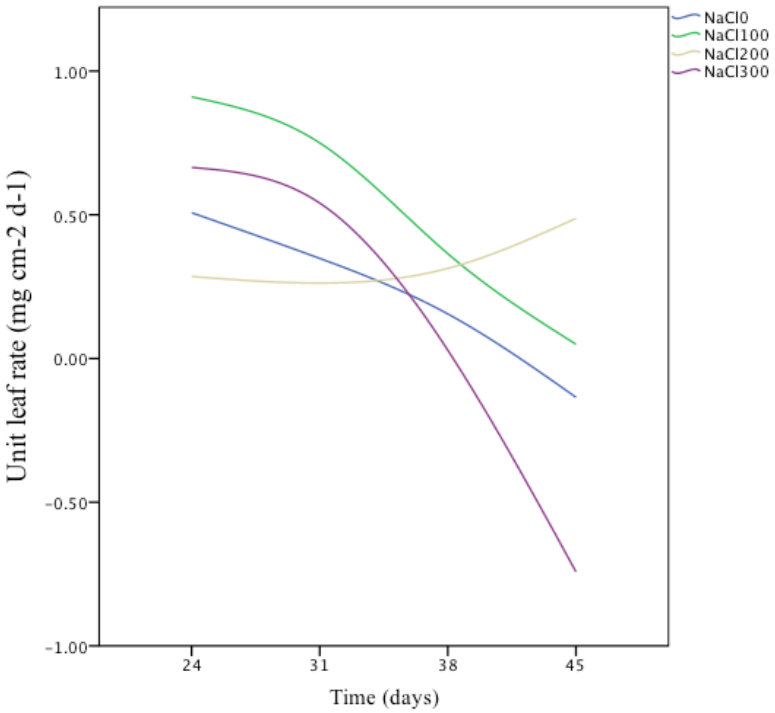
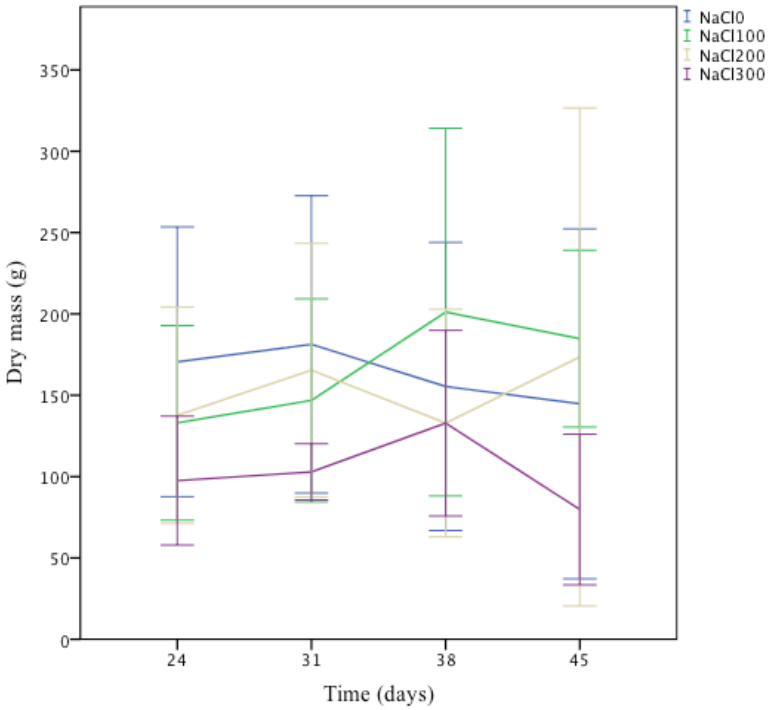


Figure 6.12. Growth analysis of *T. aestivum* TW161 in response to treatment with 0, 100, 200 and 300 mM NaCl in a glasshouse experiment: (a) ln dry mass; (b) Relative growth rate; (c) Leaf area ratio; (d) Unit leaf rate. Estimating equations are presented in Table 6.1.

The trends of total dry mass and total leaf area with time for *Triticum aestivum* W9940 at the four salinities are shown in Figure 6.13. As growth in this variety was particularly poor, significant trends of ln dry mass and ln leaf area with time were not found in most treatments; the exceptions were at 100 mM NaCL, where there were linear fits for both variables, and at 300 mM, where there was a linear only for ln leaf area; the estimating equations, where applicable, are given in Table 6.1. Consequently no useful growth analysis can be presented for this variety.

a)



b)

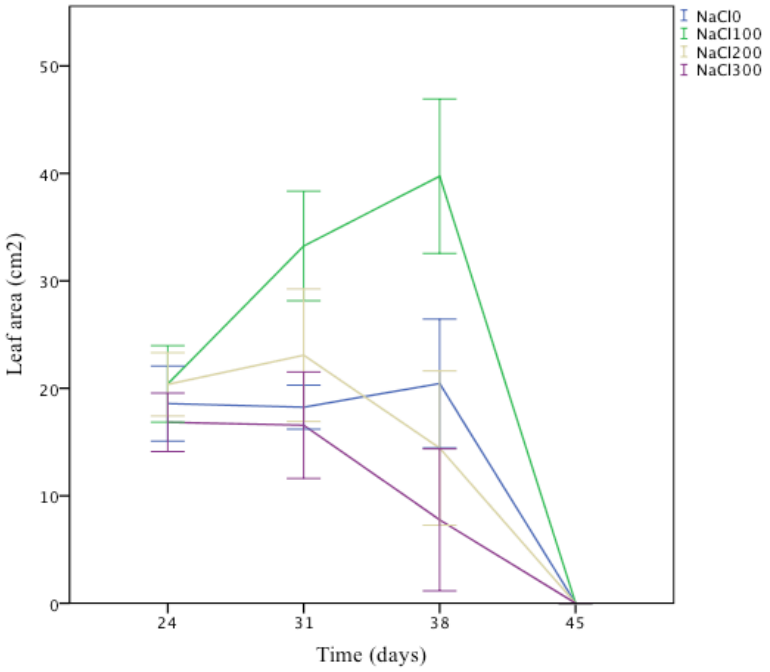


Fig. 6.13. Growth of *Triticum aestivum* W9940 in response to treatment with 0, 100, 200 and 300 mM NaCl in a glasshouse experiment: (a) dry mass; (b) leaf area.

Table.6.1. Log-polynomial estimating equations, derived from HPcurves, relating dry mass (Y) and leaf area (Z) to time (T) for harvests of four varieties of *Triticum aestivum* at weekly intervals between 24-45 days of age.

Variety	NaCl (mM)	Dry mass	Leaf area
Triticum aestivum Meyeh	0	ln(Y) = +3.537810 +0.051347T	ln(Z) = +2.461973 +0.020586T
	100	ln(Y) = +3.673793 +0.049871T	ln(Z) = +2.191139 +0.039492T
	200	ln(Y) = +4.368809 +0.031017T	ln(Z) = +2.801591 +0.021231T
	300	ln(Y) = +4.617566 +0.014009T	ln(Z) = *** -1.652025T +0.051111TT -0.000513TTT
Triticum aestivum Karachi	0	ln(Y) = +3.411023 +0.055765T	ln(Z) = *** -2.721137T +0.079168TT -0.000748TTT
	100	ln(Y) = +2.834204 +0.079454T	ln(Z) = +2.326014 +0.041521T
	200	ln(Y) = +2.928345 +0.071040T	ln(Z) = +2.646499 +0.028684T
	300	ln(Y) = +3.552399 +0.042391T	ln(Z) = +2.192447 +0.029247T
Triticum aestivum TW161	0	ln(Y) = +2.078135 +0.153267T -0.001818TT	ln(Z) = -0.082306 +0.174519T -0.002492TT
	100	ln(Y) = -0.710521 +0.301619T -0.003298TT	ln(Z) = *** -0.869318T +0.031983TT -0.000350TTT
	200	ln(Y) = +3.807989 +0.041982T	ln(Z) = -0.390215 +0.201314T -0.002679TT
	300	ln(Y) = -2.461895 +0.390360T -0.005078TT	ln(Z) = *** -1.707077T +0.056922TT -0.000604TTT
Triticum aestivum W9940	0	No significant fit	No significant fit
	100	ln(Y) = +3.502008 +0.055090T	ln(Z) = +2.021188 +0.043857T
	200	No significant fit	ln(Z) = +4.223420 -0.044329T
	300	No significant fit	No significant fit

*** Indicates an intercept too large for output in the format used by HPcurves.

A summary comparing the relative growth rates of all four varieties over the whole experimental period is presented in Table 6.2.

Table 6.2. Summary of average relative growth rates (mg mg^{-1} d^{-1}) for the whole experimental period of four varieties of *Triticum aestivum* grown in four salinity treaments.

Variety	Treatment (mM NaCl)			
	0	100	200	300
Meyeh	0.051	0.050	0.031	0.014
Karachi	0.056	0.080	0.071	0.042
TW161	0.028	0.074	0.042	0.040
W9940	0	0.055	0	0

TW161 decline throughout the experiment because it is sensitive to the growing conditions.

6.5 Discussion

Overall, growth was unexpectedly poor in this experiment. The highest overall relative growth rates under any conditions were only c. 0.08 mg mg^{-1} d^{-1} (Table 6.2). British wild grasses at optimal temperatures over a similar period from germination can achieve and R of c. 0.2 mg mg^{-1} d^{-1} (Grime and Hunt, 1975) and individually potted cereal plants may have an RGR up to 1.2 mg mg^{-1} d^{-1} even in artificially lit (130 μmol m^2 s^{-1}) growth rooms (e.g. Mallott and Davy, 1978). Relatively restricted growth is likely to have been the result of suboptimal conditions for wheat in the glasshouse environment. The experiment was carried out in late autumn/winter when ambient light valus were low in a glasshouse with heating but limited supplementary lighting. Consequently photosynthesis was almost certainly limited by conditions that were too warm, with consequently low atmospheric relative humidity, for the light energy available. These varieties also showed considerable variability within replicates and this, at least in the case of Meyeh, might be partly due to the genetic variability typically found within land races (Hassan 1979).

However, the main purpose of this experiment was to compare the growth and salt tolerance of a typical Saudi Arabian land-race (Meyeh) with types of previously known performance. Under control conditions, Meyeh grew very similarly to Karachi 65, the variety with established salt tolerance, but it showed substantially less tolerance than Karachi as salinity was increased; Karachi actually increased its RGR up to 200 mM NaCl and at 300 mM its RGR was not much lower than the control, whereas the RGR of Meyeh declined progressively with increasing salinity. Karachi was clearly the most salt-tolerant variety in the trial, as it was able to maintain both the LAR and ULR components of RGR at high salinity, unlike the othere three types. W9940, a known salt-sensitive type, was probably the least salt-tolerant in the experiment, although its growth was so poor and erratic, even in the control treatment, this cannot be stated with confidence. The performance of Meyeh at increased salinity was not obviously any better than that of TW161, another salt-sensitive variety but, again the data for TW161 showed much variability and its performance declined thoughout the experiment in all treatments.

Although it is not possible to ascribe particular salt tolerance to Meyeh in the vegetative growth phase of its life history, it did show features that are consistent with selection for its native habitat in the semi-arid regions of Saudi Arabia. First, its overall growth was not seriously impaired up to a salinity of 200 mM NaCl; this represents a salinity of about 40% that of oceanic seawater and would be very high in comparison with a conventional agricultural system. Second, it was able to maintain its LAR, even at the highest salinity. This implies that it was able to achieve sufficient water uptake, in spite of the low osmotic potential of its root environment, to develop the turgor required for leaf cell expansion. This is a feature of true halophytes, such as *Atriplex portulacoides* (Redondo-Gomez et al., 2007), and in itself could represent an adaptive response to salinity, as high salinity has been shown to affect RGR by reducing turgor pressure and cell wall extension in other species (Peter et al., 1998). The declining growth with increased salinity appears to have stemmed mainly from effects on unit leaf rate. ULR, the rate of dry mass increase per unit leaf area, is essentially analogous to a measure of average net photosynthesis (Evans 1972). Hence we should look to effects on photosynthesis (and possibly respiration, although it is quntitatively less important) for an explanation of the response to salinity. A declining photosynthetic rate with increased external salinity is also a feature of some halophytes and has been associated with declining stomatal conductance (and hence CO_2 uptake) rather than adverse effect on photosynthetic biochemistry (and hence CO_2 fixation) in *Atriplex portulacoides* (Redondo-Gomez et al., 2007). This may also be the case in Saudi-Arabian wheats but further work would be needed to establish it.

Chapter 7. Synthesis and General Discussion

The Arabian Peninsula harbours many local races of crop species (land races) that have been selected and preserved by generations of indigenous farmers. Because of the local selection for productivity, they are likely to be well adapted to the prevailing environmental conditions. Much of the Arabian Peninsula experiences arid or semi-arid conditions with hot, dry summers and erratic rainfall in winter. The resulting net evapotranspiration can cause an accumulation of salts in the surface layers of the soil and hence saline root environments. Plant growth can be severely affected by the osmotic effects of salinity on water availability and the directly toxic effects of high concentration of ions (Flower et al., 1977) and this can be a major constraint on crop productivity (Flowers et al., 1995). However, the climate of the huge area of Saudi Arabia is far from uniform. Hence the local land races would be expected to have, to a greater or lesser extent, different tolerances to drought, high temperatures and salinity. Thus they represent a largely unknown and unexploited source of germplasm that could be of value for crop breeding in a world where food security demands greater productivity in areas considered sub-optimal, or even unsuitable, for cultivation, and projected climate change suggests that growing conditions will become increasingly extreme. The overall aim of the work described in this thesis was to examine some of the tolerances of land races of a range of cereal species that are widely cultivated by artisanal farmers in the Kingdom of Saudi Arabia and deposit representative material for preservation in national and international germplasm banks. For practical reasons, work has focused on seed storage, germination and the early stages of growth, because success at these stages is a prerequisite for any potential crop value, although of course success at later stages of the life cycle would also be necessary for overall crop potential.

The viability of seed collections from the arid Al-Qassim area, as judged from tetrazolium testing, proved to be very high, indicating that it was suitable for germplasm banking and therefore potential use in future plant breeding (Munns et al. 2006). The work in Chapter 2 demonstrated remarkable overall tolerance of germination to high salinity in a broad range of cereal species (Poaceae), *Triticum aestivum* (Arabic local name, meyeh), *Triticum durum* (Arabic local name, logemei), *Hordeum vulgare* (Arabic local name, saear arubi), *Panicum miliaceum* (Arabic local name, mlessa) and *Pennisetum glaucum* (Arabic local name, sudany). Such genetically based variation for

salinity tolerance in plants has been reported in many previous studies. Salt tolerance of crops may also vary with their growth stage (Iqbal and Ashraf, 2013) and difference among genotypes for salt tolerance may occur at different growth stages (Shabala et al., 2013). Germination in *Triticum aestivum* and *Hordeum vulgare* proved to be extremely tolerant to salinity, whereas *Pennisetum glaucum* in particular was found to be relatively salt sensitive. Remarkably, seeds of *T. aestivum* and *Hordeum vulgare* showed some germination up a sodium chloride concentration of 600 mM, a salinity significantly greater than oceanic seawater; even *Pennisetum glaucum* showed germination at 300 mM NaCl, a response more typical of a coastal halophyte (Woodell, 1985; Ungar, 1987) than a crop plant. In fact halophytes themselves normally germinate best in fresh water, with increased dormancy and mortality as salinity increases (Ungar, 1987; Redondo-Gomez et al., 2008). All of the species collected germinated as well in 100 mM NaCl as in distilled water; more remarkably, all of them achieved some germination at 500 mM NaCl, a salinity similar to that of sea water. This substantial tolerance notwithstanding, these experiments were carried out at a typical average ambient temperature and did not consider the effects of the extremes of temperature that would be expected on the Arabian Peninsula.

The more detailed work described in Chapter 3 employed the thermogradient and incubator technology of the Kew Millennium Seed Bank at Wakehurst Place to examine not only the effects of temperature, but also the interactions of salinity with temperature, on germination in the same land races of these five species. Furthermore, it allowed the development and evaluation of seed-testing protocols appropriate for this material that were applied to work described in later chapters. The same land races of the same five species were examined but, for comparison, a commercial variety of winter wheat (*Triticum aestivum* Istabraq) was also introduced. The fine resolution of temperature provided by the thermogradient plate (necessarily limited to a subset of the land races) was complemented by incubator experiments, providing fewer temperature treatments but embracing the full range of species. As before (see Chapter 2) and as expected, germination declined with increasing salinity. Furthermore in general it was apparent the temperature had relatively little effect on final germination percentage in the absence of salinity. However, increasing salinity disproportionately reduced germination towards both extremes of temperature, creating sharper temperature optima. Nevertheless, all the land races showed considerable salt tolerance and the germination of *Triticum aestivum* from Qassim was less severely affected by salinity

and temperature than the commercial variety Istbraq. The use of the thermogradient plate allowed exploration of the methodology for examining basal, optimal and ceiling temperatures - from the relationships between the reciprocal of time to 50% germination and temperature. Where these cardinal temperatures could be determined they were generally consistent with the extremes of average temperature seen in Qassim (see Chapter 4). Non-dormant seeds respond to continually varying temperature conditions in the field but their temperature responses can be characterized from these constant temperature germination experiments if the concept of thermal time is employed. Germination below the optimal temperature is generally modelled as a linear response to accumulated day-degrees above the basal or threshold temperature (e.g. Garcia-Huidoboro et al., 1982a,b) to allow extrapolation to (and from) field conditions. The current investigation validated the use of a basal, optimal and ceiling temperature model derived from constant-temperature investigations (Hardegree et al., 1999) for use with the Saudi Arabian wheat land races. Taking seedling mass 3 days after germination on the thermogradient plate as an indicator of vigour (Butterfield et al., 2013) confirmed that, despite salinity tolerance, the greatest seedling vigour was in the non-saline treatments around the central temperature of the range (19-20 °C).

The experiments in Chapter 4 sought to extend the work by examining the germination responses of land races of *Triticum aestivum* from different environments in Saudi Arabia: the continental, arid climate of Qassim and the less extreme climate of Al-Baha, also taking into account possible differences in the chemical and physical composition of their soils. The most distinctive property of land races from the Kingdom of Saudi Arabia is their perceived ability to grow and crop in environments that would often be regarded as too arid and saline for cereal crop productivity. Chemical and physical analyses of soils samples collected with the plant samples confirmed this general tolerance (Quaye et al., 2013). All of the soils were essentially sandy, with inevitably poor water-retention. All had a high pH and electrical conductivity, indicating a high ionic status that could be explained by the particularly high concentrations of extractable calcium, sodium and chloride ions (Raz and Fluhr 1992). Salinization is the inevitable consequence of evapotranspiration exceeding precipitation in the long term. By agricultural standards, all of the soils were extremely deficient in phosphorus and very poorly supplied with nitrogen. These crops are therefore possibly amongst the most stress-tolerant cereals growing anywhere in the world, a fact that underlines their potential value as sources of genetic variation for the

development of new varieties that might be appropriate for large areas increasingly aridity as a result of predicted global climate change (Rosenzweig and Hillel 1995). Meteorolgical data from the two sites confirmed that the two land races experience distinctly contrasting climates. Although both sites have similarly hot summers, there is virtually no rainfall at Al-Qassim from May to October (and little in October itself), whereas low to moderate rainfall is maintained throughout the summer at Al-Baha. The winters, when most growth occurs, have similar rainfall and mostly mild temperatures. This has implications for the timing of germination and harvest. Sowing of these land-races in Saudi Arabia can be from mid-September to early January (see Chapter 6). Clearly, irrigation would be needed if sowing was before November at Al-Qsaim and by then temperature would be lower than at Al-Baha; however, as irrigation can be used, selection may not necessarily be expected to have favoured germination at the lower temperatures. Given the constraints of drought at the end of the growing season, early establishment at Al-Qassim might be advantageous.

The overall germination responses of these two land races of *Triticum aestivum* on the thermogradient plate confirmed the findings of Chapter 3, as final germination percentage was nearly insensitive to temperature in the absence of salinity but increasing the salinity to 500 mM NaCl (similar to that of sea water) sharply narrowed the response curve to give an optimum of c. 19 °C. Thus the results of this experiment also support an important finding also seen in the work described in both chapters 3 and 5: a progressive narrowing of the range of temperature at which germination can occur as salinity was increased; as elsewhere this narrowing was evident at the higher extreme temperatures at moderate salinity and also at the lower extreme temperatures at higher salinity. However, these overall responses masked subtle differences between land races from the two sites. Land-races collected from two different locations certainly reacted differently to salinity stress, with a highly significant interaction between land-race and salinity; most extraordinarily, Al-Baha seeds did not germinate at all at 500 mM NaCl. Whereas those from Al-Qassim, which had much higher salinities in their soil of origin (cf. Chapter 4) showed up to 70% germination at optimal temperatures (c. 23 °C). Even at 250 mM NaCl, Al-Qassim seeds consistently germinated faster (lower values of t_{50}) and had a broader range of temperature tolerance than those from Al-Baha. Nevertheless, once again seedling masses 3 days after germination indicated greatest seedling vigour in the absence of salinity and after germination at 18-23 °C. Other studies have also found that different

wheat cultivars have different responses to salinity stress, resulting in differing grain yields (Richards et al., 1987; Slavich et al., 1990; Albarih 2010). In a large-scale study of 103 wheat genotypes from across Europe, Asia and the Middle East, genotypes from locations in Pakistan were among the most and least salt tolerant (El-Hendawy et al., 2005).

Having established and quantified the salt tolerance of germination, the aims of next part of the study were related to seed germination in the context of the agronomic practice of the artisanal farmers in the field. The progression of seed ageing during storage becomes progressively more rapid with unavoidable trends in viability (Thomas 2013). Seeds stored at low moisture content and temperatures can resist ageing and maintain viability for long periods (e.g. Suma et al., 2013). A seed moisture content of lower than 10% is generally regarded as necessary for secure storage of seeds, even for a short period (Banks et al., 1998). On the other hand, land races only persist because farmers have stored their own seed collections, individual batches often for several years, in conditions that are far from optimal for the maintenance of seed viability. Thus a deterioration in seed quality with storage is to be expected and it is important to know what its effects might be. This was the aspect addressed in chapter 5. The approach was to subject seeds from the two land races of *Triticum aestivum* from contrasting climatic zones represented by the Al-Qassim and the Al-Bahah regions, to accelerated ageing and investigate their subsequent ability to germinate under saline conditions. The assumption was that aged seeds of poorer quality would be less salt tolerant. A supplementary objective was to investigate the practical value of electrical conductivity (EC) measurements of electrolyte leakage in predicting relative seedling emergence for these land-races of *Triticum aestivum* under different salinity conditions, where average germination lay in the commercially conventional range. As shown in previous chapters, germination of both races showed substantial salt tolerance by the standards of wheat varieties and even in comparison with halophytes. The accelerated ageing treatment (60% Relative Humidity at 45 °C) was extremely effective, as has been shown previously, with declining viability at both salinities during the 30 days of ageing. Interestingly, the Al-Bahah land race was less severely affected by the ageing treatment than the Al-Qassim one, possibly suggesting that it was better adapted for storage in the more humid summers there. However the Al-Qassim race maintained its tolerance of salinity better after aging, reflecting the higher salinities in its soils of origin (cf. Chapter 4). The electrolyte leakage tests for seed vigour, carried out in a few

hours, proved to be good indicators for the germination response to salinity in the full-scale experiment. In particular, the greater electrolyte leakage from seeds experiencing 500 or 1000 mM NaCl during imbibition was in agreement with the failure of all seeds to germinate at these salinities.

Following the detailed studies of seed viability and germination tolerances, a preliminary attempt was made to extend our understanding of the land races into the vegetative stage of growth in the context of the local agronomic practices of the Al-Quassim region (Chapter 6). The focus again was on a local winter wheat land race (meyeh) but for comparative purpose, three other varieties were introduced into the study: a known salt-tolerant variety (Karachi 65) and two relatively salt-sensitive varieties (W9940 and TW161) supplied by the John Innes Centre, Norwich. The approach taken was a glasshouse experiment using soil-free cultivation and the well-established methods of growth analysis with frequent sampling and stepwise log-polynomial regressions of plant mass and leaf area on time. Overall the growth rates recorded were low, probably because of insufficient supplementary lighting at a time of the year when natural light levels were very low, combined with relatively high temperatures. Consequently, when extrapolating to the field situation, the results should be interpreted with caution, but some useful insights were obtained nevertheless. As expected, NaCl salinity had clear inhibitory effects on number of plant growth parameters (cf. Singh et al., 2014). Reduction in dry biomass would be expected because of energy consumption for osmotic adjustment (Hood et al., 2013) and loss of photosynthetic production associated with water stress (Redondo-Gomez et al., 2007). Wheat is a moderately salt tolerant crop (Ahmad et al., 2013) but there were differences between varieties in response to salt; salt-sensitive genotypes showed more decline in dry biomass production in comparison with salt-tolerant genotypes. Similar differences between genotypes of wheat in response to salinity and also hypoxia have been recorded previously (Saqib et al., 2013). Meyeh from Al-Qasssim generally grew similarly to Karachi 65, the variety with established salt tolerance, although it showed substantially less tolerance than Karachi as salinity was increased; Karachi actually increased its RGR up to 200 mM NaCl and at 300 mM its RGR was not much lower than the control, whereas the RGR of meyeh declined progressively with increasing salinity. Although it is not possible to ascribe particular salt tolerance to meyeh in the vegetative growth phase of its life history, it did show features consistent with selection for its native habitat in the semi-arid regions of Saudi Arabia (see Chapter 4), particularly that overall

growth was not seriously impaired up to a salinity of 200 mM NaCl, a feature more characteristic of a halophyte. In addition, it was able to maintain its LAR, even at the highest salinity. This is consistent with its relative salt tolerance at the seed germination stage (discussed previously). It is generally considered that plants with better germination and seedling growth under salt stress will also be more stress-tolerant at maturity and give greater production yields (Ahmadi and Arkedani, 2006). RGR appears to have the potential to provide valuable information needed for comparing parallel morphological and physiological variation among genotypes of wheat landraces of differing salt tolerance. There is great need to generate new breeding material for developing salt tolerant wheat cultivars. Wheat land-races are a main source of genetic diversity in wheat (Asif et al., 2014). However, a range of potential criteria for screening of genotypes for salt tolerance are needed. Salt tolerance plant is not equivalent at all growth stages as it differs relatively over the life cycle. Furthermore single criteria may not be good predictors of plant performance in field conditions. The present study has revealed sufficient genetic variation for salt tolerance at two stages of the life cycle to be of interest for breeding. The salt tolerant Saudi-Arabian land races of *T. aestivum* had different characteristics from other cultivars studied and may offer novel value to breeders.

The aim of this research was to investigate the germination and growth characteristics of endemic land-races of cereal species collected from Saudi Arabia and also to identify material suitable for preserving in the germplasm collection of the Kingdom's rare and indigenous crops for future generations, thus providing plant breeders with the genetic resources necessary for developing stress-resistant cultivars in the future. This work has successfully identified appropriate local populations of five cereal species in rural farms of the Al-Qassim and Al-Baha regions. Bulk collections of seed have been made in a fashion that would be expected to represent the genetic diversity within these land races of cereal species. This will need to be confirmed, preferably by molecular genetic analyses, in the future development and use of the germplasm bank (Van et al., 2002). Thus an important outcome of this work is that fully documented seed collections have been made, dried-down to less than 8% moisture content and placed in long-term storage at -20 °C, with the objective of securing them for the benefit of future research and exploitation. The validity of the methodology and the utility of the germplasm-banking resources established in the Kingdom of Saudi

Arabia have therefore been established. However bulk duplicate samples of seeds also have been stored at Millennium Seedbank, Wakehurst Place (Royal Botanic Gardens, Kew).

The utility of screening seed germination for tolerance to extreme environments, particularly salinity and temperature, has been established as an efficient, rapid precursor to more time-consuming studies of the relevant tolerances for growth and agricultural productivity. Enhanced understanding of physiological processes underpinning salt tolerance would be of advantage in breeding programs, since multiple characters could be combined in the screening for salt tolerance (Munns et al., 2006). Selection will be improved only when there are more detailed descriptors for salt tolerance, and plant physiologists will be able to improve the salt tolerance of crops by specifying precise genes or characteristics for breeders to exploit (Faghani et al., 2014).

There are many ways in which a broad study such as this could be developed and extended. First and foremost, we need to corroborate the outcomes in field environments. There are number of research recommendations that could be followed to assist similar work in the future; these involve modifying the sampling techniques to assess environmental factors and plant performance over longer periods that cover all the life-history stages of populations of these species: survival, growth, and productivity. In addition the current study should encourage molecular assessment approaches using sophisticated techniques in order to reveal the genetic factors underlying the variations among those populations. More broadly, it will also be necessary to give higher priority to the conservation of land races of crop species from arid and semi-arid lands, particularly of the Arabian Peninsula. Global climatic change and food security for a rapidly growing world population suggest that this will become an urgent issue. Specific recommendations include:

1. Expand facilities for long-term storage of germplasm at low temperatures in Saudi Arabia to facilitate increased representation of domesticated species as well as wild ones; collaborate with The Royal Botanic Gardens, Kew, a world famous scientific organisation that is internationally respected for its outstanding living collections of plants and world-class Herbarium, as well as its scientific expertise in plant diversity, conservation and sustainable development in the UK and around the world.

2. Increase the scale of collections, along the lines established by this scoping study; this should include more geographically extensive and larger collections of the cereal species investigated and a wider range of indigenous, locally adapted crops such as sorghum and rice (Gepts, 2006).

3. Carry out molecular genetic analysis of the variation captured by such seed collections for each species as well as detailed eco-physiological work to establish a more comprehensive picture of their environmental tolerances; these should be integrated with the range of approaches tested in this work, with the collection of detailed environmental data, voucher specimens, and an experimental analysis of germination biology as an aspect of standard germplasm-banking methodology (Fourcaud, 2008).

4. Establish botanic gardens, herbaria and nurseries for the cultivation of rare land races and to preserve them from extinction. It is believed that the draft World Bank Protectorate (MAB), pilot project will help to protect the plants, since it includes research on natural methods of breeding threatened species and the establishment of nurseries for plant reproduction (Maloupa et al., 2003).

5. Prepare Red Lists of indigenous cropland races of species whose populations are threatened or endangered, providing legal protection for them in Saudi Arabia.

6. Engage the attention of all parties interested in conservation in Saudi Arabia, such as the Environmental Protection Council, research centres and universities; accelerate the establishment of nature reserves, as recommended by many international and Arab organizations and local communities, especially the study by the General Authority for the Protection of the Environment and Protected Areas proposed for protection of threatened plant species (Rodrigues et al., 2004).

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