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Simulated field environment with combined salt and drought stresses as a platform for phenotyping plant tolerance to salinity

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…..life is short…..

Table of Contents

List of Figures

List of Tables

Zusammenfassung

Salinität ist ein Schlüsselfaktor, der die weltweite agrarische Produktion limitiert und oft gleichzeitig mit Trockenstress vorkommt. Generell sind klassische Selektionsverfahren unter Salzstressbedingungen wenig erfolgreich, da die natürlichen, salinen Böden eine hohe Variabilität aufweisen. Daher ist eine präzise und kostengünstige Beurteilung von salz- und trockengestressten Pflanzen bedeutend für eine schnelle und zuverlässige Selektion in der Pflanzenzüchtung. Spektrale Reflexions- und Temperaturmessungen bieten eine Möglichkeit zur raschen Bestimmung von Pflanzeneigenschaften und Stresserkennung. Eine feld-ähnliche Container- und eine künstlichere Topf- Wachstumsplattform sind bislang noch nicht ausgiebig unter den Wachstumsumwelten von Salz- und Trockenstress beurteilt worden. Daher wurden im Rahmen dieser Arbeit zwei Sommerweizensorten (*Triticum aestivum* L.*)*, die sich in ihrer Salztoleranz unterscheiden, unter Salz-, Trockenstress und deren Kombination in beiden Plattformen durch das Erfassen von Biomasse- und weiterer Pflanzenstressparameter mit spektralen und thermischen Messungen verglichen. Container- und Topfexperimente wurden in Gewächshäusern an der Forschungsstation in Dürnast der Technischen Universität München in den Jahren 2009 und 2010 durchgeführt.

Salinität und deren Kombination mit Trockenstress hatte Einfluss auf Biomasse- und Stressparameter von Weizen, wobei das Ausmaß unter anderem von der Wachstumsplattform und Wachstumsumwelt abhängig war. Agronomische Parameter von Pflanzen, unabhängig von den beiden Sorten, waren stärker mit den aus aktiven und passiven Sensormessungen stammenden spektralen Indizes in den Containern als in den Töpfen korreliert. Die Bestandestemperatur als ein Indikator für Salz- und Trockenstress bei Pflanzen, die sowohl mit Thermographie als auch mit IR Thermometrie gemessen werden kann, zeigte zu verschiedenen Wachstumsstadien der Weizenpflanzen Unterschiede zwischen den Stressbehandlungen und den Weizensorten in den Containern.

Die Ergebnisse unserer Studie weisen darauf hin, dass Topfexperimente im Vergleich zu feldnahen Containerexperimenten im Hinblick auf die Auswahl von salztoleranten Weizensorten zu unterschiedlichen Ergebnissen führen können. Spektrale und thermische Messgeräte bieten gute Möglichkeiten für eine Hochdurchsatzphänotypisierung und ermöglichen es tolerantere Genotypen bei kombiniertem Salz- und Trockenstress und unter Berücksichtigung der Wachstumsplattform (Container vs. Topf) auszulesen.

Summary

Salinity is a key factor limiting the agricultural production worldwide and occurs often simultaneously with drought stress. Classical selection under saline conditions has generally been unsuccessful, partly due to the high variability of naturally saline soils resulting from the different salinity and drought status. Therefore, a precise and cheap assessment of salinity and drought stress to plants is relevant to physiological and morphological studies, which can be used for a rapid selection of cultivars in plant breeding. Spectral reflectance and thermal measurements offer a possibility to rapidly determine plant traits and recognize plant stress. A realistic stress protocol simulating a field platform in close-to-field container and more artificial pot platform has not extensively been evaluated under the growth environments of salinity and drought stress. Thus, we compared two spring wheat cultivars (*Triticum aestivum* L.), differing in their salt tolerance, grown in containers and pots under salinity, drought and combined salinity+drought by assessing biomass and plant stress parameters with spectral and thermal measurements. Container and pot experiments in greenhouses were conducted at the Research Station Dürnast of the Technische Universität München, Germany, in 2009 and 2010.

Salinity alone and combined with drought had different impacts on biomass and stress parameters of wheat depending on the growth platform and environment. Agronomic parameters were generally more closely related to the spectral indices derived from both active and passive sensors for plants grown in containers than in pots regardless of cultivar. Canopy temperature as an indicator of plant´s salt and drought stress obtained by using thermography and IR thermometry showed differences among treatments and between the two cultivars at different growth stages of the wheat plants grown in containers.

Our results suggest that pot experiments may lead to different conclusions for screening salt tolerant wheat genotypes as compared to experiments in close-to-field container platforms. We conclude from our study that spectral and thermal devices offer a great potential for high-throughput phenotyping, which will help breeders to select genotypes being more tolerant to salinity combined with drought stress which is considered as best growth environment, with consideration of the growth platform (container vs. pot).

1 Introduction

1.1 Salinity and drought – an increasing challenge

The world with its increasing population is facing tremendous challenges in producing enough food on a continuously reducing agronomic producing area. Besides manmade losses of areas due to progressive urbanization and rural depopulation, natural reasons due to changing climatic trends cause extensive losses to agricultural production worldwide and an increase in the scarcity of water in the near future (IPCC 2007). Water and salt stress due to drought and soil salinity are the most significant abiotic stresses to limit the production of the world´s stable food crops (Munns 2011).

Soil salinity is a serious threat to global agriculture limiting the agricultural production worldwide (FAO 2008; Tavakkoli et al. 2010), and occurs in 6% of the world's land area and 20% of irrigated land (Munns 2005). In addition to a natural increase in saline soils especially in arid and semiarid regions due to limited rainfall, high temperatures and evapotranspiration, an inadequate freshwater management and the clearing of land for dryland agriculture contribute to an increase in salinity stress to plants. Because salinity is particularly relevant for arid and semiarid areas and due to the increasing frequency of dry periods in many other regions of the world, salinity stress often occurs simultaneously with drought stress (Schmidhalter and Oertli 1991; Hu et al. 2006). Both abiotic stresses reduce the soil water potential and the ability of plants to take up water resulting in reducing the rate of cell expansion in growing tissues, the stomatal conductance and consequently the photosynthetic rate (Munns 2011; Munns and Tester 2008). While drought additionally reduces the nutrient availability, the nutrient uptake by the roots and the transport from the roots to the shoots, saline soils further reduce plant growth at most during the vegetative stage due to specific ion toxicities and ionic imbalances (Hu and Schmidhalter 2005) which was proposed as the two-phase model of salt stress (Munns 1993).

In the near future there will be a need for higher-yielding crops on continuously reducing agronomic producing areas and for crops tolerant to abiotic stresses that are growing in less favourable environments to meet the increasing world food requirement (Passioura and Angus 2010). While conventional plant breeding has doubled the production of crops in regions that are not limited by water, less improvements could be reached in breeding and selecting for

plants tolerant to abiotic stresses (Richards et al. 2010). In addition to the proposed use of molecular markers in physiological breeding (Cattivelli et al. 2008; Richards et al. 2002), accurate and quick sensing methods to screen for plant tolerance under the best and less elaborate screening platform and environment for abiotic stresses could contribute in satisfying the world food requirement.

1.2 Growth platforms and stress scenarios to assess salt tolerance of wheat

Since salinity is particularly relevant for arid and semiarid areas (Ashraf 1994; Hollington 1998; Hu and Schmidhalter 2005), salinity stress often occurs simultaneously with drought stress (Schmidhalter and Oertli 1991; Mittler 2006). Global climate change and increasing fresh water scarcity due to the increasing population will not only lead more often to the occurrence of salt stress combined with drought periods, but also aggravate the severeness of a combined stress. Therefore the traditional approach of comparing the consequence of drought and salinity separately seems to be no longer suitable (Katerji et al. 2009) and plant responses to the combined stresses is of considerable interest (Hao and de Jong 1988). Since crops are routinely subjected to a combination of different abiotic stresses in the field (Moffat 2002), classical selection under saline conditions has generally been difficult, due to the high variability of natural saline soil, the lack of effective evaluation methods for salt tolerance to screen genotypes in breeding programs, low selection efficiency using overall agronomic parameters, and the multigenic nature and complexity of salt tolerance involving morphological, physiological and biochemical parameters among genotypes (Flowers and Flowers 2005). To tackle these problems simplified growth platforms, stress scenarios and stress protocols have been used frequently under controlled and uniform conditions (Hao and de Jong 1988; Singh Greal 2010; Tavakkoli et al. 2010) that make it difficult to extrapolate to real field and more heterogeneous conditions (Homaee and Schmidhalter 2008) due to differences in soil temperature, rates of soil drying, the rootable volume and the availability of nutrients (Townend and Dickinson 1995). This fact may have led to oversimplifications by investigating salinity in differently small-sized pots under controlled conditions representing either a hydroponic situation or soil-based systems and by disregarding the nature of salinity, the type of salinity and the co-existence of drought. Further as drought usually exists in real saline conditions, the osmotic stress as a function of the salt concentration might frequently

exert only a minor effect compared with drought due to decreased soil matric potentials as a function of soil water content (Shalhevet and Hsiao 1986; Schmidhalter and Oertli 1991; Singh Grewal 2010). Both, additive (Wadleigh and Ayers 1945; Hanks et al. 1976; Stark and Jarrell 1980; Shalhevet and Hsiao 1986; Broadbent et al. 1988; Frenkel et al. 1990) and non-additive effects (Hao and de Jong 1988; Schmidhalter and Oertli 1991; Dean-Knox et al. 1998; Shani and Dudley 2001) on plant growth of different cereal and vegetable cultivars have been reported. These effects, however, cannot be tested reasonably in salinized nutrient solutions due to differences in the availability of nutrients, the pore size distribution and the plant-water relations (Tavakkoli et al. 2010). Surrogate media including a matricum such as PEG do not really eliminate this complexity. Maintaining constant temporal and spatial salinity in soil is experimentally attractive, but does not mimic a real saline situation under field conditions (Homaee and Schmidhalter 2008). Even if a uniform salinization can be created in pots, the distribution of salts starts to vary as soon as the roots start taking up soil water (Passioura 2010). This indeed will be accompanied by changes in the soil matric potentials that need to be taken into account when investigating the effects of salinity. Although the above mentioned difficulties were long known, the use of unrealistic stress protocols, such as those for mimicking salinity/drought stress, is the norm rather than the exception in biotechnological studies (Mittler 2006), with nearly each experiment varying in soil and solution characteristics, pot size and many other factors. The search for the best medium and growth platform for growing plants to impose a controlled water deficit with or without salinity has been going on for decades, with the conclusion that there is no perfect medium (Munns et al. 2010).

1.3 Spectral assessment of wheat plants grown in pots and containers under saline conditions

Classifying cultivar characteristics and screening for plant tolerances to various biotic or abiotic stresses is mostly still done manually by classical phenotypic evaluation of cultivars in pot or field-plots. Because classical screening of destructive biomass parameters is expensive and time consuming, indirect parameters that are easy and rapid to use for screening genotypes in a relatively short time are sought. Although field measurements are ideal for realistically assessing salinity stress on plants, small-scaled pot and container platforms offer

the advantage of requiring reduced effort and being highly controlled in terms of salt content and watering. While large containers simulate a close-to-field platform, small pots may suffer from salt that is leached to the bottom (Passioura 2010) and provide a smaller measuring area due to limited plant numbers. The size of pots used for experiments may have an influence on the physiological plant parameters (Poorter et al. 2012) and the spectral reflectance patterns.

No direct comparison between pot and container studies related with spectral measurements under saline conditions has been previously reported. Applying spectral measurements in the field may be more accurate and more natural than in small pots with regard to the spectral sensors' footprints, differences in the canopy area and stress intensity in addition to the influence of the water and nutrient supply on plant growth. Despite these difficulties in small-scaled controlled platforms, salinity is better generated and controlled in pots or containers than in the field, and the results of these smaller scaled experimental systems help to develop our understanding of findings in the field experiments.

Spectral methods may allow for assessing biomass and plant water status by light absorption of water at certain visible and near-infrared wavelengths (Peñuelas et al. 1997b). Spectral sensors providing relative values work either passively or actively using sunlight as the source of light or their own light emitted in specific waveband regions, respectively. Although passive sensors hyperspectrally measure a number of wavelengths in the visible (VIS; approximately 400-700 nm) and near-infrared (NIR; approximately 700-2500 nm) ranges for calculating different vegetation indices (VIs), active spectral sensing is more flexible in terms of timeliness and illumination conditions but limited to few possible selected spectral indices. However, active spectral sensing is mostly restricted to a limited number of wavelengths and indices (Erdle et al. 2011). Furthermore, active sensing, rather than passive sensing, may be better suited to the variable light intensities and qualities in a greenhouse environment.

Various studies have demonstrated that plants exposed to drought or salinity can be differentiated by spectral indices in pots (Rud et al. 2011; Elmetwalli et al. 2012), containers (Elsayed et al. 2011) or in the field (Leone et al. 2001; Leone et al. 2007). Therefore, spectral sensing holds potential for characterising the effect of stresses on plants (Major et al. 2003). Using advanced non-destructive, high-throughput sensors (Rajendran et al. 2009; Thoren and Schmidhalter 2009; Golzarian et al. 2011; Arvidsson et al. 2011) in different platforms for precision phenotyping may enable scientists to accurately estimate the key traits of plants under different abiotic stresses.

Non-destructive spectral analyses of various plant parameters have been previously used in studies relating spectral indices to plant biomass (Mistele and Schmidhalter 2008; 2010), crop water status (Claudio et al. 2006; Winterhalter et al. 2011a,b) and leaf water potential (Peñuelas et al. 1993; Gutierrez et al. 2010; Elsayed et al. 2011). Reflectance has been further used to assess salinity effects on barley (Peñuelas et al. 1997b), eggplants (Leone et al. 2007) and alfalfa and tall wheatgrass (Poss et al. 2006), suggesting that the normalized difference vegetation index (NDVI) and water band index (WBI) allow the response of plant growth to salinity to be studied. Thermal reflectance in infrared wavebands was also applied to successfully differentiate between salt- and non-stressed treatments and wheat cultivars in a container based experiment (Hackl et al. 2012).

Furthermore, new spectral indices that may be more sensitive to high biomass and stress conditions have been developed to better estimate various agronomic parameters. These newly developed and previously tested indices, including R_{780}/R_{740} (Mistele et al. 2004), R_{760}/R_{730} (Erdle et al. 2011) and R_{780}/R_{550} (Takebe et al. 1990), concentrate on a combination of visible and near-infrared light to better identify the green vegetation even at high crop density by the absorbance capacity of chlorophyll. Salinity alters the leaf chlorophyll content of wheat plants (El-Hendawy et al. 2005). A significant correlation between chlorophyll and NDVI values under salinity stress caused by sodium chloride has been shown in a pot experiment (Turhan et al. 2008). Salinity in combination with water deficiency results in a significant decrease in the NIR reflectance and an increase in the VIS reflectance (Poss et al. 2006), which can be explained by decreased absorption by chlorophyll and carotenoid pigments (Peñuelas et al. 1997b; Wang et al. 2002) as well as by the breakdown of the internal cell structure (Elmetwalli et al. 2012).

The reflectance spectrum of vegetation offers broad information about its health, nutritional status and stress condition. Depending on the growth platform spectral measurements are differently well suited for evaluating plant stands.

1.4 Techniques available for measuring plant and leaf surface temperatures

Beside spectral measurements of crops to screen for plant tolerances to abiotic or biotic stress, canopy temperature has long been recognized to be an indicator of plant stress. Its use to quantify drought or salt stress in plants is based upon the assumption that plant temperatures

increase as water becomes limiting because transpiration becomes reduced (Jackson et al. 1988).

Two main methods are currently available for measuring plant temperatures. Thermometry, i.e. IR thermometry, has been used successfully in such contexts for decades. Second one is thermal imaging, which has become increasingly widespread and more intensively investigated over the last decade because of improved technology and decreasing costs. Thermometry and thermal imaging in particular have proven to be especially valuable in screening for crop water stress and water use (Blum et al. 1982; Babar et al. 2006), salt stress (Howell et al. 1984; Sirault et al. 2009) and for plant phenotyping (Inagaki and Nachit 2008; Winterhalter et al. 2011a,b).

Porometers are widely used and still remain the method of choice to measure stomatal conductance in the field or greenhouse (Grant et al. 2006), and it has been found empirically to be related to the canopy temperature as measured with thermography (Möller et al. 2007; Sirault et al. 2009) or infrared thermometry (Jones 1999a). Thermistor-based leaf surface temperature measurements, assessed as by-product in this study as well, may differ considerably from those obtained using an infrared thermometer depending on the air vapour-pressure deficit, the air temperature, and the temperature difference between the leaf and porometer cup (McDermitt 1990; Idso and Allen 1988; Meyer et al. 1985).

Close relationships between stress conditions and the leaf or stem water potential have already been shown using both thermometry (Blum et al. 1982) or thermography individually (Meron et al. 2010; Cohen et al. 2005). These relationships become closer as the intensity of the stress increases. The latter trend is also true for the differentiation of genotypes by leaf temperature (Blum et al. 1982), as well as for the detection of plant stress (Fuchs 1990; Kumar and Tripathi 2008). Similarly, the quality of the relationships with biomass and grain yield under water-stressed and non-stressed conditions also differs. Altogether, however, apart for Blum et al. (1989), generally good relationships using the absolute temperature have been found for the measurements deriving from both thermometry (Rashid et al. 1999; Babar et al. 2006) and thermography (Inagaki and Nachit 2008). Finally, Selige and Schmidhalter (2001) and Winterhalter et al. (2011a,b) have shown a great potential of thermal infrared measurements to assess biomass fresh weight of field-grown maize plants as compared to NDVI-based reflectance measurements.

Yet, despite the promise of thermography and thermometry as useful tools for phenotyping and for crop water-stress mapping, measurements must be made under stable conditions that

exclude as many influencing factors as possible, including the time of day, the spatial resolution and angle of view of the instrument (see Alchanatis et al. 2010; Munns et al. 2010). In addition, the soil itself can often have a distorting influence: the lower the soil coverage, the higher the difference between the average thermal image temperature and the real canopy temperature is, depending on the difference between the soil and canopy temperatures (Rodriguez et al. 2005). Thus, it has been suggested to reduce the potential impact of the soil by measuring at an angle between 25 and 35° to the horizontal (Blum et al. 1982; Rashid et al. 1999) as well as by further processing of the thermal images to exclude the soil and other non-plant parts via thresholds based on special reference surfaces such as a background (Sirault et al. 2009) or by combining digital colour photos with thermal images (Möller et al. 2007; Wang et al. 2010). Finally, differences in canopy temperature have also been found between the centre and boundary areas of a plant stand, with better relationships observed between stomatal conductivity and canopy temperatures when measurements are taken in the centre of the canopy (Möller et al. 2007). As long as the influencing parameters on temperature measurements can be excluded previously and subsequently, canopy temperature seems to be a reliable indicator of plant stress.

1.5 Objectives of this study

The three main objectives of this study were:

Section I: The first section compared soil and plant parameters in a realistic stress protocol simulating a field platform in large containers with salinity alone, drought and combined salinity+drought stress and a pot platform including the same scenarios by using two cultivars, Sakha 61 and Sakha 93, known to differ in their salt tolerance. Our hypothesis are that firstly different growth platforms with different volumes of available soil cause different effects on plant biomass, and secondly the effects of the salinity- and combined salinity+drought stress environment on plants differ between pot and container experiments dependent on the salt tolerance of the used cultivars. The objectives of the first section are to evaluate: (1) different growth platforms with a different soil volume (pot vs. container); (2) moderate salinity stress alone using an equimolar combination of NaCl and $CaCl_2$; and (3) combined salinity+drought stress to screen for salt tolerance.

Section II: The second section represents the first direct comparison of a pot and a close-to-field container platform by applying active and passive spectral measurements under salinity stress conditions. Specifically, we sought to assess spectral measurements by relating well-known and tested spectral indices to the fresh weight, the water content of the above-ground biomass, and the water potential and relative water content of the youngest fully developed leaf for the treatments of control, salinity and salinity combined with drought at selected growth stages. In particular, the second section evaluates: (1) a pot and a close-to-field container platform using active and passive spectral sensors at different growth stages in 2009 and 2010; (2) the potential use of various spectral indices for the non-destructive assessment of biomass and plant stress parameters under salt stress; and (3) the spectral stability to differentiate two Egyptian wheat cultivars differing in their salt tolerance under combined salt and drought stress and control conditions.

Section III: The third section represents the first direct comparison of canopy and leaf surface temperatures measured with thermometry, thermography and thermistor porometry. We sought to assess these non-destructive techniques in a container experiment comparing drought-, salinity- and combined salinity+drought stress treatments to evaluate the advantages and disadvantages of their applications and the quality of the obtained canopy and leaf surface temperature measurements. In particular, the third section evaluates: (1) two spring wheat genotypes that differ in their salt tolerance; (2) treatments of salinity, drought and salinity combined with drought; (3) the influence of the soil on the thermal images during different growth stages; and (4) the potential for non-destructive thermal measurements to replace the highly time-consuming and destructive measurements of leaf water potential and biomass parameters in order to have high-throughput measurements and genotyping.

2 Materials and Methods

2.1 *Study site and growth conditions*

Experiments were conducted at the Dürnast Research Station of the Chair of Plant Nutrition (Technische Universität München) in Freising, Germany. The first experiment was carried out in a heated greenhouse during the winter months of 2009 from September 17, 2009 through February 16, 2010. The second experiment was conducted in the summer months of 2010 from April 13, 2010 through August 2, 2010 in a non-heated, comparable adjoining greenhouse with a removable roof. While the roof was always closed during the winter experiment, it was predominantly in the open position during the summer experiment, except for rainy days, thereby exposing the plants to nearly ambient temperature and radiation conditions characteristic for nearby fields.

Two Egyptian spring wheat cultivars differing in their salt tolerance, Sakha 61 and Sakha 93, with the latter being the more salt tolerant one (El-Hendawy et al. 2005, Hackl et al. 2012), were grown in 376-L plastic containers (LxWxD of 95 cm x 55 cm x 70 cm and soil surface area of 0.52 m²) and in 15-L pots (depth of 29.5 cm and soil surface area of 0.049 m²) placed on a steel carrier 1 m above concrete floor. Two hundred sixty four seeds of each cultivar were sown in six rows in the containers filled with 350 kg of sandy soil, which consisted of 66.5% sand, 20.5% silt and 13% clay. Twenty-eight seeds, which resulted in a similar seed density (approximately 510 seeds/m^2) compared to the containers, were sown in the pots filled with the same soil. Both cultivars were tested under four treatments as follows: control, salinity, drought and combined salinity+drought. Each treatment was repeated three times in the pot experiment resulting in 24 block-placed pots and six times in the container experiment resulting in 48 block-placed containers to conduct both non-destructive spectral and thermal analysis and destructive measurements.

Air temperature and relative humidity were recorded throughout the experiment (Model OAK, Toradex, Horw, CH), with the sensors isolating using polyurethane and aerated with a ventilator. Four sensors were distributed over the entire experiment and were located at a height between 1.5 and 2 m above the greenhouse floor. A fifth sensor unit, positioned at the canopy surface within the plant stand, also included sensors to record total solar radiation

(Model Pyr total solar radiation sensor, Decagon Devices, Inc., Pullman, WA, USA) in addition to air temperature and humidity (Model Humidity/Temp sensor with radiation shield, Decagon Devices, Inc., Pullman, WA, USA). All meteorological data were acquired every ten minutes and recorded with a data logger (Model Em50 digital/analogue data logger, Decagon Devices, Inc., Pullman, WA, USA).

Soil salinization was achieved by leaching the soil in both platforms five times with an equimolar solution of 0.12 M NaCl and $CaCl_2$ before sowing until an electrical conductivity (EC) between 12 to 14 dS m^{-1} in the soil solution was reached. To avoid an osmotic shock for seedling emergence, a 7-cm layer of non-saline soil was placed on the top of the salinized soil. During the growth period, the EC was detected in situ at soil depths of 20 cm in the pots and at depths of 20 and 50 cm in the containers using soil salinity sensors (5000L10, Soilmoisture Equipment Corp., Santa Barbara, USA). Tensiometers (T3, UMS, Munich, Germany) were installed at soil depths of 25 and 50 cm with oblique angles of 51° and 66°, respectively, at the side of the containers and at a soil depth of 20 cm in the pots for measuring the soil matric potential. The drip irrigation system with 14 pressure compensated arrow drippers arranged in three rows per container was automatically turned on for 7.5 minutes to provide 5 L of tap water per container when the soil matric potential reached a threshold value of -0.035 MPa. The pots were weighed daily and the water loss was replaced by adding tap water by hand to an adjusted soil water capacity of 70%. To ensure an optimal nutrient supply, the NPK(S) compound fertilizer “Hakaphos Blau” (Compo, Münster, Germany) was applied four times at different growth stages in the same amounts per area in the containers and pots. Its compositions are: 15% (N), 10% (P2O5), 15% (K2O), 2% (S), 2% (MgO), 0.01% (B), 0.02% (Cu), 0.075% (Fe), 0.05% (Mn), 0.001% (Mo) and 0.015% (Zn).

Salinity and drought stress relevant growth stages occur during vegetative and reproductive periods. Thus, two drought cycles were induced in vegetative and reproductive growth stages by withholding irrigation water for the salinity+drought treatment (Table 1). These two drought periods were imposed to study whether spectral relationships can be established at different growth stages and different levels of stress, the latter also being due to differences in biomass.

The first drought cycle started at ZS 15-21 (seedling growth with 5 leaves unfolded to the beginning of tillering) and ended at ZS 39-65 (end of stem elongation to half-way anthesis) (Zadok´s scale, Zadoks et al. 1974) in both growth platforms in 2009, and started at ZS 14-20 (seedling growth with 4 leaves unfolded to the beginning of tillering) and ended at ZS 50-59

(beginning of florescence emergence to completed emergence of florescence) in both growth platforms in 2010. The second drought cycle started at ZS 65-71 (half-way anthesis to kernel watery ripeness) and ended at ZS 75-85 (medium milk to soft dough development) in both growth platforms in 2009, and started at ZS 65-70 (half-way anthesis to beginning of milk development) and ended at ZS 75-83 (medium milk to early dough development) in both growth platforms in 2010 (Table 1).

Drought stress cycles were terminated based on clearly visible drought symptoms of plants, which indicated symptoms of moderately severe drought stress and were further based on tensiometric values.

Table 1. Climate data and time schedule of the winter experiment in 2009 and summer experiment in 2010, i.e. at days after sowing (DAS), Zadok's stage (ZS) or growth stage (GS), air temperature (AT), solar radiation (SR) and vapour pressure deficit (VPD). The measurements for AT, SR and VPD, carried out at noon at 2 m above-ground, are shown at the beginning and end of both drought cycles in both years. No data (n/a) for the SR and the VPD were available at the beginning and end of drought cycle 1 in 2010.

	2009							2010						
	Date	DAS	ZS	GS	AT	SR	VPD	Date	DAS	ZS	GS	AT	SR	VPD
					— °C —	– lux –	– kPa –					— °C —	– lux –	– kPa –
Sowing	September 17	0						April 13	0					
Beginning of drought cycle 1 container/pot	October 30	43	15 - 21	Seeding growth - Tillering	20.3	9566	1.3	May 11	27	14 - 20	Seeding growth - Tillering	18.7	n/a	n/a
End of drought cycle 1 pot	November 11	55	39 - 47	Stem elongation - Booting	28.3	5070	2.5	June 8	55	50 - 59	Florescenc emergence	28.2	n/a	n/a
End of drought cycle 1 container	November 19	63	47 - 65	Booting - Anthesis	23.3	12800	1.7	June 10	57	53 - 59	Florescenc emergence	36.5	> 65535	4.2
Beginning of drought cycle 2 pot/container	December 1	75	65 - 71	Anthesis – Kernel watery ripeness	30.4	5179	3.0	June 16	63	65 - 70	Anthesis – Beginning of milk development	16.4	5223	0.4
Middle of drought cycle 2 pot/container	December 15	89	71 - 75	Kernel watery ripeness – Medium milk	29.6	4794	3.2	June 28	75	70 - 73	Beginning of milk development – Early milk	33.4	> 65535	1.2
End of drought cycle 2 pot/container	December 23	97	75 - 85	Medium milk – Soft dough development	24.3	8308	1.7	July 8	85	75 - 83	Medium milk – Early dough development	30.8	> 65535	3.3

2.2 *Section I: What are reliable platforms and stress scenarios to assess salt tolerance of wheat plants?*

The soil and plant measurements recorded in both growth platforms only during the summer experiment of 2010 were used for section I.

2.2.1 Soil sampling and analysis

During the growth period of 2010, the EC in the salinized containers was detected in-situ at soil depths of 20 and 50 cm, and in the salinized pots at 20 cm using the previously mentioned soil salinity sensors. Soil osmotic potential in MPa for treatments salinity and salinity+drought was calculated by MPa = -0.036 x EC (dS m^{-1}).

Soil matric potentials were recorded daily using the above mentioned tensiometers in the containers at 25 and 50 cm and in the pots at 20 cm soil depth.

Soil samples were taken with a soil probe at four soil depths for containers and two for pots with an interval of 15 cm at each destructive biomass sampling (May 11, June 8 and July 6). Volumetric soil water content was calculated based on the soil fresh and dry weight. Oven dried samples of the soil were sieved through a 2 mm sieve for determination of their Na^+, Ca^{2+} and Cl^- content. To determine the Na^+, Ca^{2+} and Cl^- content of the soil, dried soil samples were suspended with 1:1 soil-to-H_2O and shaken for one hour. Soil suspensions were centrifuged with the centrifuge GS-6 (Beckman Instruments GmbH, Munich, Germany) and filtered. Chloride was determined using an ion chromatography analyzer (LC20-1, Dionex, Sunnyvale CA, USA). Sodium and calcium were determined with the flame photometer (ELEX 6361, Eppendorf, Hamburg, Germany).

2.2.2 Destructive biomass sampling

Twenty plants of each container and two plants of each pot were harvested for the determination of the aerial biomass dry weight (DW) at the beginning and end of the first drought cycle and at the end of the second drought cycle. Fresh weights (FW) of shoots at destructive biomass samplings were determined, and then plant materials were dried at 65 °C for 48 h to determine their dry weights.

2.2.3 Final harvest parameters and analysis of the ion contents in the shoots

At maturity, plants were harvested and above-ground dry weight, grain dry weight per m^2, thousand grain weight (TGW) and the grain number per ear were determined. Dried straw samples of the shoots were stored for ion analysis. For ion analysis of plant materials at final harvest, oven dried straw was ground into a fine powder by passing them through a 0.5-mm diameter sieve. For the determination of the Na^+, K^+ and Ca^{2+} content, 300 mg of ground plant materials was digested by adding 3 mL concentrated HNO_3 (65%) and 2 mL H_2O_2 (30%) for 30 min at 2600 kPa (80 psi) in a MDS-2100 microwave oven (CEM Corporation, Matthews NC, USA). After digestion, each sample was brought up to a 50 mL final volume with distilled-deionised water. The concentration of Na^+ and K^+ was determined with an inductively coupled plasma emission spectrometer (ICP, Liberty 200, Varian Australia, Mulgrave, Australia). The Ca^{2+} content was determined with a flame photometer. For Cl^- analysis, 100 mg of sample was extracted with 50 mL distilled water and was shaken for one hour and then filtered. Chloride was determined as described above for soil samples.

2.3 *Section II: Spectral assessment of wheat plants grown in pots and containers under saline conditions*

The EC and the soil matric potential were measured according to *Section II*. Soil and selected plant parameters of both experimental years were used for the analysis.

2.3.1 Above-ground biomass sampling and determination of leaf water relations

The water potential of the youngest, fully developed and sun-exposed leaf was simultaneously measured with a pressure chamber (PMS instruments, Corvallis, OR, USA) (Schmidhalter et al. 1998) when spectral measurements were performed (Table 2). The relative water content of the youngest fully developed leaf was determined by taking its fresh, saturated and dry weights. For each measurement day, one to three leaves from each container were sampled depending on the stability of the ambient radiation conditions to perform all measurements under comparable and similar radiation conditions. Three destructive biomass samplings were performed (beginning of drought cycle 1, end of drought cycle 1 and end of drought cycle 2) using 20 plants from each container and 2 plants from each pot at each sampling date. The fresh weight and water content of the above-ground biomass were determined and further related to the calculated spectral indices. Spectral reference measurements were performed on the same day or one day before/after the destructive biomass samplings.

2.3.2 Spectral reflectance measurements

Passive and active optical sensor systems were mounted on a moveable vehicle that allowed passing by the containers and pots within 5-10 minutes per spectral sensor. Passive sensor systems depend on sunlight as the source of light, whereas active sensors are equipped with light-emitting components providing radiation in specific waveband regions. However, both types of sensor systems, passive and active, measure the amount of light reflected by the crop by converting the light signal into electrical output. The measurements were taken between noon and the early afternoon to provide the best possible conditions for passive recordings. While clear sky conditions prevailed at measurements during the summer experiment, the weather was predominantly cloudy during the winter experiment. All devices were used at a nadir position of approximately 60 cm above the plant stand in the containers and 40 cm

above the plant stand in the pots for both seasons to constantly sense the central areas and to observe the different spectral sensor footprints.

A bi-directional radiometer (HandySpec® Field Spectrometer, tec5, Oberursel, Germany) was used as a passive device to enable hyperspectral readings. This bi-directional radiometer (BDR) contained two Zeiss MMS1 silicon diode array spectrometers with a spectral detection range of 300-1150 nm and a bandwidth of 3.3 nm (Mistele and Schmidhalter 2010). One unit was linked to a diffuser detecting solar radiation as a reference signal. The second unit simultaneously measured the canopy reflectance with a 22° field of view (FOV) of circular shape with a diameter at half signal maximum of 23.5 or 15.5 cm at a distance to the canopy of 60 or 40 cm, respectively. The bi-directional radiometer was calibrated with a PTFE white standard (Spectralon® Target, Labsphere, Inc., New Hampshire, USA). Spectral indices were calculated according the formulas shown in Table 3.

The two active devices used are commercially available sensor systems (GreenSeeker RT100®, NTech Industries, Inc., Ukiah, CA; and Crop Circle ACS-470®, Holland Scientific, Inc., Lincoln, NE). The GreenSeeker uses two LEDs as a light source and detects the reflection of each in the VIS (656 nm with an approximate 25 nm band width) and NIR (774 nm with an approximate 25 nm band width) spectral regions. The FOV of this device was a narrow strip of approximately 61 cm x 1.5 cm (0.009 m^2) at a height of 66-112 cm above the plant canopy (NTech Industries 2007) with a strip length at half signal maximum of 20.6 or 14.7 cm at a distance to the canopy of 60 or 40 cm, respectively.

The Crop Circle device operates similarly to the GreenSeeker device, but it allows for more flexibility in the wavelengths detected, because it emits white light and offers a choice of selectable interference filters. Filters for the 670, 730, and 760 nm wavelengths were selected to record reflectance data. The FOV of the Crop Circle was an oval with a range of approximately 32° x 6° (Holland-Scientific 2008) with a diameter of the long side of the oval at half signal maximum of 26 or 15.5 cm at a distance to the canopy of 60 or 40 cm, respectively (Kipp *et al.* 2012, unpublished). The active sensors were calibrated before delivery according to the manufacturers' information, and no additional calibration was further required. While the GreenSeeker automatically calculates the NDVI index from the reflection values at 656 and 774 nm, the Crop Circle derived R_{760}/R_{670} index needs to be calculated from the relevant reflected wavelengths (Table 3).

Table 2. Climatological conditions and differences in measurement times indicated by days after sowing (DAS) for the leaf water relations (LWRM; water potential and relative water content of the youngest fully developed leaf) and spectral measurements (Spectral) of the winter experiment in 2009 and summer experiment in 2010. The air temperature (AT), solar radiation (SR) and vapour pressure deficit (VPD) were measured at 2 m above-ground. No data were available for the VPD at 55 DAS in the pots in 2010 (n/a).

Container									
	LWRM	Spectral	Time of day	AT		SR		VPD	
	DAS			min	max	min	max	min	max
			h	°C		lux		kPa	
2009									
	61	61	12:30-14:00	28.9	31.4	6467	> 65535	2.0	2.8
	89	89	13:00-15:00	28.6	30.5	1784	5269	2.9	3.4
	95	95	12:30-14:30	30.3	31.1	2350	8080	3.3	3.4
2010									
	57	57	12:30-14:00	37.8	40.3		> 65535	4.5	5.8
	75	75	12:30-14:30	31.0	34.5		> 65535	1.0	1.3
	85	85	12:30-14:30	30.8	32.1		> 65535	3.4	3.9

Pot									
	LWRM	Spectral	Time of day	AT		SR		VPD	
	DAS			min	max	min	max	min	max
			h	°C		lux		kPa	
2009									
	54	54	12:30-13:30	31.9	33.6	4079	7272	3.0	3.5
	89	89	13:00-15:00	28.6	30.5	1784	5269	2.9	3.4
	95	95	12:30-14:30	30.3	31.1	2350	8080	3.3	3.4
2010									
	55	55	12:30-14:00	29.3	30.5		> 65535	n/a	n/a
	75	75	12:30-14:30	31.0	34.5		> 65535	1.0	1.3
	85	85	12:30-14:30	30.8	32.1		> 65535	3.4	3.9

Eight spectral vegetation indices were calculated in the VIS and NIR ranges out of the passive spectral data that offer narrow band reflectance values (Table 3). One vegetation index, which was comparable to the passive vegetation index, was selected for each active spectral device (Crop Circle, R_{760}/R_{670}; and GreenSeeker, NDVI using R_{656} and R_{774}). Active and passive sensor devices were applied throughout the plant life cycle in both experiments in 2009 and 2010, except for the GreenSeeker device, which was not available before the end of drought cycle 1 in 2009.

Table 3. Spectral indices used and assigned to the used spectral sensors (HandySpec (HS), Crop Circle (CC), GreenSeeker (GS)).

Index name	Formula	Reference	Sensor type
Simple ratio (SR)	R_{760} / R_{670}	Pearson and Miller 1972	HS; CC
NDVI	$(R_{800} - R_{680}) / (R_{800} + R_{680})$	Peñuelas *et al.* 1997*a*, Claudio *et al.* 2006; Mistele and Schmidhalter 2008	HS; GS
WBI/NDVI	$(R_{900} / R_{970}) / [(R_{800} - R_{680}) / (R_{800} + R_{680})]$	Claudio *et al.* 2006; Peñuelas *et al.* 1997	HS
NIR/NIR	R_{780} / R_{740}	Mistele *et al.* 2004; Mistele and Schmidhalter 2010	HS
NIR/NIR	R_{760} / R_{730}	Erdle *et al.* 2011	HS
NIR/Green	R_{780} / R_{550}	Takebe *et al.* 1990; Mistele *et al.* 2004; Mistele and Schmidhalter 2008	HS
Water Band Index (WBI)	R_{900} / R_{970}	Peñuelas *et al.* 1993; Claudio *et al.* 2006	HS
REIP (Red edge inflection point)	$700 + 40((R_{670}+R_{780})/2-R_{700})/(R_{740}-R_{700})$	Guyot *et al.* 1988	HS

2.4 *Section III: A comparison of plant temperatures as measured by thermal imaging and infrared thermometry*

The EC was measured according to *Section I* and *II*. Soil and chosen plant parameters of the container experiment only in the summer experiment of 2010 were used for the analysis.

2.4.1 Measurements of canopy and leaf surface temperature

Average canopy temperatures were measured using thermography and thermometry. Measurements were taken on four days throughout the summer experiment in 2010 (Table 4) to assess the influence of the soil coverage and the different stress treatments on the plant growth parameters of the two cultivars.

Table 4. Measurements conducted at selected growth stages, corresponding to Zadoks stages (ZS) and time of day, for canopy and leaf surface temperatures using thermography (TG), thermometry (TM) and thermistor (porometer) (TP) measurements under the prevailing conditions (air temperature (AT), canopy temperature (CT) and vapour pressure deficit at canopy level (VPD)).

Date	Explanation	Used technique	Days after sowing	ZS	Time	AT (°C)	CT (°C)	VPD (kPa)
May 11	Beginning of drought cycle 1	TG	28	12-22	09:30-10:00h		16.2-17.0	0.83-0.87
May 26	Middle of drought cycle 1	TG, TM, TP	43	31-33	14:00-14:30h	28.3-28.9	24.8-25.6	1.35-1.41
June 10	End of drought cycle 1	TG, TM, TP	58	49-61	15:00-15:30h	38.3-38.7	31.8-32.2	2.02-2.04
July 8	End of drought cycle 2	TG, TM, TP	86	73-85	11:00-11:30h	32.9-33.7	28.9-29.7	2.23-2.33

Thermometry measurements used a hand-held infrared thermometer (Model AG-42, Telatemp Crop, Fullerton, CA, USA) with a resolution of 0.1 °C, an accuracy of +/- 0.2 °C and a field of view of a 4° cone. To minimise any influence of the exposed soil, the instrument was held so as to view the crop at an angle of 55-60° from the nadir at a distance of approximately 1 m away from and 70 cm above the canopy at the long side of the container. In addition, the measurements were recorded in the central part of each container capturing 2 plant rows in the

field of view (target spot size at given angle and distance to the canopy was an ellipsoidal area of approximately 300 cm²).

Thermal images were obtained with an uncooled infrared thermal camera (Model T335, FLIR Systems, Oregon, USA) that operates in a wavelength range of 7.5-13 µm with a thermal resolution of 0.05 °C; it produces a spatial resolution of 320 x 240 pixels. A lens with a field of view of 45° x 34° was used. The emissivity was set to 0.96, which differs slightly but negligibly from the emissivity of plant leaves (approximately 0.98; Jackson et al. 1981). The thermal camera was mounted on a mobile carrier vehicle in the nadir position directly above the containers (similar to the active and passive spectral measurements) with a fixed distance to the plant stand (approximately at 0.5 to 0.8 m above the top of the canopy) regardless of the development stage or plant height. The vertically directed position of the thermal camera, in contrast to the oblique view of the infrared thermometer, was chosen to assess the mixed soil-plant effects and to evaluate possible container boundary effects on the average thermal image readings.

Both instruments, thermal camera and hand-held IR thermometer, were calibrated against a black body (Model IRS-350 Blackbody, Voltcraft, Hirschau, Germany) with deviations from fixed 30 °C of 0 °C and -0.1 °C, respectively.

Finally, the leaf surface temperature was measured with a porometer (Model LCi portable photosynthesis system, ADC BioScientific Ltd, Hoddesdon, UK) using a thermistor directly attached to the abaxial leaf surface. The youngest, fully developed top leaves (being fully exposed to solar radiation) from the central part of the container were used.

Porometer thermistor measurements were made simultaneously with the thermometry and thermography measurements within a short time period (30 minutes) to avoid any perturbing effects due to changing conditions, which would be particularly relevant for the first measurement date in 2010. The weather conditions during the measurement days were highly stable with regard to temperature, sunlight and zenith angle.

2.4.2 Analysis of thermal images

Data export was performed using the software FLIR QuickReport 1.2 SP 1, with their subsequent statistical analysis being performed using “R” version 2.12 (R development Core Team 2010).

Because bare soil and the other non-plant parts are significantly hotter than the vegetation itself (Luquet et al. 2003), we sought to separate these areas on the thermal images from those covered by plants using a threshold approach.

Threshold values were determined visually, according to observed valleys or inflection points in the observing histograms or density curves. The areas selected on the basis of these thresholds were visualized and corrected as needed to optimize the separation of areas covered by plants. A strip of 60 pixel rows (25%) was cut from the image of the long container side that was not adjacent to other containers to minimize the influence of any boundary effects on the processed thermal images.

2.4.3 Measurements of water potential and plant biomass

Measurements of the water potential of the youngest, fully-developed, sun-exposed leaf were conducted of plants in the containers according to the procedure described in *Section II*. Measurements were performed simultaneously with the canopy and leaf surface temperature measurements (Table 5). For each measurement day, one or two leaves in each sampled container were assessed.

Three destructive biomass samplings were done in the containers according to the description in *Section II*, using twenty plants from each container at each sampling date, beginning on the left side of each container and excluding plants directly at the container boundaries. This procedure was adapted to secure an intact plant stand on the adjacent side of the container for the temperature measurements. At the end of the experiment straw and grain yield were determined in all containers.

2.4.4 Evaluation of the mixed soil-plant pixel error

The influence of the soil was determined by comparing the unprocessed with the processed thermal image and was normalized by referring to the plant temperature range at a given day. Hence, a mixed soil-plant pixel error (A, in %) was calculated as:

$A = 100*[|(T_{ws} - T_{wos})|/(\text{Max. } T_{wos} - \text{Min. } T_{wos})]$, where

T_{wos} and T_{ws} represent the thermal image temperatures without and with soil, respectively. Max. T_{wos} and Min. T_{wos} represent the maximum and minimum plant canopy temperature without soil influence of all images taken at a given day. Differences can thus be better

visualized instead of using the absolute difference between processed and unprocessed images.

Table 5. Details of destructive and non-destructive measurements including sampling dates for the final harvest of the drought (d), salinity+drought (s+d), control (ctr) and salinity (s) treatments.

Date	Days after sowing	Temperature measurements	Pressure chamber measurements	Destructive biomass sampling	Final harvest
May 11, 2010	28	X		X	
May 26, 2010	43	X	X		
June 10, 2010	58	X	X	X	
July 8, 2010	86	X	X	X	
July 15, 2010	93				X (d, s+d)
August 2, 2010	111				X (ctr, s)

2.5 Statistical analysis of data

All data were tested for normality and homogeneity of variances using the Kolmogorov-Smirnov and Levene's tests, respectively, using IBM SPSS Statistics 19.0 (IBM, Armonk, New York, USA).

Section I: Stress parameter, growth platform and cultivar effects on the biomass parameters and ion contents of the straw were tested by using the multivariate GLM analysis (ANOVA). Multiple comparisons using Duncan´s test were performed whenever the multivariate GLM analysis indicated significant differences ($P < 0.05$).

Section II: The influence of the year (encompassing different season and growth conditions) on the biomass- and water-related parameters was tested using a multivariate GLM analysis. For the comparison of the two cultivars with regard to the agronomic parameters and spectral indices, a t-test for independent samples was used. The significance of all linear relationships for the different parameters was tested using a coefficient of determination from the Pearson Product-moment correlation coefficient. Calculations of the relationships were done using treatments control, salinity and salinity+drought, only.

Section III: Stress parameter and cultivar effects as well as interactive effects on the canopy and leaf temperature were tested by using an ANOVA analysis. The comparison of the canopy and leaf surface temperatures among treatments and measuring methods (thermography, thermometry and thermistor) used a Duncan test, whereas the comparison of the two cultivars used a t-test for independent samples. The significance of all the relationships for the different parameters was tested by calculating the coefficient of determination from the Pearson Product-moment correlation coefficient. Unless stated otherwise, all statistical analyses used a nominal alpha of $p < 0.05$.

3 Results

3.1 Section I: What are reliable platforms and stress scenarios to assess salt tolerance of wheat plants?

3.1.1 Soil salinity and soil water status depending on the treatment and growth platform

In-situ measured soil salinities, expressed as soil osmotic potential, at 20 cm soil depth were initially at about -0.5 MPa in the pots and containers for the two salinized treatments and remained almost stable until the beginning of the first drought cycle in 2010 (Figure 1).

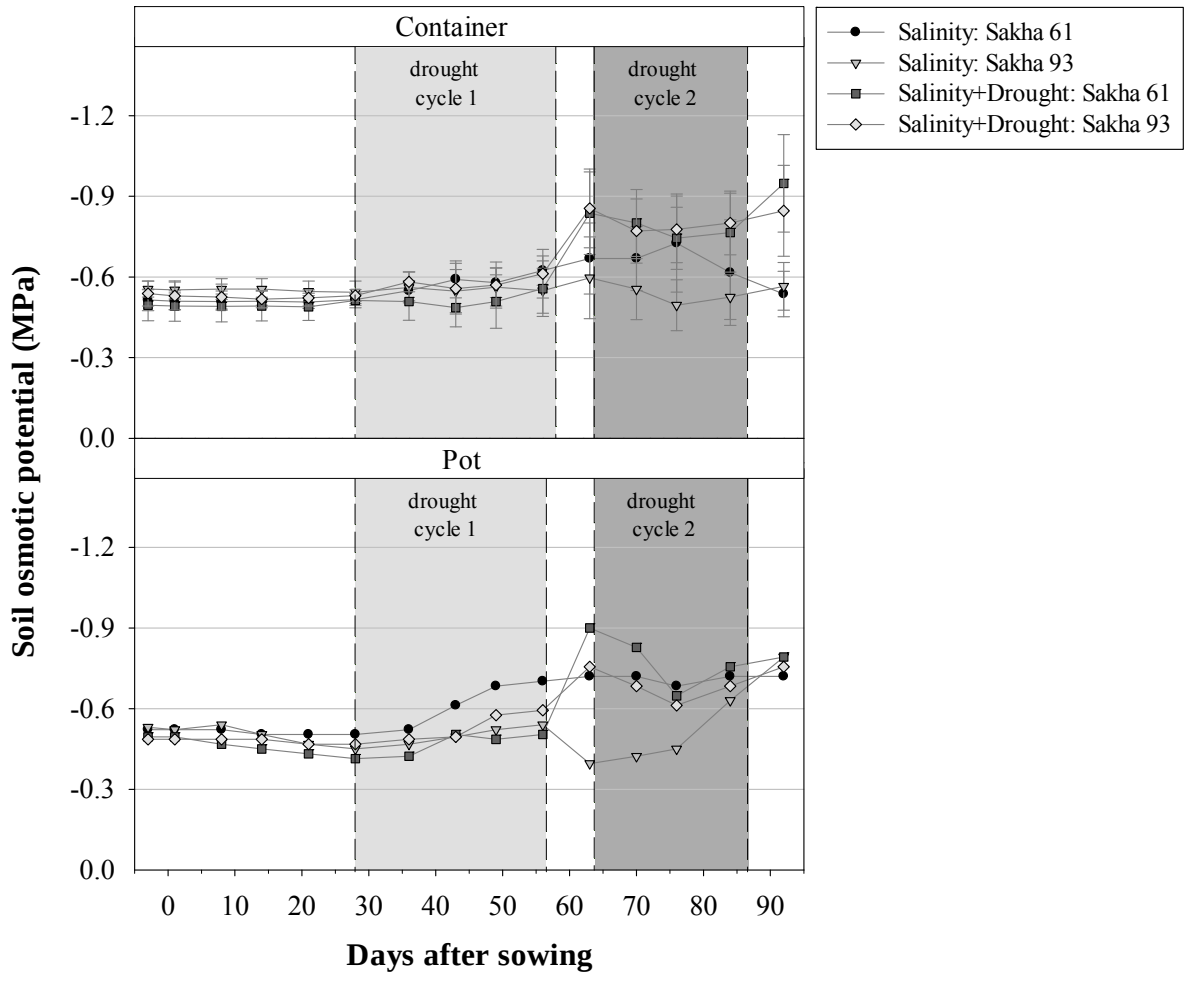

Figure 1. Osmotic potential of the soil for the treatments salinity alone and salinity+drought in 2010. The osmotic potential was calculated according to the EC values, which was measured weekly with in situ salinity sensors at a soil depth of 20 cm and 50 cm in the containers and of 20 cm in the pots. Highlighted areas indicate drought cycles (light grey = drought cycle 1; and dark grey = drought cycle 2).

Withholding water in both drought stress cycles only slightly decreased the soil osmotic potential within the combined stress in pots and containers for both cultivars. After both drought cycles, combined salinity+drought resulted in markedly decreased values of about -0.8 to -1.0 MPa for both cultivars and growth platforms. However, comparable decreases in the soil osmotic potential were observed for Sakha 61 under salinity alone, particularly in the pots.

The irrigation amounts at given periods and related to this, changes in the soil matric potential and in the volumetric soil water contents at given dates in pots and containers are shown in Table 6, Figures 2 and 3, respectively. Determined by the soil matric potentials in the containers, each cultivar received the highest irrigation amount for treatment control (212.8 and 216.1 L), closely followed by salinity alone (197.6 and 180.8 L) and much less for the two drought-exposed treatments. Consequently treatments control and salinity alone had far less negative values for the soil matric potential compared to both drought-stressed treatments, with values beyond the measurement range of the tensiometers. Whereas soil water contents for treatment salinity in the containers and in the pots were comparable to the control treatment, withholding water decreased the water contents to 4-9% in the drought and the combined stress treatments in pots and containers. Drought in the pots decreased water contents slightly more at the soil depth <15 cm compared to the combined stress with a difference of around 2% at the end of drought cycle 1. Soil water contents were more reduced in the containers than in the pots under both drought treatments down to a soil depth of 30 cm. Whereas the soil water content for treatment drought in the containers was almost similar throughout all 4 soil layers at the end of both drought cycles (6-9%), it significantly increased towards the lower soil depths, varying between 4-5% at the upper and 15-20% at the lowest soil layer for the combined stress treatment.

Table 6. Time schedule in 2010 indicated as days after sowing (DAS) and Zadok's stage (ZS). The air temperature (AT) measurements collected at noon at 2 m above-ground are shown at the beginning and end of both drought cycles in both years. No data for the AT were available at sowing and at final harvest (n/a). Irrigation amounts of the containers are given from sowing till final harvest, separated in the four treatments, control (ctr), salinity (s), drought (d), and combined salinity+drought (sd), in certain time periods and summed-up for the whole time of the plant life cycle.

Time period	DAS	ZS	AT	Irrigation amounts							
			— (°C) —	— (L box-1) —							
				Sakha 93				Sakha 61			
				ctr	s	d	sd	ctr	s	d	sd
Sowing - Beginning of drought cycle 1	0 - 27	0 - 14/20	n/a - 18.7	34.5	34.5	34.5	34.5	34.5	34.5	34.5	34.5
Beginning of drought cycle 1 - End of drought cycle 1	27 - 55/57	14/20 - 50/59	18.7 - 28.2/36.5	72.2	67.1	0.0	0.0	63.8	60.4	0.0	0.0
End of drought cycle 1 - Beginning of drought cycle 2	55/57 - 63	50/59 - 65/70	28.2/36.5 - 16.4	15.2	10.1	32.3	24.5	32.0	20.2	25.6	21.9
Beginning of drought cycle 2 - End of drought cycle 2	63 - 85	65/70 - 75/83	16.4 - 30.8	70.7	65.7	0.0	0.0	65.7	55.6	0.0	0.0
End of drought cycle 2 - Final harvest	85 - 93/110	75/83 - 92	30.8 – n/a	20.2	20.2	20.2	20.2	20.2	10.1	20.2	20.2
Sum				212.8	197.6	87.0	79.2	216.1	180.8	80.3	76.6

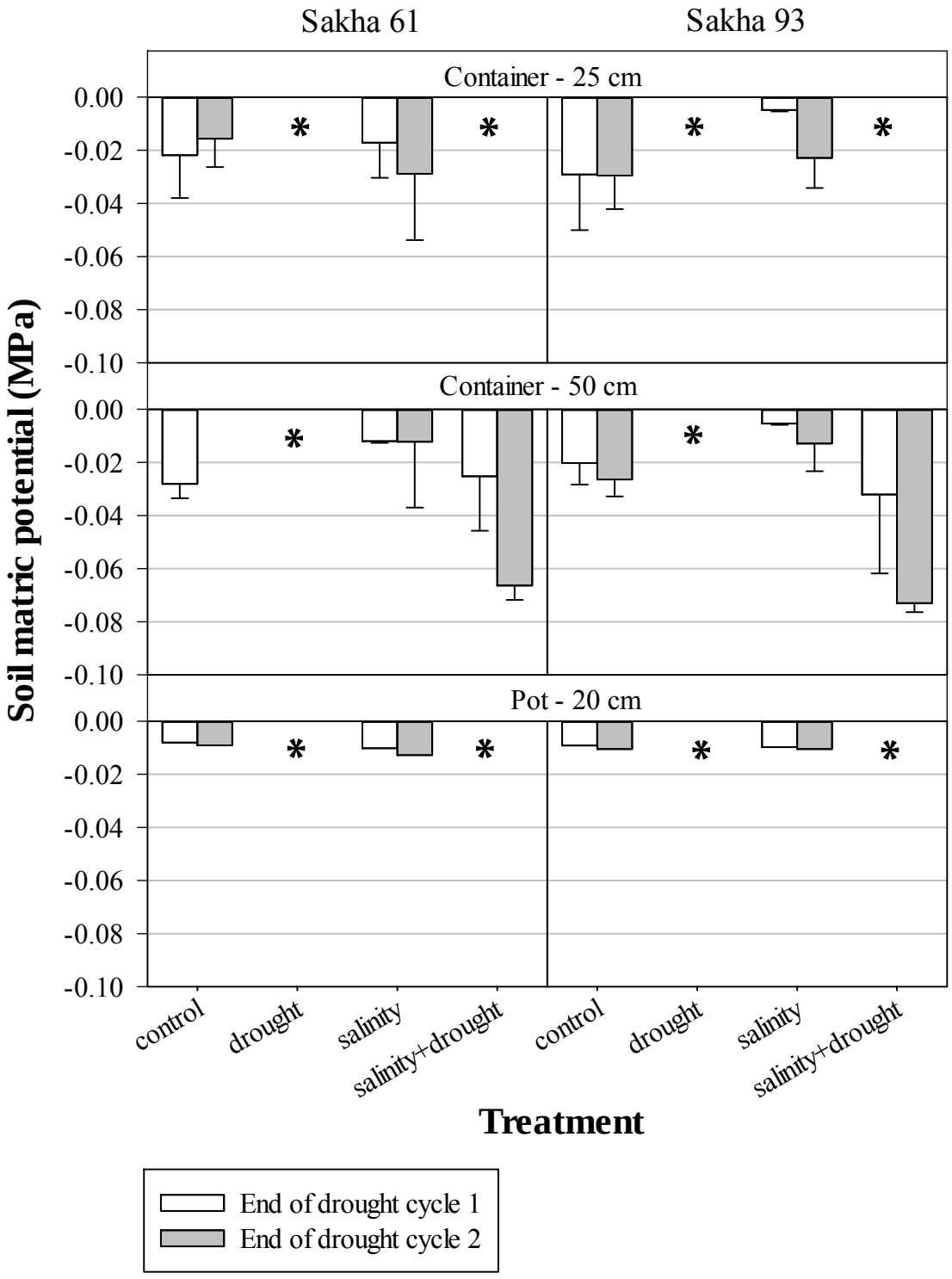

Figure 2. Soil matric potential recorded with tensiometers in the containers and pots at the end of both drought cycles in 2010 for the control, drought, salinity and salinity+drought treatments. The measurement range of the tensiometers was restricted to values higher than -0.08 MPa (*).

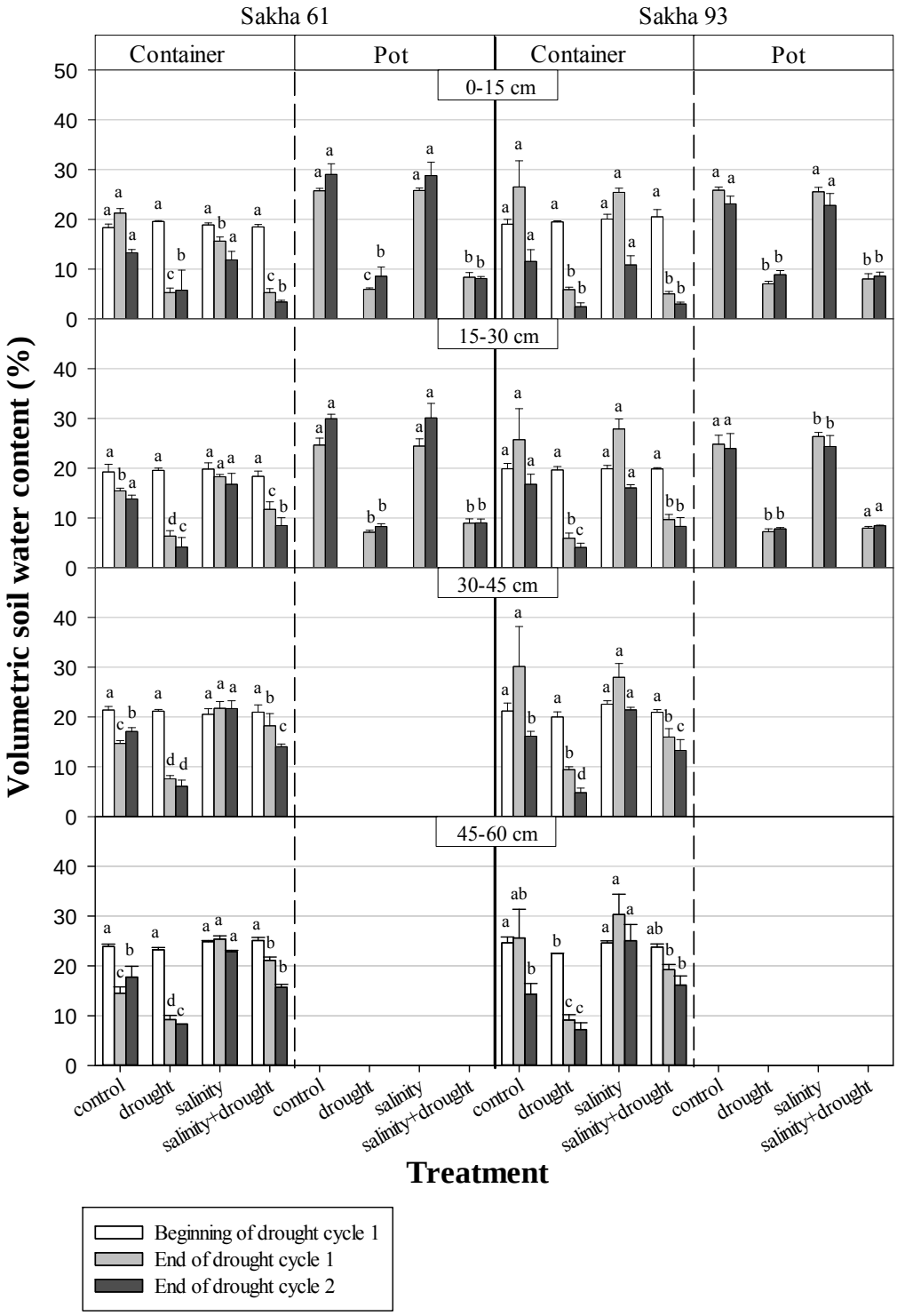

Figure 3. Volumetric soil water content of the four treatments control, drought, salinity and salinity+drought, two cultivars, Sakha 61 and Sakha 93, and certain soil depths as indicated by error bars at different dates in the containers and pots. Each soil layer has a depth of 15 cm, consequently four layers and two layers were sampled for the containers and pots, respectively. Duncan tests were made separately within every cultivar, growth platform and date of the summer experiment 2010 (lower case letters).

Table 7. Average ion contents of the soil over the whole container (0-60 cm) and pot depth (0-30 cm) for Na^+, Ca^{2+} and Cl^-, respectively. Every pot and destructive sampling container were sampled and the ion contents were indicated in (mmol kg^{-1} soil DW) per container and pot, separated in the two cultivars, Sakha 61 and Sakha 93, and in the four treatments, control (ctr), drought (d), salinity (s) and combined salinity+drought (sd). Duncan tests are indicated per cultivars, growth platforms and dates of the summer experiment 2010 (small letters), respectively.

	Sakha 61								Sakha 93							
	Container				Pot				Container				Pot			
Time	ctr	d	s	sd	ctr	d	s	sd	ctr	d	s	sd	ctr	d	s	sd
							Na^+ (mmol kg^{-1} soil DW)									
Sowing	0.2 b	0.2 b	6.1 a	5.6 a	___	___	___	___	0.2 b	1.2 b	6.4 a	5.5 a	___	___	___	___
Beginning of drought cycle 1	0.3 b	0.3 b	6.8 a	7.2 a	___	___	___	___	0.3 b	1.5 b	6.3 a	6.5 a	___	___	___	___
End of drought cycle 1	0.4 b	0.7 b	7.7 a	8.7 a	0.5 c	0.7 c	7.7 b	10.0 a	0.4 c	1.7 c	5.8 b	10.7 a	0.5 b	0.7 b	8.9 a	9.4 a
End of drought cycle 2	0.4 b	0.5 b	7.4 a	8.8 a	0.4 b	0.6 b	9.5 a	9.8 a	0.5 c	3.1 c	6.6 b	10.9 a	0.4 b	0.5 b	9.5 a	8.0 a
Final harvest	0.6 c	0.5 c	6.3 b	10.2 a	0.6 c	0.6 c	11.2 a	9.1 b	0.4 c	0.6 c	6.2 b	9.6 a	0.8 c	0.7 c	11.8 a	5.4 b
							Ca^{2+} (mmol kg^{-1} soil DW)									
Sowing	0.8 b	0.8 b	3.5 a	3.1 a	___	___	___	___	0.7 b	1.3 b	3.8 a	3.0 a	___	___	___	___
Beginning of drought cycle 1	1.3 c	1.4 c	4.7 b	5.7 a	___	___	___	___	1.4 d	2.9 c	4.8 b	6.0 a	___	___	___	___
End of drought cycle 1	1.5 c	3.9 b	5.2 b	8.0 a	1.8 c	4.0 bc	5.7 ab	7.7 a	1.8 c	4.3 b	3.7 b	9.6 a	2.0 b	2.4 b	6.2 a	6.4 a
End of drought cycle 2	1.0 c	2.2 c	5.1 b	8.6 a	0.8 b	1.9 b	7.2 a	7.4 a	1.0 c	3.7 b	3.5 b	10.2 a	0.8 b	1.7 b	7.8 a	5.8 a
Final harvest	1.7 b	1.7 b	4.4 b	10.1 a	1.3 b	2.3 b	9.5 a	8.0 a	1.0 c	2.4 c	4.2 b	9.9 a	1.7 c	2.2 c	10.5 a	4.9 b
							Cl^- (mmol kg^{-1} soil DW)									
Sowing	0.2 b	0.2 b	12.9 a	11.4 a	___	___	___	___	0.2 b	2.4 b	13.9 a	11.4 a	___	___	___	___
Beginning of drought cycle 1	0.2 b	0.2 b	13.9 a	15.9 a	___	___	___	___	0.2 c	2.8 c	12.3 b	14.2 a	___	___	___	___
End of drought cycle 1	0.2 c	0.4 c	16.4 b	23.7 a	0.1 c	0.2 c	15.8 b	22.2 a	0.2 c	2.8 c	11.3 b	27.9 a	0.1 b	0.2 b	19.1 a	20.0 a
End of drought cycle 2	0.1 b	0.2 b	15.8 a	23.5 a	0.2 b	0.2 b	22.0 a	21.7 a	0.2 c	6.2 c	13.1 b	29.3 a	0.1 c	0.1 c	22.6 a	15.8 b
Final harvest	0.1 c	0.3 c	14.4 b	27.3 a	0.2 c	0.3 c	28.0 a	21.3 b	0.1 c	0.4 c	13.4 b	25.2 a	0.2 c	0.3 c	29.2 a	11.3 b

Duncan test: $P \leq 0.05$

Soil Na^+ contents under salinity alone remained stable at any sampling time in the containers, whereas increased values were observed at the end of both drought cycles in the pots (Table 7). Increased and similar values were also found in the combined stress within the pots and containers. Similar observations could be made for the Cl^- contents, which were in general two-to-three fold higher compared to the Na^+ contents. While soil Ca^{2+} contents were comparable in the pots either being salinized or salinized+droughted, higher soil Ca^{2+} contents were observed in the combined stress as compared to salinity alone in the containers. No significant differences in the soil ion contents were observed between the two cultivars.

3.1.2 Influence of the soil osmotic and soil matric potential on various plant parameters during the plant life cycle of two differently salt tolerant cultivars grown in different growth platforms

Whereas at the end of the first drought cycle, the stress factor drought alone significantly reduced the aerial biomass dry weight for both cultivars only in the pots, the combined stress resulted in the highest reduction in the aerial biomass dry weights in both growth platforms (Figure 4).

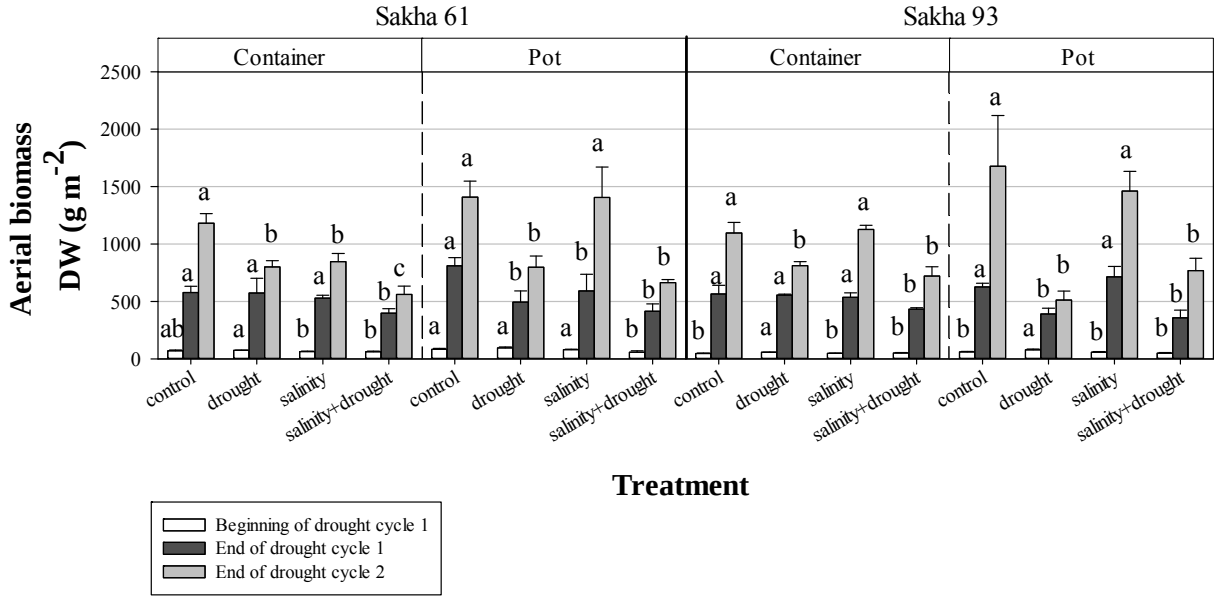

Figure 4. Comparison of the aerial biomass dry weight per m² (DW) of the summer experiment 2010, at three different dates (beginning of drought cycle 1, end of drought cycles 1 and 2), separately indicated for the containers and pots, the two cultivars, Sakha 61 and Sakha 93, and the four treatments, control, drought, salinity and combined salinity+drought. Duncan tests were made separately within every cultivar, growth platform and date of the summer experiment 2010 (lower case letters).

Table 8. ANOVA results (F values and its significance) of the aerial biomass dry weight at three destructive biomass samplings (at the beginning of drought cycle 1 - May 11, 2010; end of drought cycle 1 – June 8, 2010; and 2 – July 6, 2010, respectively); straw and grain dry weight (DW), thousand grain weight (TGW) and grain number per ear at final harvest and ion contents of the straw at final harvest for effects of the parameters, drought, salinity, growth platform, cultivar and its interactions are shown.

	Aerial biomass DW at destructive biomass sampling		
	May 11	June 8	July 6
Growth platform	18.9***	0.0	39.5***
Cultivar	80.7***	0.7	1.8
Salinity	47.9***	17.5***	3.6
Drought	1.7	73.7***	156.2***
Growth platform * Cultivar	0.7	3.9	0.3
Growth platform * Salinity	9.9**	1.2	1.8
Growth platform * Drought	1.5	28.9***	31.1***
Cultivar * Salinity	3.0	7.9**	3.3
Cultivar * Drought	3.9	0.3	2.2
Salinity * Drought	27.7***	2.1	0.8

	Final harvest			
	Straw DW	Grain DW	TGW	Grain number per ear
Growth platform	227.7***	145.3***	17.9***	6.9*
Cultivar	86.3***	181.0***	204.2***	279.0***
Salinity	69.6***	9.2**	87.9***	14.4***
Drought	928.3***	3233.1***	239.3***	522.7***
Growth platform * Cultivar	14.6***	19.4***	3.3	6.0*
Growth platform * Salinity	12.3***	15.7***	31.5***	19.7***
Growth platform * Drought	48.3***	564.9***	33.2***	80.5***
Cultivar * Salinity	5.6*	75.1***	20.5***	38.2***
Cultivar * Drought	31.4***	140.1***	1.4	42.8***
Salinity * Drought	3.1	112.7***	6.5*	43.4***

	Ion contents of the straw at final harvest			
	Na^+	Ca^{2+}	K^+	Cl^-
Growth platform	31.3***	3.1	395.8***	17.8***
Cultivar	3.2	0.5	5.3*	1.3
Salinity	279.1***	382.5***	80.0***	1644.9***
Drought	8.3**	31.8***	108.4***	104.6***
Growth platform * Cultivar	10.9**	1.3	0.4	1.7
Growth platform * Salinity	26.4***	12.9***	72.1***	41.0***
Growth platform * Drought	0.0	15.5***	1.5	0.0
Cultivar * Salinity	3.5	0.1	5.2*	6.5*
Cultivar * Drought	0.1	1.6	6.8*	0.1
Salinity * Drought	9.7**	12.7***	5.2*	91.2***

*, **, *** indicate significance at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$

At the end of both drought cycles, control and salinity treatments did not differ for Sakha 93 either grown in pots or containers, whereas the aerial biomass was significantly reduced for Sakha 61 at the end of drought cycle 1 and 2 in the pots and containers, respectively. Confirmed by ANOVA analysis, the stress parameter drought had the biggest influence by far on the aerial biomass dry weight at the end of both drought cycles (Table 8). While salinity had a highly significant influence on the aerial biomass dry weight at the end of the first drought cycle, the growth platform had a highly significant influence at the end of the second drought cycle.

Straw dry weight at final harvest and grain yield of the pot-grown cultivar Sakha 93 were comparable in the treatments control and salinity alone and excelled all other combinations among treatments, cultivars and growth platforms (Figure 5).

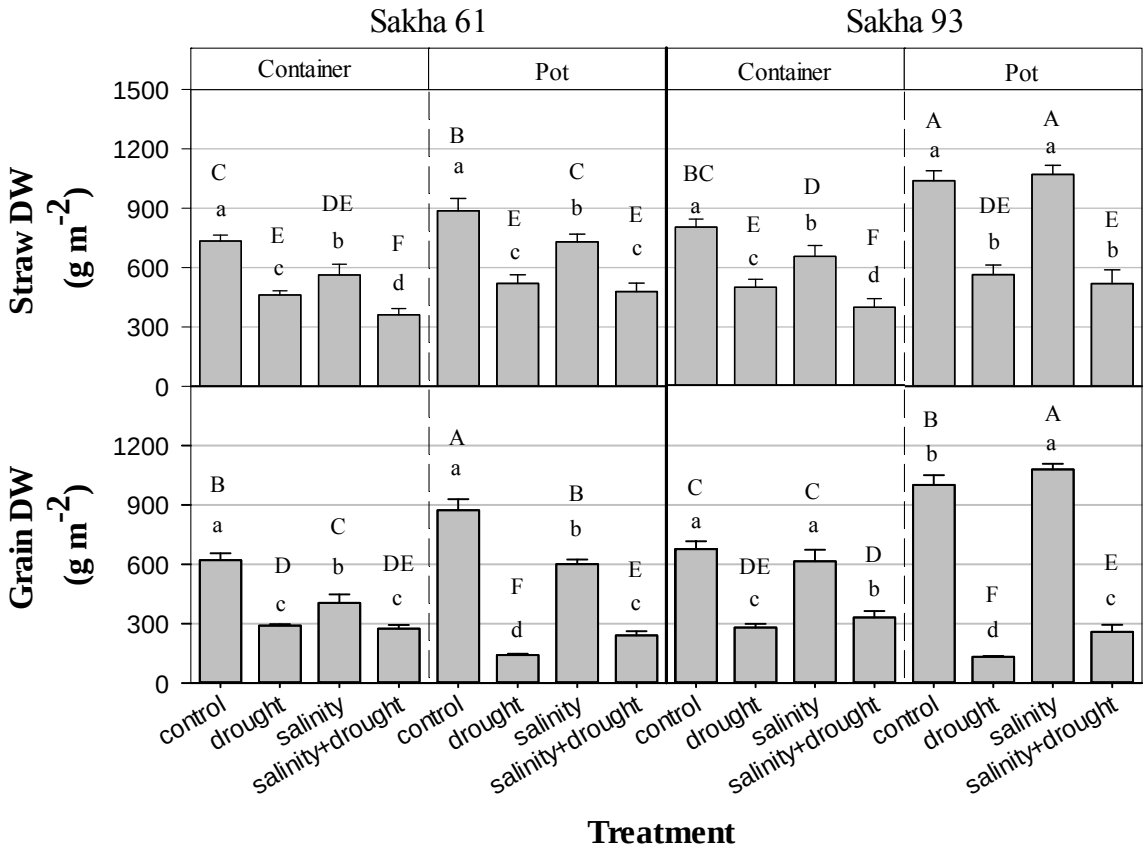

Figure 5. Straw and grain dry weight (DW) at final harvest of the summer experiment 2010, separately indicated for the containers and pots, the two cultivars, Sakha 61 and Sakha 93, and the four treatments, control, drought, salinity and salinity+drought. Duncan tests were made separately within every cultivar and growth platform, pot and container (lower case letters) and for every cultivar together for both growth platforms (capital letters).

Treatments control and salinity alone also resulted in the highest values of the straw dry weight and grain yield in the pots for Sakha 61, but with a significant decrease for the salinity treatment. Whereas the straw dry weight within the combined salinity+drought stress and the solely drought stress treatment hardly differed among the cultivars and growth platforms, there was even a higher grain yield of both cultivars in pot experiments for the combined stress than for drought alone. The combination of salinity+drought and especially to an even higher degree drought alone reduced the grain yield of the pot-grown plants of either cultivar significantly more than for the container-grown plants, reflecting the highest influence of the parameter drought and furthermore a highly significant influence of the growth platform (Table 8). Grain yield of the salt tolerant cultivar Sakha 93 was not different between control and the salinity treatment for both the pot and the container grown plants, although at a different level. In contrast salinity alone significantly decreased the straw dry weight and grain yield of the less tolerant cultivar Sakha 61 in the pots and containers. Whereas still a slightly higher grain yield was observed for the more salt tolerant cultivar as compared to the less salt tolerant one in the containers and subjected to the combined stress, there were no differences for the pot grown plants.

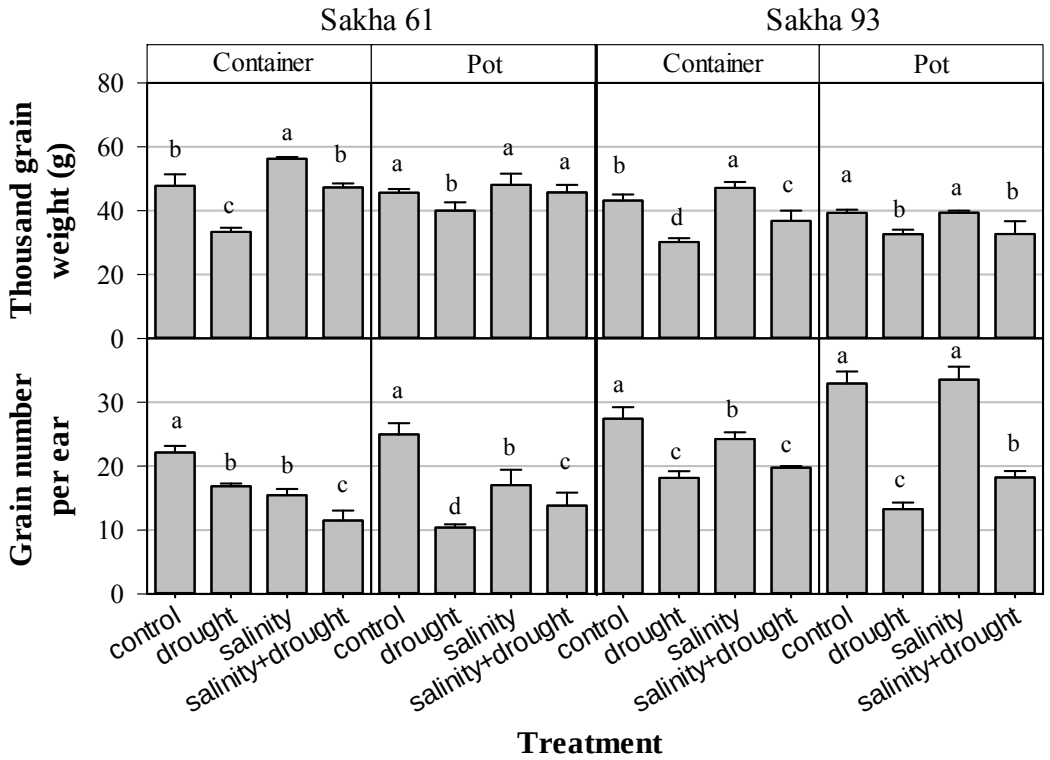

Figure 6. Thousand grain weight and grain number per ear at final harvest of the summer experiment 2010, separately indicated for the containers and pots, the two cultivars, Sakha 61 and Sakha 93, and the four treatments, control, drought, salinity and salinity+drought. Duncan tests were made separately within each cultivar and growth platform, pot and container (lower case letters).

The thousand grain weight was similar for both cultivars in the pots for the treatments control and salinity alone, whereas it was significantly higher for salinity alone in the containers (Figure 6). While the thousand grain weight for Sakha 61 in both platforms was similar for treatments control and the combined stress, it was significantly reduced for Sakha 93 exposed to the combined stress. This reflected the highly significant influence of the cultivars, which closely followed the highly significant influence of the stress parameter drought (Table 8). While the thousand grain weight for Sakha 93 tended to be lower for every treatment compared to Sakha 61, it was the other way round for the grain number per ear. Both droughted treatments resulted in the lowest grain numbers per ear for both cultivars and platforms, leading to the highest influence of the treatment drought (Table 8).

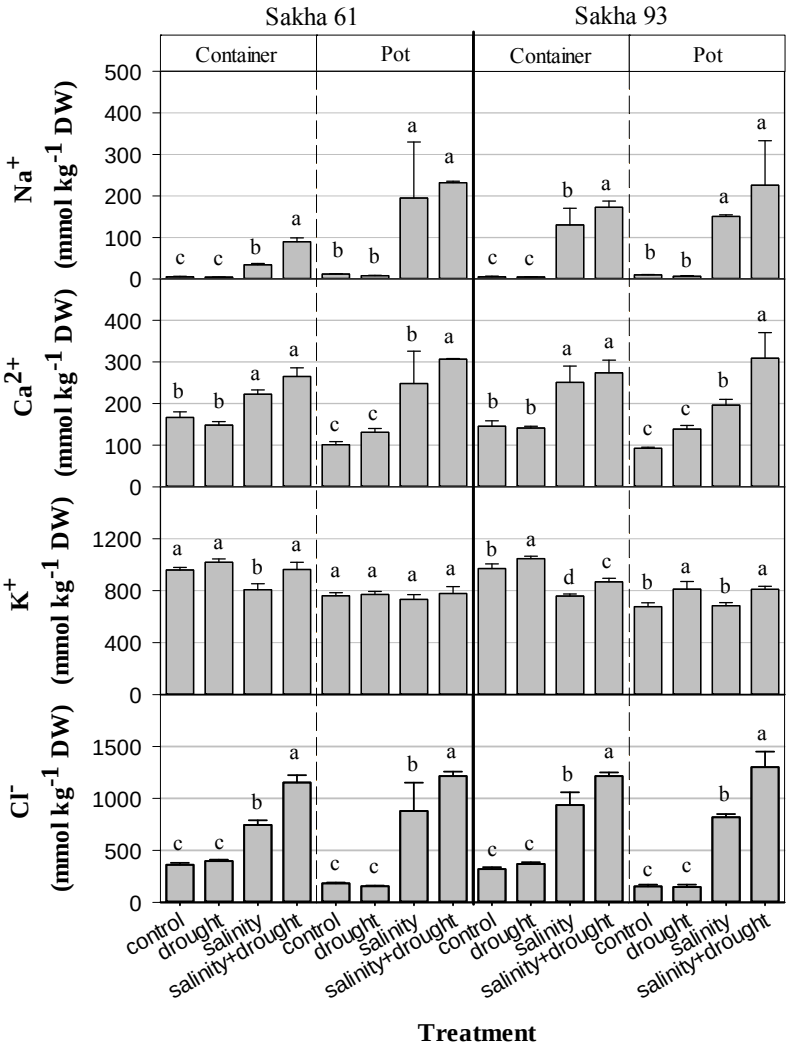

Figure 7. Comparison of the ion contents of Na^+, Ca^{2+}, K^+ and Cl^- of the shoots at the final harvest of the summer experiment 2010, indicated for the containers and pots, the two cultivars, Sakha 61 and Sakha 93, and the four treatments, control, drought, salinity and salinity+drought, respectively. Duncan tests were made separately within each cultivar and growth platform, pot and container (lower case letters).

While the Na^+, Ca^{2+} and Cl^- contents of the shoots were highly significantly influenced by the stress scenario salinity, K^+ was highly influenced by the growth platform (Table 8). Pot and container grown plants had similar shoot ion contents within every treatment, although both cultivars differed in their salt tolerance. Plants exposed to salinity stress alone had slightly to significantly lower Na^+, Ca^{2+} and Cl^- contents compared to the combined salinity+drought stress (Figure 7).

3.2 Section II: Spectral assessment of wheat plants grown in pots and containers under saline conditions

3.2.1 Soil salinity and soil matric potential

While in situ-measured soil salinities at a 20 cm soil depth remained almost stable until the beginning of the first drought cycle in both years, withholding water during the first drought stress cycle only slightly increased the EC of the soil with the combined stress in pots and containers for both cultivars (Figure 8). After the first drought cycle, the salinity+drought treatment resulted in markedly increased salinity levels of approximately 20-24 mS cm^{-1}, except in the pots in 2009.

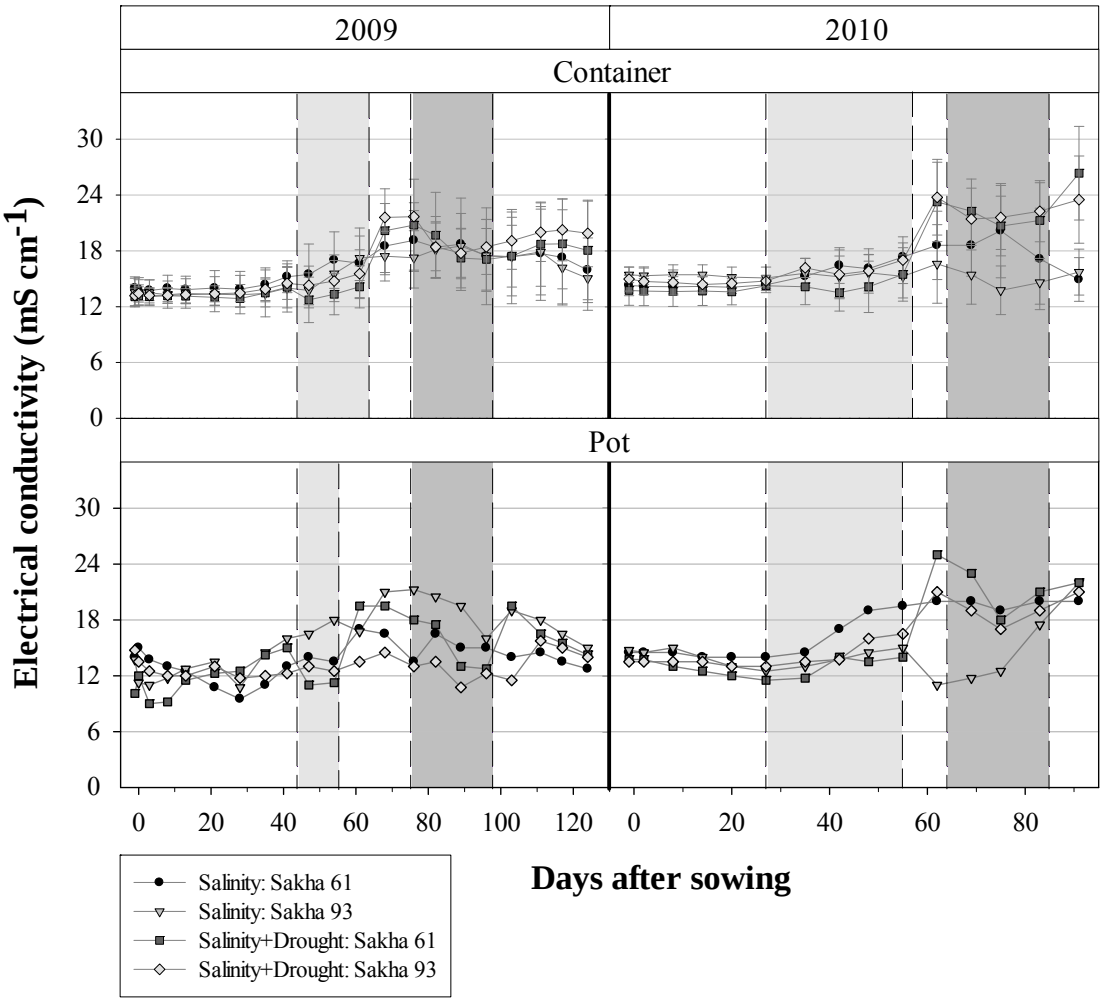

Figure 8. Electrical conductivity of the soil for the salinity and salinity+drought treatments during the winter experiment in 2009 and summer experiment in 2010. The electrical conductivity was measured weekly with in situ salinity sensors at a soil depth of 20 cm in the containers and pots. Highlighted areas indicate drought cycles (light grey = drought cycle 1; and dark grey = drought cycle 2).

The soil matric potentials indicated a marked water stress for the salinity+drought treatment at the end of both drought cycles in both years in the upper soil layer in the containers and pots in addition to a significantly lower water stress in the 50 cm soil depth of the containers (Figure 9). No water stress at any time was indicated in the salinity and control treatments for both growth platforms and depths.

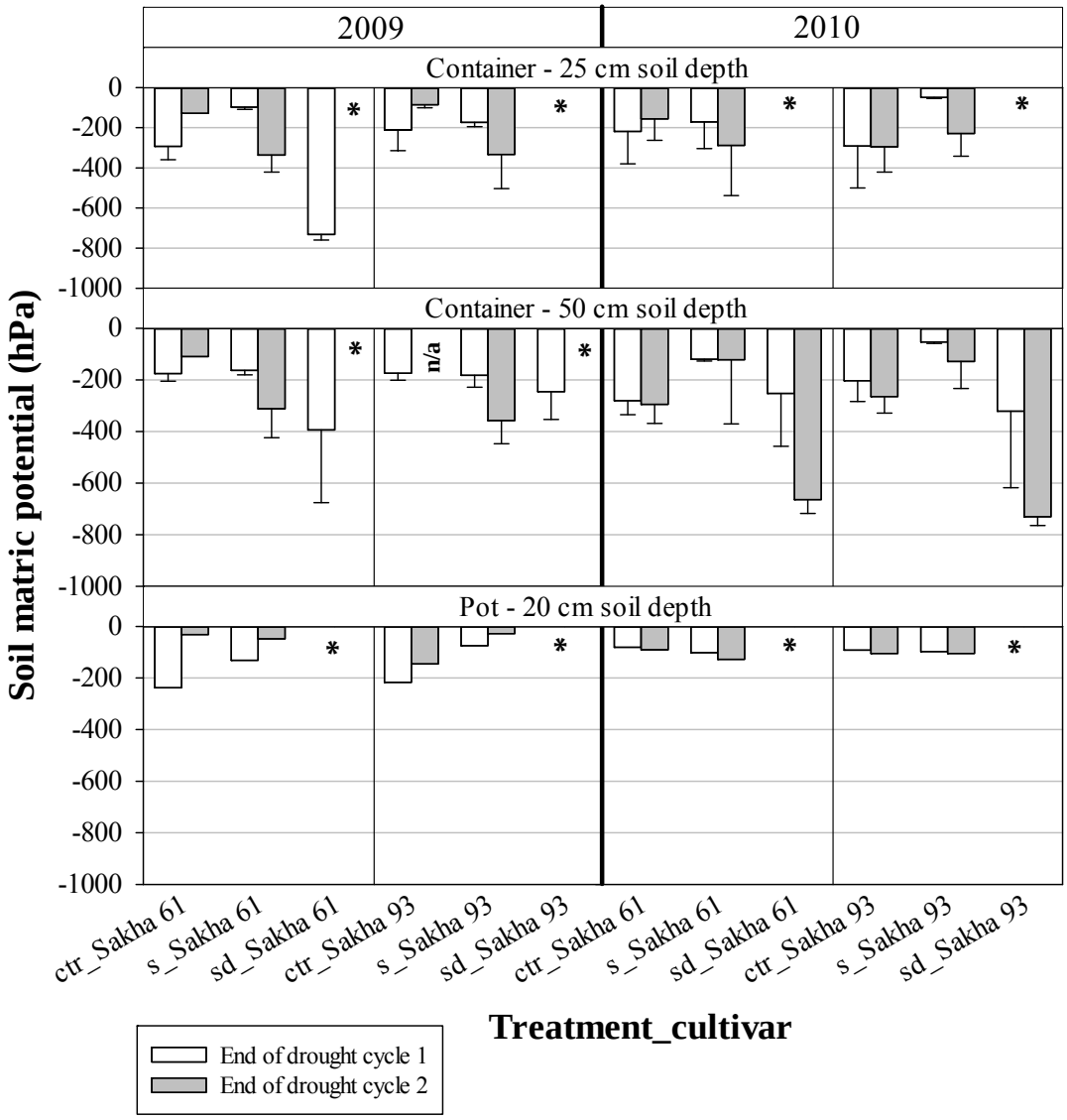

Figure 9. Soil matric potential recorded with tensiometers in the containers and pots at the end of both drought cycles in the winter experiment in 2009 and summer experiment in 2010 for the control (ctr), salinity (s) and salinity+drought (sd) treatments. The measurement range of the tensiometers was restricted to values higher than -800 hPa (*). At the end of drought cycle 2, the tensiometers did not work for control treatment and Sakha 93 (n/a).

3.2.2 Relationships between spectral indices and agronomic parameters

A multivariate GLM analysis revealed significant to highly significant influences of years (seasons) on all agronomic and physiological parameters at almost every measuring date (Table 9). Consequently, the coefficients of determination were calculated for each experimental year separately (Tables 10 and 11). Since plants under field conditions can be exposed to salinity and to differing degrees of drought under saline conditions as well as no stress, the specific objective of this study was to evaluate whether spectral assessments could be applied under such co-occurring conditions and therefore the treatments were combined for the analysis.

The fresh weight and water content of the above-ground biomass were weakly related to the chosen spectral indices at the beginning of drought cycle 1 with only a few exceptions for Sakha 61 in the container experiment in 2009 and for Sakha 93 in the pot experiment in 2010 (Table 10). In general, the spectral relationships to the fresh weight and water content of the above-ground biomass were significantly better for the containers compared to the pots at the end of both drought cycles, with slightly better correlations in 2010. The coefficients of determination were similar for both cultivars in the containers at the end of drought cycle 1 (Figures 10 and 11) and 2 in the containers. The best spectral indices for differentiating the fresh weight and water content of the above-ground biomass in the containers in 2009 and 2010 were the R_{780}/R_{550} index, the R_{760}/R_{670} index and the NDVI. The slopes of the linear regression lines were significantly steeper for the container-grown plants compared to the pot-grown plants, except for the NDVI.

Table 9. Results of the multivariate GLM analysis (F values and significance) for the year effects (winter 2009 and summer 2010) on the agronomic parameters, including fresh weight and water content of the above-ground biomass, as well as the water potential and relative water content of the youngest fully developed leaf, for the control, salinity and salinity-drought treatments at selected dates.

Sources of variation	Beginning of drought cycle 1	End of drought cycle 1	Middle of drought cylce 2	End of drought cycle 2
Year (df=1)				
FW	226.1***	13.3***	n/a	13.0***
WC	13.8***	6.2*	n/a	5.9*
WP	n/a	3.9*	21.0***	1.8
RWC	n/a	6.9*	2.4	16.2***

*, **, *** indicate significance at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$

* n/a, data not available

Table 10. Coefficients of determination (R^2) for the relationships between the fresh weight or the water content of the above-ground biomass and selected spectral indices of two active sensors (Crop Circle ACS-470® and GreenSeeker RT100®) and one passive sensor (HandySpec® Field Spectrometer) for the control, salinity and salinity-drought treatments for the two cultivars (Sakha 61 and Sakha 93) and for the two growth platforms (container and pot) for the respective dates during the winter experiment in 2009 and summer experiment in 2010.

		2009											2010									
			Crop Circle	GreenSeeker	HandySpec								Crop Circle	GreenSeeker	HandySpec							
		N	760/670	NDVI	760/670	NDVI	WBI	WBI/NDVI	780/740	760/730	780/550	REIP	N	760/670	NDVI	760/670	NDVI	WBI	WBI/NDVI	780/740	760/730	780/550	REIP
Fresh weight of the above ground biomass																						
Begin of drought cycle 1																						
Container	Sakha 61	9	0.75**		0.70**	0.58*	0.03	0.38	0.57*	0.54*	0.64**	0.57*	9	0.01	0.14	0.17	0.23	0.57*	0.11	0.45*	0.33	0.45*	0.16
	Sakha 93	9	0.37		0.55*	0.46*	0.51*	0.57*	0.27	0.40	0.50*	0.28	9	0.26	0.12	0.31	0.33	0.01	0.34	0.12	0.35	0.21	0.00
Pot	Sakha 61	9	0.00		0.07	0.07	0.34	0.02	0.07	0.08	0.03	0.03	9	0.35	0.23	0.67**	0.62*	0.19	0.65**	0.79**	0.71**	0.76**	0.02
	Sakha 93	9	0.05		0.07	0.22	0.13	0.00	0.17	0.09	0.08	0.15	9	0.03	0.05	0.10	0.11	0.34	0.07	0.03	0.09	0.10	0.07
End of drought cycle 1																						
Container	Sakha 61	9	0.55*		0.71**	0.74**	0.58*	0.23	0.62*	0.67**	0.74**	0.66**	9	0.95***	0.89**	0.97***	0.85***	0.86***	0.29	0.92***	0.90***	0.95***	0.93***
	Sakha 93	9	0.77**		0.78**	0.77**	0.55*	0.25	0.57*	0.63*	0.80**	0.51*	9	0.91***	0.77**	0.85***	0.75**	0.92***	0.46*	0.74**	0.78**	0.79**	0.75**
Pot	Sakha 61	9	0.41		0.57*	0.51*	0.83***	0.33	0.31	0.43	0.35	0.01	9	0.38	0.62*	0.74**	0.69**	0.78**	0.60*	0.69**	0.69**	0.78**	0.15
	Sakha 93	9	0.56*		0.90***	0.80**	0.56*	0.72**	0.86***	0.89***	0.91***	0.06	9	0.77**	0.94**	0.95***	0.95***	0.92***	0.91***	0.93***	0.96***	0.97***	0.04
End of drought cycle 2																						
Container	Sakha 61	9	0.76**	0.79**	0.90***	0.95***	0.92***	0.84***	0.94***	0.95***	0.93***	0.48*	9	0.67**	0.60*	0.84***	0.69**	0.97***	0.61*	0.81***	0.81***	0.78**	0.28
	Sakha 93	9	0.97***	0.86***	0.87***	0.83***	0.67**	0.72**	0.76**	0.81***	0.88***	0.77**	9	0.95***	0.95***	0.92***	0.96***	0.95***	0.92***	0.95***	0.96***	0.94***	0.92***
Pot	Sakha 61	9	0.00	0.11	0.24	0.39	0.14	0.53*	0.68**	0.54*	0.47**	0.10	9	0.47*	0.04	0.32	0.31	0.64**	0.23	0.18	0.10	0.17	0.50*
	Sakha 93	9	0.02	0.06	0.22	0.20	0.65**	0.29	0.67**	0.60*	0.52**	0.79**	9	0.81***	0.65**	0.70**	0.71**	0.69**	0.64**	0.22	0.62*	0.54*	0.06
Water content of the above ground biomass																						
Begin of drought cycle 1																						
Container	Sakha 61	9	0.69**		0.67**	0.58*	0.00	0.25	0.40	0.43	0.68**	0.42	9	0.01	0.19	0.34	0.37	0.76**	0.20	0.44	0.37	0.59*	0.11
	Sakha 93	9	0.51*		0.63*	0.42	0.06	0.19	0.51*	0.38	0.62*	0.48*	9	0.21	0.59*	0.06	0.03	0.41	0.08	0.12	0.01	0.09	0.00
Pot	Sakha 61	9	0.00		0.00	0.01	0.02	0.00	0.02	0.03	0.00	0.05	9	0.01	0.06	0.06	0.02	0.07	0.03	0.16	0.02	0.07	0.05
	Sakha 93	9	0.00		0.13	0.19	0.67**	0.45*	0.28	0.20	0.19	0.26	9	0.04	0.01	0.03	0.02	0.23	0.00	0.00	0.01	0.02	0.01
End of drought cycle 1																						
Container	Sakha 61	9	0.92**		0.88***	0.79**	0.36	0.63*	0.28	0.38	0.67**	0.23	9	0.94**	0.91**	0.97***	0.90***	0.89***	0.33	0.94***	0.96***	0.98***	0.96***
	Sakha 93	9	0.89**		0.86***	0.75**	0.42	0.36	0.33	0.41	0.69**	0.29	9	0.97**	0.87**	0.95***	0.86***	0.95***	0.60*	0.88***	0.88***	0.90***	0.88***
Pot	Sakha 61	9	0.43		0.59*	0.73**	0.51*	0.71**	0.31	0.46*	0.40	0.00	9	0.60*	0.80**	0.76**	0.89***	0.84***	0.85***	0.87***	0.88***	0.91***	0.23
	Sakha 93	9	0.74**		0.88***	0.93***	0.78**	0.81***	0.73**	0.82***	0.84***	0.00	9	0.79**	0.97**	0.87***	0.91***	0.92***	0.87***	0.84***	0.87***	0.87***	0.11
End of drought cycle 2																						
Container	Sakha 61	9	0.86**	0.96**	0.87***	0.84***	0.55*	0.86***	0.67**	0.73**	0.75**	0.39	9	0.90**	0.84**	0.97***	0.90***	0.97***	0.83***	0.87***	0.94***	0.94***	0.35
	Sakha 93	9	0.98**	0.81**	0.89***	0.84***	0.66**	0.76**	0.75**	0.81***	0.89***	0.73**	9	0.93**	0.94**	0.92***	0.95***	0.99***	0.89***	0.98***	0.98***	0.96***	0.95***
Pot	Sakha 61	9	0.04	0.03	0.02	0.07	0.01	0.10	0.30	0.15	0.24	0.01	9	0.55*	0.02	0.48*	0.47*	0.82***	0.38	0.21	0.14	0.11	0.46*
	Sakha 93	9	0.09	0.11	0.08	0.10	0.54*	0.18	0.62*	0.51*	0.44	0.33	9	0.85**	0.80**	0.79**	0.86***	0.84***	0.83***	0.50*	0.84***	0.66**	0.25

*, **, *** indicate significance at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$

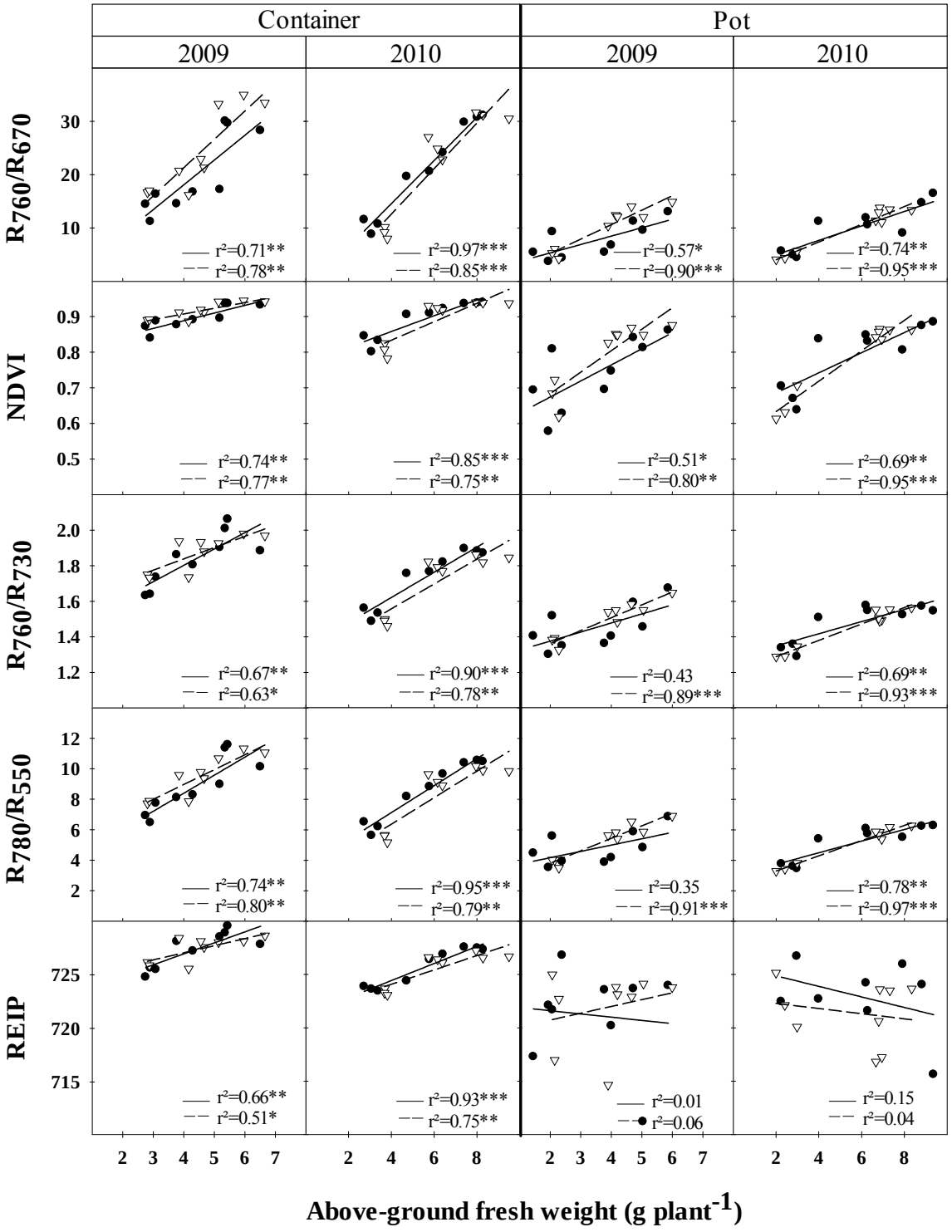

Figure 10. Relationships between the above-ground fresh weight at partial harvests and selected spectral indices as detected with the HandySpec® Field Spectrometer passive sensor for the control, salinity and salinity+drought treatments for each of the two cultivars (Sakha 61 "——" and Sakha 93 "– –") at the end of drought cycle 1 in both years.

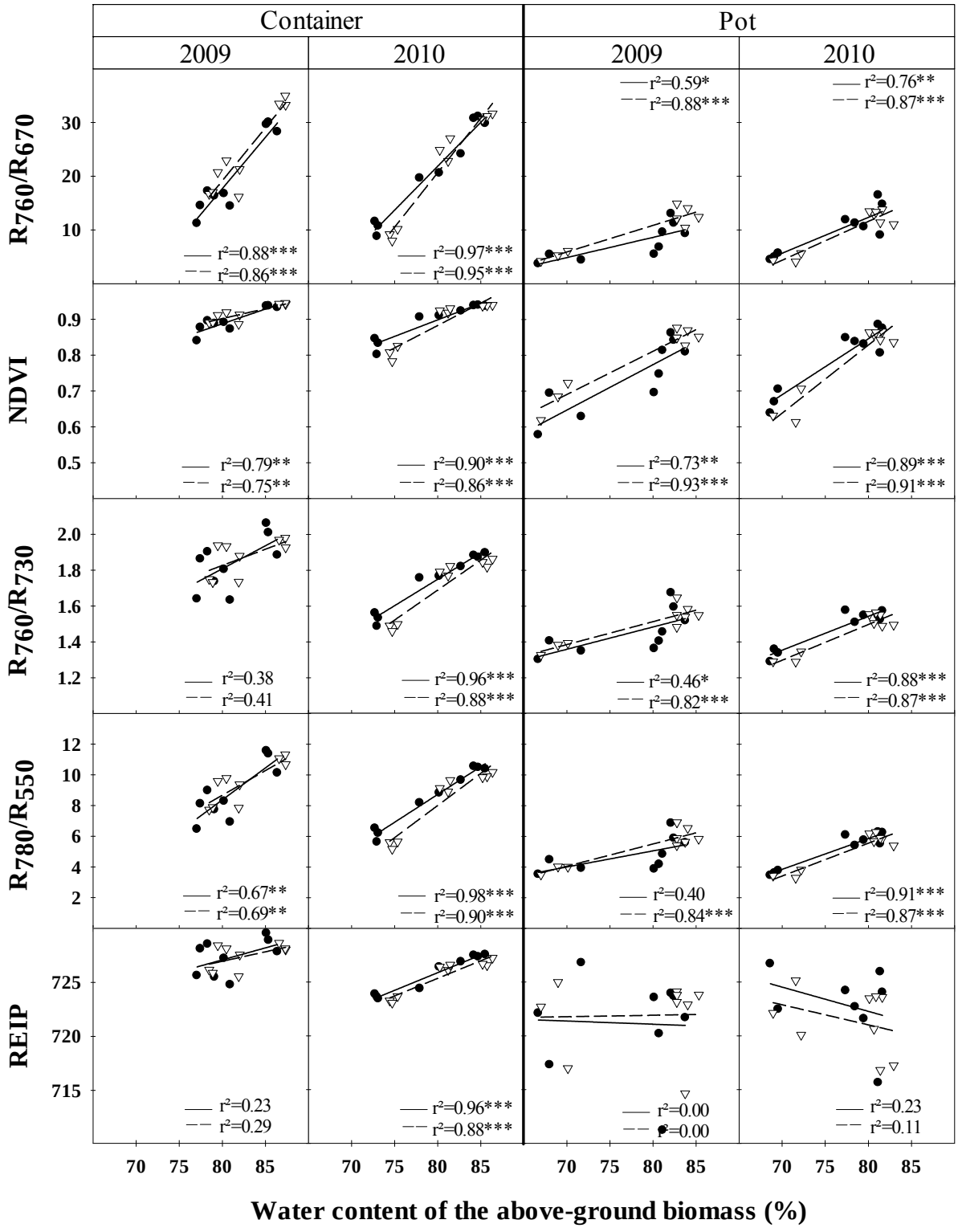

Figure 11. Relationships between the water content of the above-ground biomass at partial harvest and selected spectral indices as detected with the HandySpec® Field Spectrometer passive sensor for the control, salinity and salinity+drought treatments for each of the two cultivars (Sakha 61 "——" and Sakha 93 "– –") at the end of drought cycle 1 in both years.

The water potential of the youngest fully developed leaf was highly significant and well-related to most of the chosen spectral indices at the end of both drought cycles and the middle of drought cycle 2 with obvious differences in the significance and quality of the relationships between containers and pots (Table 12).

The best spectral indices to detect the water potential of the container-grown plants at the end of both drought cycles in 2009 and 2010 were the R_{760}/R_{670} spectral index and the NDVI; the R_{780}/R_{550} spectral index was only slightly less efficient than these indices. Similar to the above described agronomic parameters, the slopes of the linear regression lines of the containers were generally significantly steeper compared to those in the pots, thus indicating stronger correlations. In contrast, closer relationships were found for Sakha 93 in the pots at the end of drought cycle 1. The quality of the relationships in the containers and at the end of drought cycle 1 in the pots for both cultivars was similar, regardless of time (Figure 12).

The relative water content of the youngest fully developed leaf did not correlate well with the given spectral indices at any time with the most significant results found for Sakha 93 in the pots at the end of drought cycle 1 within both years (Table 11).

Similar relationships between the spectral indices and the water potential of the youngest fully developed leaf, the fresh weight of the above-ground biomass and the water content of the above-ground biomass at the end of both drought cycles can be attributed to high autocorrelations among these agronomic parameters (Table 12).

The significantly weaker relationships between the spectral indices and the relative water content of the youngest fully developed leaf were reflected in decreased coefficients of determination compared to the other parameters.

The t-test analysis, which included all three treatments (control, salinity and salinity+drought) revealed the opportunity for cultivar differentiation of the chosen agronomic parameters and spectral indices at almost every date and for each growth platform (Table 13).

Table 11. Coefficients of determination (R^2) for the relationships between the water potential or the relative water content of the youngest fully developed leaf and selected spectral indices of two active sensors (Crop Circle ACS-470® and GreenSeeker RT100®) and one passive sensor (HandySpec® Field Spectrometer) for the control, salinity and salinity-drought treatments for the two cultivars (Sakha 61 and Sakha 93) and growth platforms (container and pot) for the respective dates during the winter experiment 2009 and the summer experiment 2010.

		2009											2010									
			Crop Circle	GreenSeeker	HandySpec									Crop Circle	GreenSeeker	HandySpec						
		N	760/670	NDVI	760/670	NDVI	WBI	WBI/NDVI	780/740	760/730	780/550	REIP	N	760/670	NDVI	760/670	NDVI	WBI	WBI/NDVI	780/740	760/730	780/550	REIP
												Water potential of the youngest fully developed leaf										
End of drought cycle 1																						
Container	Sakha 61	9	0.74**		0.92***	0.88***	0.67**	0.31	0.66**	0.74**	0.90***	0.62*	27	0.91***	0.87***	0.88***	0.80***	0.01	0.00	0.80***	0.86***	0.85***	0.81***
	Sakha 93	9	0.91***		0.98***	0.98***	0.58*	0.44	0.67**	0.74**	0.95***	0.64**	27	0.95***	0.86***	0.93***	0.84***	0.00	0.00	0.87***	0.87***	0.89***	0.85***
Pot	Sakha 61	6	0.51		0.70*	0.83*	0.61	0.80*	0.46	0.59	0.53	0.17	18	0.49**	0.53***	0.62***	0.74***	0.62***	0.76***	0.66***	0.68***	0.73***	0.34*
	Sakha 93	6	0.74*		0.85**	0.82*	0.80*	0.70*	0.67*	0.75*	0.79*	0.58	18	0.73***	0.91***	0.82***	0.81***	0.82***	0.76***	0.74***	0.77***	0.79***	0.06
Middle of drought cycle 2																						
Container	Sakha 61	9	0.85***	0.54*	0.88***	0.76**	0.17	0.92***	0.67**	0.73**	0.93***	0.35	18	0.85***	0.77***	0.83***	0.83***	0.07	0.02	0.85***	0.84***	0.85***	0.87***
	Sakha 93	9	0.92***	0.67**	0.92***	0.93***	0.21	0.37	0.76**	0.77**	0.85***	0.76**	18	0.80***	0.86***	0.76***	0.81***	0.01	0.06	0.86***	0.85***	0.82***	0.87***
Pot	Sakha 61	9	0.42	0.15	0.71**	0.75**	0.21	0.77**	0.71**	0.76**	0.59*	0.01	18	0.02	0.11	0.19	0.18	0.01	0.00	0.04	0.00	0.02	0.06
	Sakha 93	9	0.13	0.07	0.07	0.07	0.23	0.00	0.34	0.21	0.19	0.15	18	0.65***	0.63***	0.61***	0.65***	0.04	0.00	0.18	0.35**	0.53***	0.06
End of drought cycle 2																						
Container	Sakha 61	9	0.76**	0.86***	0.95***	0.95***	0.86***	0.85***	0.93***	0.95***	0.95***	0.47*	12	0.95***	0.84***	0.90***	0.85***	0.15	0.79***	0.65**	0.81***	0.70***	0.64**
	Sakha 93	9	0.86***	0.88***	0.87***	0.96***	0.83***	0.81***	0.97***	0.98***	0.96***	0.93***	12	0.75***	0.71***	0.65**	0.81***	0.20	0.78***	0.73***	0.79***	0.66**	0.76***
Pot	Sakha 61	9	0.12	0.38	0.42	0.54*	0.19	0.63*	0.85***	0.73**	0.64**	0.06	9	0.33	0.09	0.55*	0.52*	0.01	0.56*	0.14	0.51*	0.34	0.00
	Sakha 93	9	0.00	0.09	0.37	0.36	0.67**	0.48*	0.82***	0.77**	0.68**	0.62*	9	0.61*	0.43	0.54*	0.54*	0.45*	0.43	0.13	0.46*	0.33	0.27
												Relative water content of the youngest fully developed leaf										
End of drought cycle 1																						
Container	Sakha 61	9	0.27		0.18	0.19	0.03	0.34	0.00	0.02	0.10	0.01	27	0.12	0.06	0.13	0.05	0.01	0.01	0.10	0.08	0.10	0.08
	Sakha 93	9	0.05		0.02	0.01	0.00	0.01	0.00	0.00	0.00	0.00	27	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pot	Sakha 61	6	0.63		0.58	0.68*	0.54	0.62	0.41	0.49	0.44	0.13	18	0.30*	0.47**	0.18	0.19	0.21	0.16	0.27*	0.27*	0.24*	0.00
	Sakha 93	6	0.83*		0.85**	0.85**	0.81*	0.75*	0.64	0.74*	0.78*	0.52	18	0.62***	0.75***	0.67***	0.68***	0.74***	0.63***	0.61***	0.66***	0.68***	0.14
Middle of drought cycle 2																						
Container	Sakha 61	9	0.31	0.36	0.27	0.12	0.06	0.51*	0.13	0.16	0.28	0.10	18	0.09	0.14	0.06	0.11	0.04	0.10	0.07	0.09	0.07	0.03
	Sakha 93	9	0.31	0.18	0.59*	0.49*	0.09	0.22	0.20	0.26	0.48*	0.24	18	0.20	0.16	0.21	0.14	0.07	0.16	0.20	0.20	0.20	0.27*
Pot	Sakha 61	9	0.08	0.01	0.03	0.04	0.00	0.04	0.05	0.03	0.08	0.03	18	0.01	0.01	0.06	0.05	0.01	0.02	0.04	0.06	0.06	0.00
	Sakha 93	9	0.59*	0.38	0.50*	0.55*	0.08	0.49*	0.18	0.25	0.31	0.00	18	0.09	0.30*	0.14	0.15	0.09	0.03	0.02	0.07	0.07	0.01
End of drought cycle 2																						
Container	Sakha 61	9	0.21	0.33	0.11	0.07	0.06	0.06	0.07	0.08	0.08	0.14	12	0.00	0.01	0.00	0.02	0.00	0.03	0.01	0.01	0.00	0.03
	Sakha 93	9	0.08	0.23	0.01	0.04	0.02	0.25	0.01	0.02	0.02	0.02	12	0.01	0.01	0.02	0.03	0.01	0.03	0.03	0.03	0.03	0.22
Pot	Sakha 61	9	0.05	0.02	0.02	0.03	0.73**	0.03	0.26	0.11	0.16	0.06	9	0.00	0.09	0.06	0.07	0.00	0.07	0.04	0.03	0.04	0.16
	Sakha 93	9	0.51	0.47	0.25	0.27	0.65**	0.39	0.53*	0.47*	0.42	0.44	9	0.67**	0.54*	0.61*	0.66**	0.45*	0.63*	0.10	0.52*	0.34	0.56*

*, **, *** indicate significance at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$

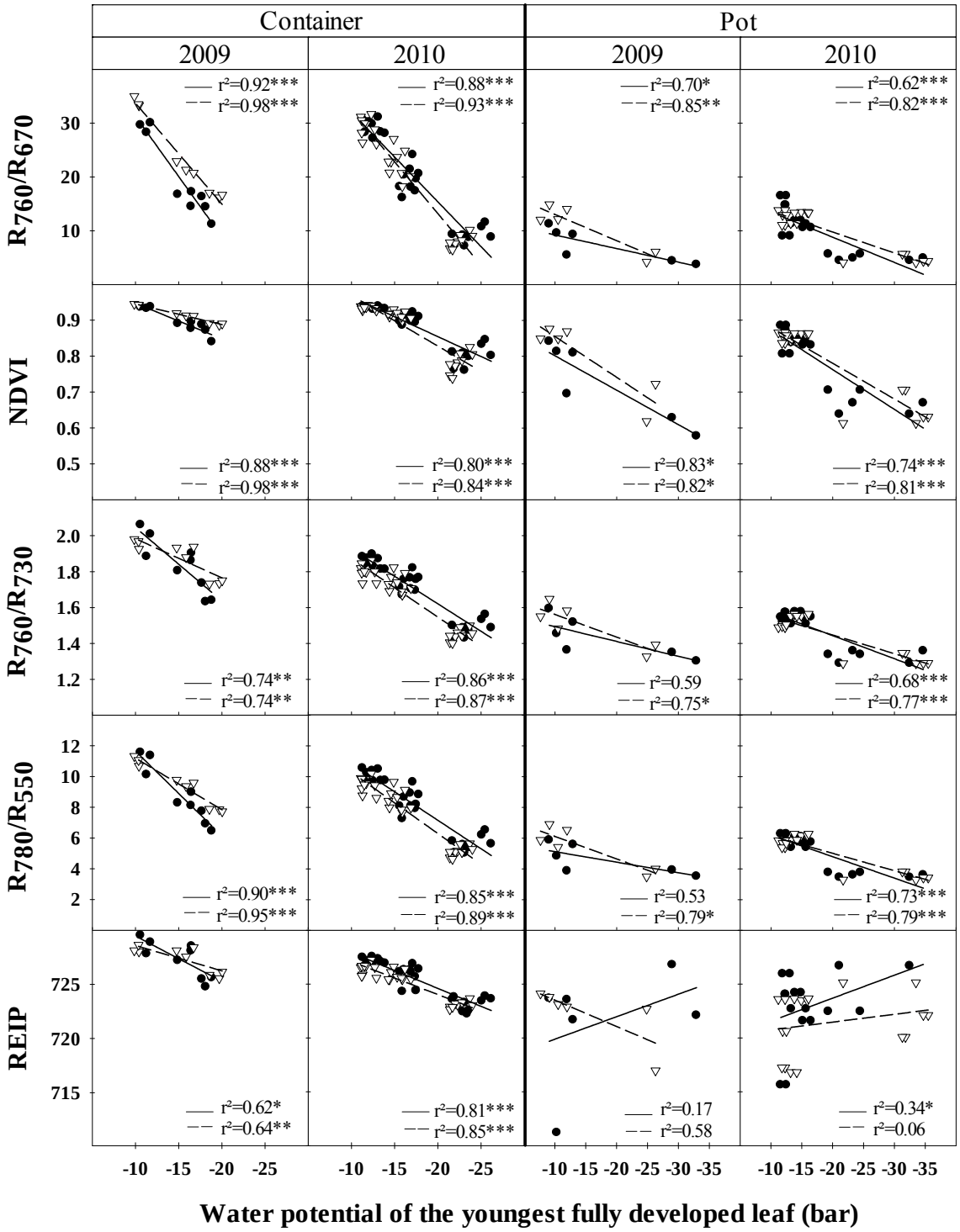

Figure 12. Relationships between the water potential of the youngest fully developed leaf and selected spectral indices as detected with the HandySpec® Field Spectrometer passive sensor for the control, salinity and salinity+drought treatments for each of the two cultivars (Sakha 61 "——" and Sakha 93 "– –") at the end of drought cycle 1 in both years.

Table 12. Coefficients of determination (R^2) for the relationships between the water potential and relative water content of the youngest fully developed leaf, as well as for the fresh weight and water content of the above-ground biomass for the control, salinity and salinity+drought treatments for the two cultivars (Sakha 61 and Sakha 93) and growth platforms (container and pot) at the end of both drought cycles during the winter experiment in 2009 and summer experiment in 2010.

			2009						2010					
			End of drought cycle 1			End of drought cycle 2			End of drought cycle 1			End of drought cycle 2		
			RWC	FW	WC	RWC	FW	WC	RWC	FW	WC	RWC	FW	WC
Container	Sakha 61	WP	0.21	0.78**	0.81***	0.15	0.93***	0.84***	0.10	0.93***	0.94***	0.00	0.91***	0.97***
		RWC		0.55*	0.22		0.11	0.11		0.08	0.11		0.00	0.01
		FW			0.55*			0.75**			0.96***			0.91***
	Sakha 93	WP	0.06	0.79**	0.81***	0.19	0.84***	0.81***	0.28	0.92***	0.95***	0.15	0.87***	0.91***
		RWC		0.01	0.03		0.12	0.13		0.18	0.16		0.07	0.09
		FW			0.80**			0.97**			0.92***			0.98***
Pot	Sakha 61	WP	0.97***	0.55	0.93**	0.35	0.82***	0.33	0.13	0.61*	0.89***	0.06	0.59*	0.77**
		RWC		0.42	0.92**		0.28	0.17		0.23	0.30		0.00	0.01
		FW			0.49*			0.50*			0.81***			0.89***
	Sakha 93	WP	0.97***	0.89**	0.91**	0.27	0.89***	0.71**	0.68**	0.73**	0.88***	0.80**	0.53*	0.44
		RWC		0.85**	0.98***		0.29	0.05		0.61*	0.82***		0.61*	0.54*
		FW			0.74**			0.56*			0.89***			0.66**

*, **, *** indicate significance at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$

Table 13. Comparison between the two cultivars (Sakha 61 and Sakha 93) with regard to the agronomic parameters (fresh weight of the above-ground biomass (FW); water content of the above-ground biomass (WC); water potential of the youngest fully developed leaf (WP); and relative water content of the youngest fully developed leaf (RWC)) and the chosen spectral indices for the three treatments (control, salinity and salinity+drought) for the winter experiment in 2009 and summer experiment in 2010. A t-test for independent samples is indicated separately for each of the parameters, dates, years and growth platforms. Significant or non-significant cultivar differences are indicated with lower case letters (a,a or a,b, respectively).

					Crop Circle	GreenSeeker	HandySpec									
	FW	WC	WP	RWC	760/670	NDVI	760/670	NDVI	WBI	WBI/NDVI	780/740	760/730	780/550	NWI-3	940/960	REIP
Beginning of drought cycle 1																
Container 2009	a,b	a,b			a,b		a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b
Container 2010	a,b	a,b			a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b
Pot 2009	a,b	a,b			a,b		a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b
Pot 2010	a,b	a,b			a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b
End of drought cycle 1																
Container 2009	a,b	a,b	a,b	a,b	a,b		a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b
Container 2010	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b
Pot 2009	a,b	a,b	a,b	a,b	a,b		a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b
Pot 2010	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b
Middle of drought cycle 2																
Container 2009			a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,a	a,b	a,b
Container 2010			a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b
Pot 2009			a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b
Pot 2010			a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b
End of drought cycle 2																
Container 2009	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b
Container 2010	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b
Pot 2009	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b
Pot 2010	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b

3.2.3 Assessment of active and passive spectral measurements

All three spectral devices differed slightly in terms of their sensor footprint and, consequently, in the measured area when placed at a certain height above the canopy. Nevertheless, the two indices, R_{760}/R_{670} recorded with the Crop Circle ACS-470® and NDVI recorded with the GreenSeeker RT100®, identified qualitatively similar relationships for all the above mentioned parameters, as the same indices were recorded with the passive spectral device for both years and both growth platforms (Tables 10 and 11). Furthermore, the t-test results revealed the same opportunity to differentiate among the three treatments by applying either passive or active spectral devices for both years (Table 13).

3.3 Section III: A comparison of plant temperatures as measured by thermal imaging and infrared thermometry

3.3.1 Soil parameters: Electrical conductivity (EC) and soil matric potential

The electrical conductivity (EC) in 20 cm soil depth increased at the end of and shortly after both drought periods in the summer experiment of 2010 within the salinity+drought treatment, while EC only slightly increased for the salt treatment from sowing till final harvest (Figure 13). The soil matric potential indicated markedly lowered values pointing out severe drought stress for the treatments drought and salinity+drought in the soil depth of 20 cm and 50 cm, with values mostly being beyond the measurement range of the tensiometers.

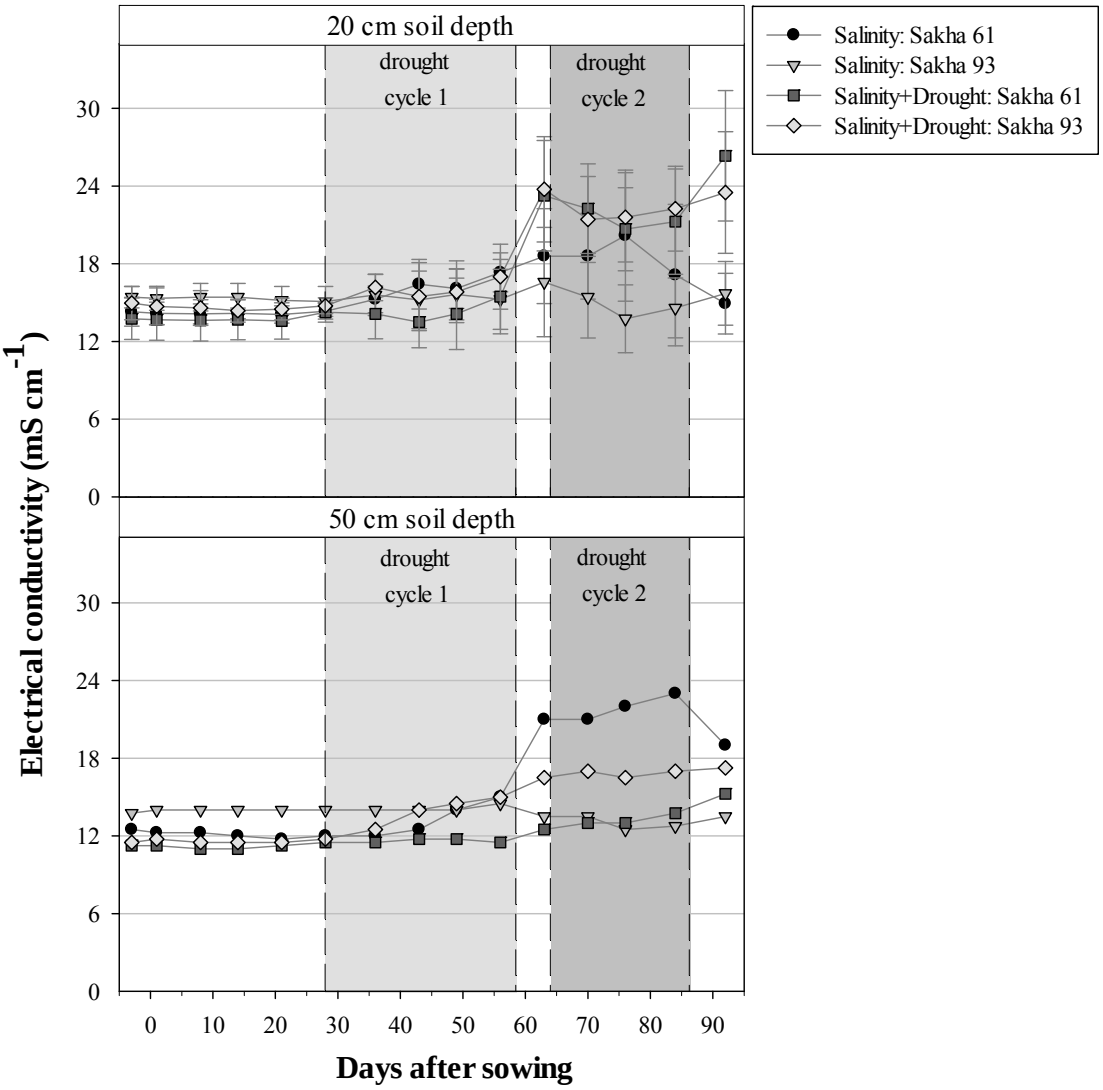

Figure 13. Electrical conductivity of the soil of the treatments salinity and combined salinity+drought measured weekly during the summer experiment of 2010 with in situ salinity sensors in a soil depth of 20 cm and 50 cm in the containers.

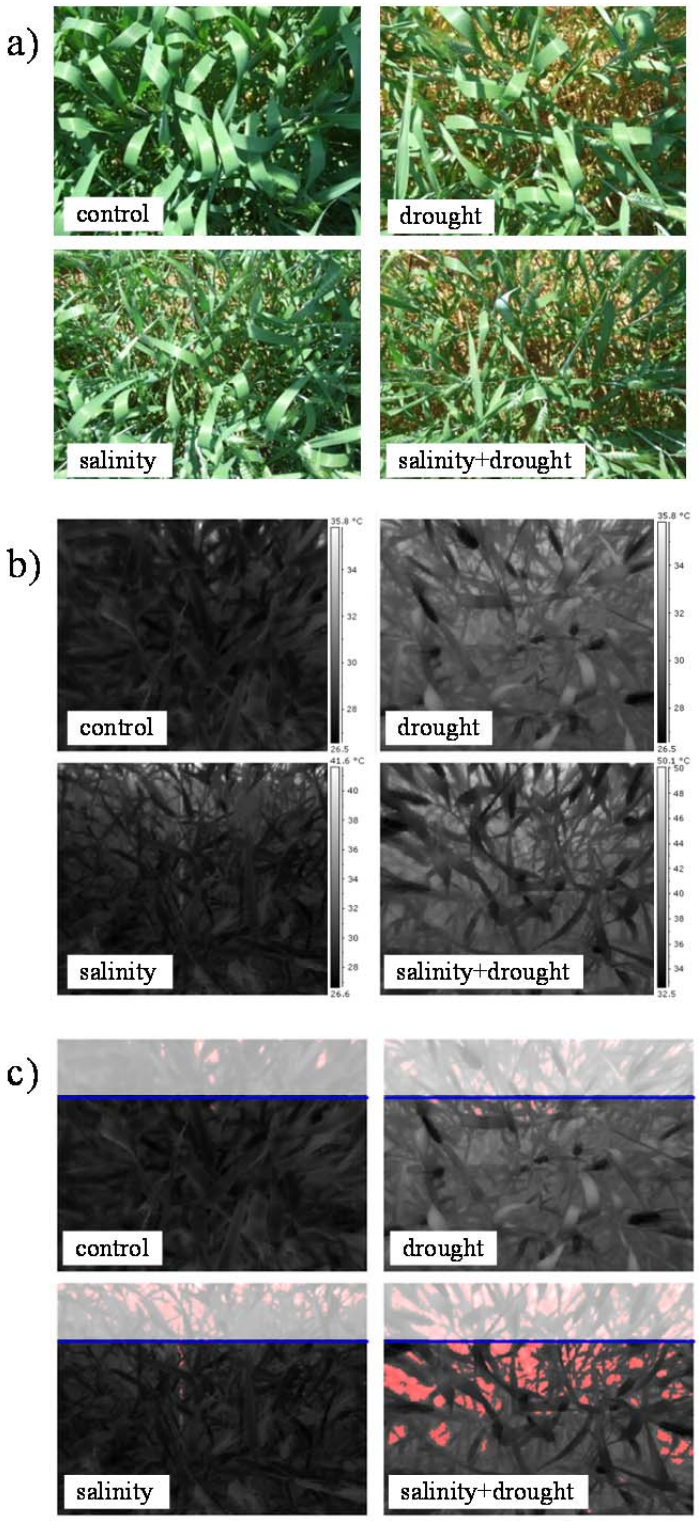

Figure 14. Images of the plant canopies for Sakha 93 taken on June 10, 2010 for each of the four experimental treatments. a) Digital colour image; b) Original thermal image; c) Processed thermal image with soil- and border-effect corrections (the brightened upper part in the thermal images consisting of 60 pixel rows represents the exposed container boundary). All images represent the temperature of all objects in that image, which covers almost the entire area of the container.

3.3.2 Thermal image interpretation

Figure 14 illustrates representative images of the plant canopies of Sakha 61 for each of the four experimental treatments on June, 2010. Obvious differences in the canopy density and the soil coverage among the treatments are apparent, with the highest and lowest values being observed in the control and salinity+drought treatments, respectively. Specific differences among the treatments result from variation in the temperatures of the canopies themselves, the visible fraction of the soil and the soil temperature. For instance, warmer, less dense crop stands were observed at the boundaries of the containers. The influence of the boundary effect on the soil and interrelated canopy temperature was markedly stronger in the combined salinity+drought stress treatment compared to the control and the single stress treatments.

3.3.3 Soil influence on thermography

The influence of the soil on the average canopy temperature as measured using thermography depended on the soil coverage associated with the growth stage as well as the density of the crop stand (Figure 15). Soil influences of approximately 20-30%, with respect to the prevailing temperature range, occurred at the growth stages seeding growth to tillering (May 11, 2010), when soil coverage was low. These values decreased to approximately 1-5% at growth stages of booting to anthesis (June 10, 2010) and growth stages of early milk to soft dough development (July 8, 2010), when soil coverages were remarkably higher. Whereas significant differences in soil influence between the combined salinity+drought stress treatment and the other treatments existed at the end of both drought periods (with the combined treatment having the higher values), no significant differences between the treatments were observed on May 11 due to similar crop densities.

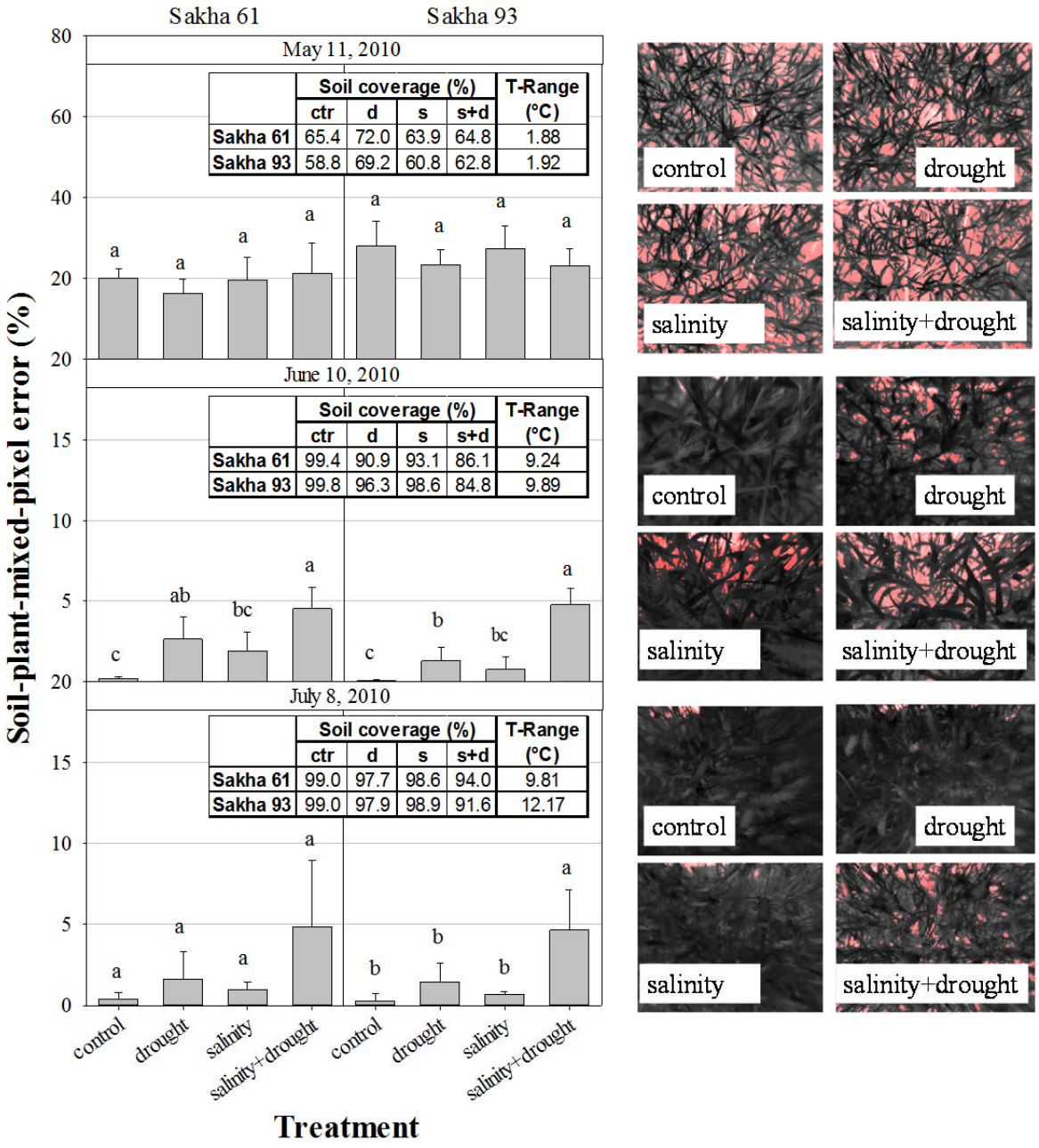

Figure 15. The relationship between the average thermal image temperature of the original and the processed image to the maximum temperature range (mixed soil-plant pixel error) for the four treatments and the two cultivars as indicated by error bars with different soil coverage at different sampling dates in 2010. Tables show the average soil coverage per treatment (ctr = control, d = drought, s = salinity and s+d = salinity+drought) and the maximal temperature range between the cultivars (T-Range). Thermal images are presented for Sakha 61 according to the four treatments. Duncan tests were made separately within each cultivar (low case letters).

3.3.4 Differences in the average canopy and leaf surface temperatures among treatments as detected by three different techniques

The average canopy and leaf surface temperatures differed among the treatments and dates, depending on the weather conditions, the intensity of the stress and the measurement method used (Figure 16). Among treatments the measurements differed greatly for thermography- and thermometry-derived measurements, but the differences were less noticeable using thermistor-based readings. Mild drought or the prevailing salt stress (May 26) caused only small differences in canopy temperature (1-2 °C) between the control and drought treatments, whereas larger, significant differences (3-5 °C) were observed between the control and salinity+drought treatments when thermography and thermometry were used. Severe drought stress (June 10 and July 8) caused large differences (7-9 °C) in canopy temperature between the control and both drought stress treatments, due to the highly significant influence of the stress parameter drought (Table 14). By contrast, differences in canopy temperatures between the control and salt stress were similar (1-3 °C) and highly significant for all measurement days. No significant interaction between the stress treatment salinity and cultivars was observed for thermometry and thermography, and only a small interactive effect was found between the stress treatment drought and cultivars. Finally, the thermistor results were more similar among treatments (1-2 °C) and differed significantly less frequently, regardless of the prevailing stress intensity. Compared to the unprocessed thermal images, the processed images indicated slightly lower canopy temperatures. Thermometry indicated similar or slightly higher canopy temperatures than thermography on May 26 but slightly lower ones at the end of both drought stress periods (June 10 and July 8), probably as a result of the different viewing angle for the former method that avoided capturing the lower canopy layers. Compared to the remainder of the plant parts thermography and thermometry reflected ear temperatures being around 1 °C warmer for the treatment control, around 0.5 °C cooler for the treatment salt and up to 2 °C cooler for the treatments drought and combined salinity+drought at the end of drought cycle 2.

3.3.5 Differences in the canopy and leaf surface temperatures between the two wheat cultivars as detected by three different techniques

Differences between the cultivars within any single treatment were small (0-2 °C; Figure 17). The largest and most significant differences between the cultivars were found using thermography and thermometry and were observed for the salinity treatments on June 10 and

July 8. This result probably reflects the differences in the salt tolerance between the two cultivars, supported by the mostly highly significant cultivar influence shown by the ANOVA analysis (Table 14). By contrast, the thermistor results did not present any obvious pattern, including any relationship associated with salt tolerance.

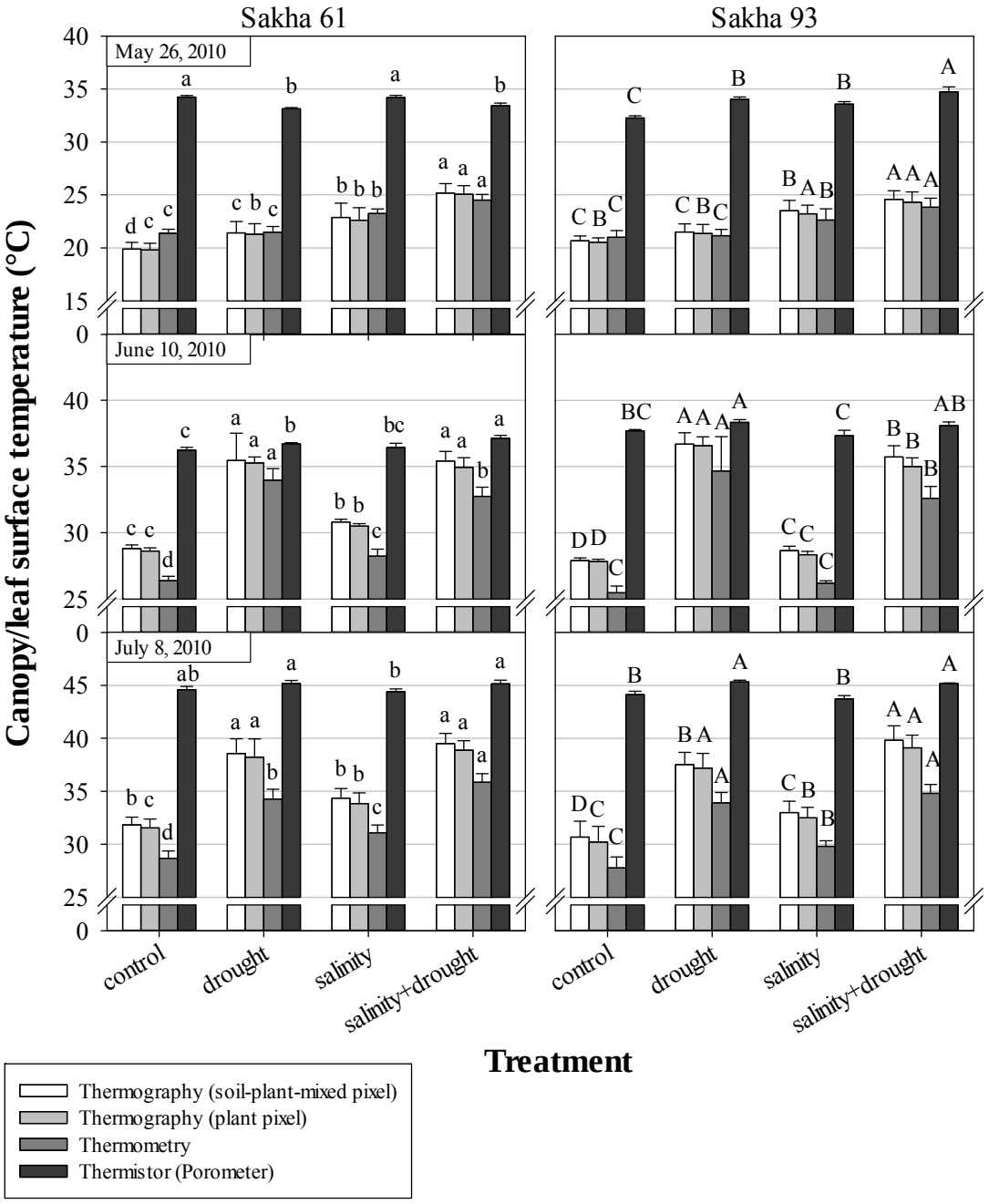

Figure 16. Comparison of the canopy and leaf surface temperatures of the two cultivars Sakha 61 and Sakha 93 for selected dates in 2010 subjected to four treatments as determined using three different measurement methods. The average temperature of the unprocessed (soil-plant-mixed pixel) and processed (plant pixel) thermal image is shown. Duncan test is indicated separately for each of the methods, dates and cultivars (low case letters = Sakha 61, capital letters = Sakha 93).

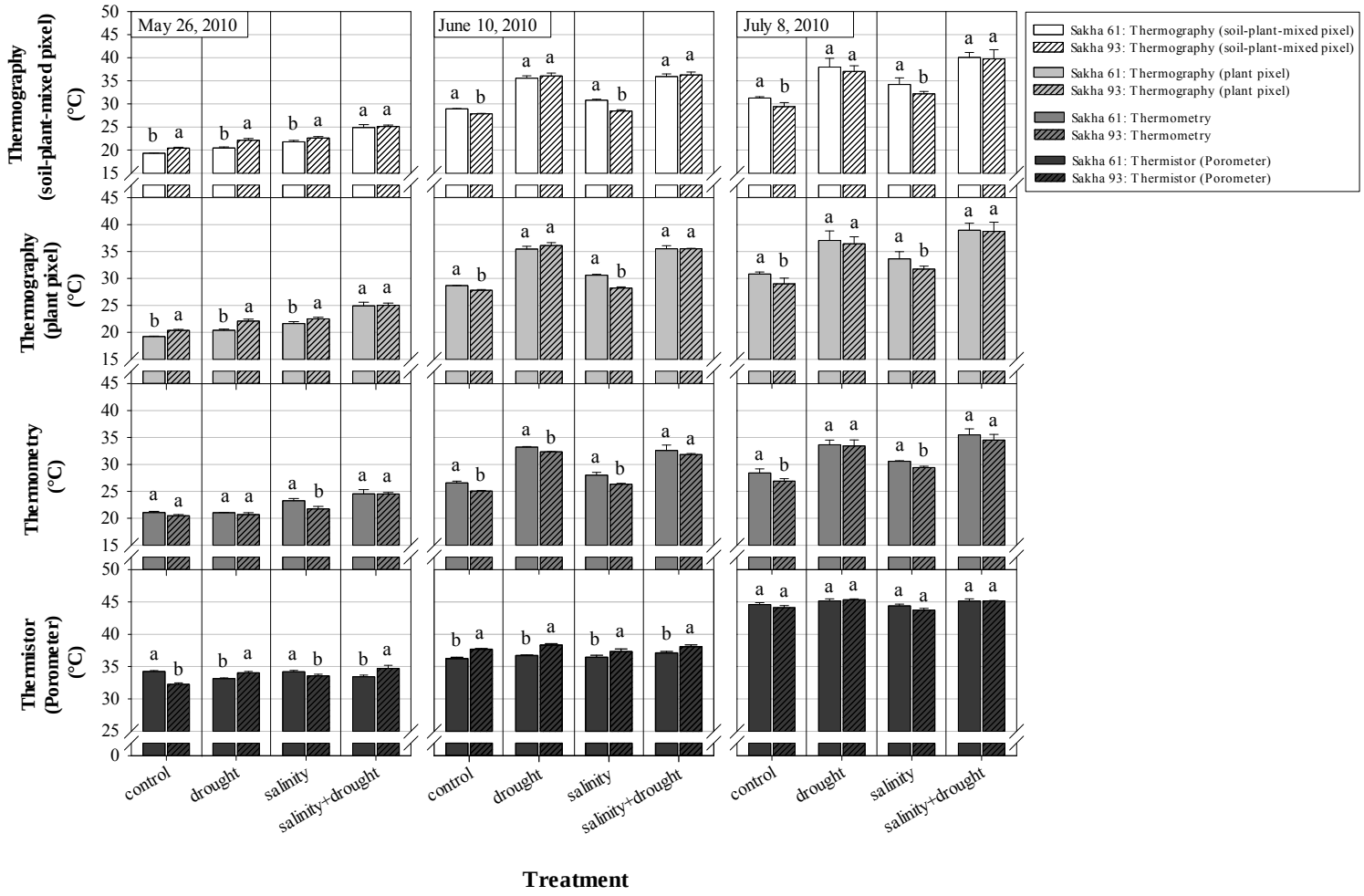

Figure 17. Comparison of the canopy and leaf surface temperature for the two cultivars for selected dates in 2010 within each treatment (control, drought, salinity and salinity+drought) regarding the canopy and leaf surface temperature with three methods (thermography, thermometry and thermistor). The temperature of the unprocessed thermal image (soil-plant-mixed pixel) and after image processing (plant pixel) was averaged. T-test for independent samples is indicated separately for each of the methods, dates and treatments (both cultivars are indicated with low case letters).

Table 14. ANOVA results (F values and its significance) for effects of the stress parameters (salinity, drought), the two cultivars and their interactions. For the thermal images, the average temperature of the unprocessed (soil-plant-mixed pixel) or processed (plant pixel) thermal images was used.

	May 11, 2010	May 26, 2010	June 10, 2010	July 8, 2010
Sources of variation	F value	F value	F value	F value
Cultivar (df=1)				
Thermography (soil-plant-mixed pixel)	0.3	46.4***	13.3**	6.0*
Thermography (plant pixel)	0.7	38.5***	13.1**	4.8*
Thermometry		15.1**	46.6***	8.8**
Thermistor (Porometer)		1.0	152.1***	4.5*
Salt (df=1)				
Thermography (soil-plant-mixed pixel)	63.2***	455.7***	18.3***	24.8***
Thermography (plant pixel)	82.3***	481.8***	11.1***	22.3***
Thermometry		274.8***	4.6*	33.8***
Thermistor (Porometer)		30.6***	0.0	3.3
Drought (df=1)				
Thermography (soil-plant-mixed pixel)	6.0*	224.4***	1541.7***	170.8***
Thermography (plant pixel)	12.1**	225.9***	1883.2***	158.1***
Thermometry		41.6***	1134.4***	275.1***
Thermistor (Porometer)		6.8*	40.6***	80.0***
Cultivar x Salt (df=1)				
Thermography (soil-plant-mixed pixel)	0.0	0.8	14.7*	0.7
Thermography (plant pixel)	0.3	10.2*	8.4*	0.0
Thermometry		0.8	0.0	0.1
Thermistor (Porometer)		17.9***	9.4**	0.6
Cultivar x Drought (df=1)				
Thermography (soil-plant-mixed pixel)	0.6	12.4*	48.4***	1.7
Thermography (plant pixel)	1.7	1.0	44.3***	2.0
Thermometry		7.1*	5.0*	1.2
Thermistor (Porometer)		137.3***	0.3	9.2**
Cultivar x Salt x Drought (df=1)				
Thermography (soil-plant-mixed pixel)	0.0	1.3	0.0	1.3
Thermography (plant pixel)	0.1	2.5	3.3	0.1
Thermometry		3.8	0.1	0.7
Thermistor (Porometer)		5.2*	0.1	0.0

*, **, *** indicate significance at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$

3.3.6 Relationships of thermal information to the leaf water potential and biomass parameters

Temperature measurements, obtained using thermography, were highly significantly related to the leaf water potential across all four treatments for both cultivars at the end of drought period 1 in 2010 (Table 15 and Figure 18). The same was true for thermometry (R^2=0.90*** for Sakha 61 and R^2=0.96*** for Sakha 93) at slightly lower temperatures. Thermistor-derived values displayed the greatest levels of significance of the three methods, but were less strongly related to the leaf water potential (R^2=0.62** for Sakha 61 and R^2=0.48* for Sakha 93) and above-ground dry weight (R^2=0.64*** for Sakha 61 and R^2=0.51** for Sakha 93) at higher temperature levels. Thermography and thermometry showed similar, highly significant results, whereas those of the thermistor data were not as strong. The relationships involving all three methods were weakened at the end of the second drought period because of the progressed senescence in the drought and salinity+drought treatments.

Both thermometry and thermography, and for either the processed and unprocessed image information, showed better relationships to the leaf water potential (a direct and immediate indication of the prevailing stress) than to above-ground dry weight (a parameter that better reflects the accumulative stress) on June 10 (Table 15). The two cultivars also differed with respect to their destructively assessed parameters, with leaf water potentials and above-ground dry weights at final harvest being higher for the salt-tolerant cultivar Sakha 93 than for the less tolerant Sakha 61 at the time of final harvest.

Above-ground dry weight and grain dry weight at final harvest showed similar relationships to the canopy and leaf surface temperatures as did the previous destructive biomass parameters, with the strongest and most highly significant relationships being found at the end of both drought stress periods (on June 10 and July 8) for all three measuring methods (Table 15). Again, however, the relationships were not as strong for the thermistor-derived values.

Overall, the relationships established by thermography (with or without processing) were the most closely correlated with the measured plant parameters; those obtained using thermometry showed a similar pattern, but were generally marginally less well related. Finally, leaf surface temperatures as measured with the thermistor technique generally presented the weakest relationships among the three methods for nearly all parameters.

Table 15. Coefficients of determination for the relationships between the leaf water potential, the destructive biomass parameters, the final harvest parameters and the canopy and leaf surface temperatures of the two cultivars (Sakha 61 and Sakha 93) over all four treatments examined (control, drought, salinity and salinity+drought) at selected dates. For the thermal images, the average temperature of the unprocessed (soil-plant-mixed pixel) or processed (plant pixel) thermal images was used. The fresh weight of the shoot at destructive biomass sampling was used as the control variable for the partial correlation between the leaf water potential and the canopy and leaf surface temperature. Canopy and leaf surface temperature measurements at different growth stages were done at the same time as the leaf water potential measurements and the destructive biomass samplings, while they were used as an estimate of the final harvest parameters.

		Leaf water potential				Destructive biomass parameters - shoot				Final harvest parameters			
		R^2		Partial R^2		Above-ground fresh weight		Water content		Above-ground dry weight		Grain dry weight	
Date	Method	Sakha 61	Sakha 93	Sakha 61	Sakha 93	Sakha 61	Sakha 93	Sakha 61	Sakha 93	Sakha 61	Sakha 93	Sakha 61	Sakha 93
May 26, 2010													
	Thermography (soil-plant-mixed pixel)	0.88***	0.77***							0.45**	0.20*	0.40**	0.08
	Thermography (plant pixel)	0.88***	0.81***							0.48**	0.18*	0.41**	0.07
	Thermometry	0.85***	0.76***							0.32**	0.17*	0.25*	0.07
	Thermistor (Porometer)	0.04	0.50**							0.46*	0.69**	0.48*	0.56**
June 10, 2010													
	Thermography (soil-plant-mixed pixel)	0.98***	0.94***	0.92***	0.87***	0.69***	0.66***	0.85***	0.79***	0.86***	0.90***	0.86***	0.94***
	Thermography (plant pixel)	0.98***	0.94***	0.93***	0.88***	0.67***	0.62***	0.83***	0.76***	0.86***	0.90***	0.85***	0.94***
	Thermometry	0.90***	0.96***	0.83***	0.94***	0.56**	0.67***	0.74***	0.83***	0.76***	0.81***	0.77***	0.88***
	Thermistor (Porometer)	0.62**	0.48*	0.04	0.68**	0.72***	0.14	0.72***	0.31	0.64***	0.51**	0.52**	0.66***
July 8, 2010													
	Thermography (soil-plant-mixed pixel)	0.55**	0.58**	0.01	0.13	0.83***	0.86***	0.66***	0.81***	0.85***	0.85***	0.81***	0.79***
	Thermography (plant pixel)	0.55**	0.61**	0.00	0.10	0.83***	0.86***	0.66***	0.83***	0.81***	0.86***	0.77***	0.81***
	Thermometry	0.55**	0.69**	0.00	0.00	0.81***	0.90***	0.67***	0.86***	0.83***	0.88***	0.77***	0.85***
	Thermistor (Porometer)	0.32	0.62**	0.07	0.00	0.30	0.77***	0.48*	0.76***	0.26	0.83***	0.26	0.92***

*, **, *** indicate significance at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$

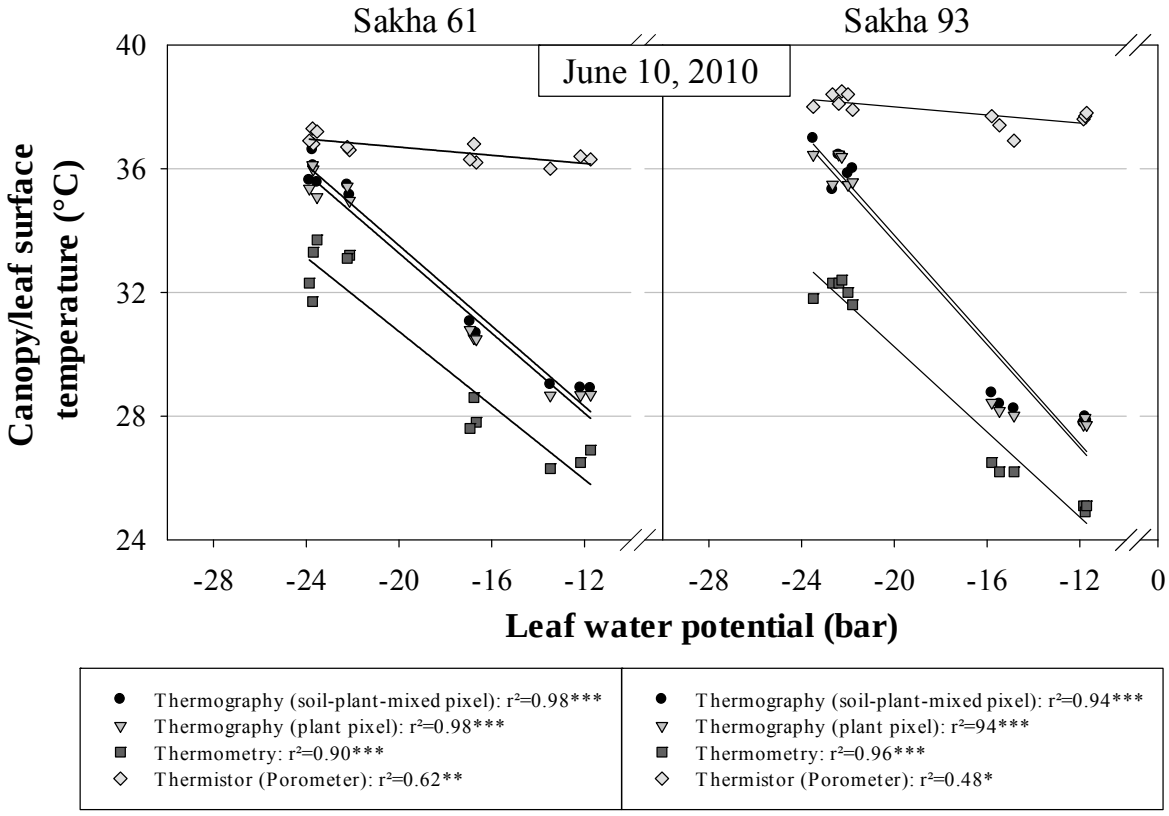

Figure 18. Relationships between the leaf water potential and the canopy and leaf surface temperatures over all four treatments for each of the two cultivars, Sakha 61 and Sakha 93, as taken on June 10, 2010 with the three different measurement methods. For the thermal images, the average temperature of the unprocessed (soil-plant-mixed pixel) or processed (plant pixel) thermal image was used.

4 Discussion

4.1 Section I: What are reliable platforms and stress scenarios to assess salt tolerance of wheat plants?

Salinity stress occurs often simultaneous with drought stress, and thus, decreased soil matric potentials need to be taken into account when investigating the effects of salinity. Currently, the use of unrealistic stress protocols, such as those for mimicking salinity/drought stress in pots, have been widely used in biotechnology studies and high-throughput systems that plant biology community currently makes a great effort to develop for plant phenotyping for abiotic stress tolerances (Mittler 2006; Granier et al. 2006; Tavakkoli et al. 2010; Poorter et al. 2012; Nagel et al. 2012). Furthermore, soil temperatures, rates of soil drying, uniformity of moisture content throughout the rooting horizon, rootable volume and availability of nutrients in pot experiments may vary with pot size and be greatly different from field conditions. Therefore, the use of unrealistic stress protocols for phenotyping of abiotic stress in research programs may lead to wrong conclusions (Mittler 2006; Poorter et al. 2012). To our knowledge, this is the first study to evaluate the different salt tolerance of wheat cultivars with salinity alone and salinity combined with drought stresses by comparing a pot experiment with large containers, which were designed to obtain a simulated field platform.

The results of this section showed that response of the grain yield of two contrasting wheat cultivars to salinity alone for both container and pot experiments was similar to our previous study (El-Hendawy et al. 2005a) with Sakha 93 being more tolerant to salinity than Sakha 61. However, the tolerance of two contrasting cultivars to salinity combined with drought was similar for both container and pot experiments but with higher grain yields in the pots. This suggests that, to screen salt tolerance of wheat genotypes in arid and/or semiarid areas, combination of salinity and drought must be considered. Surprisingly salinity combined with drought did not show any additive effect on grain yield for both cultivars compared to that under drought stress alone. There was even a higher grain yield of both cultivars in the pot experiments. In literature, both additive and non-additive effects have been reported. Soil matric and osmotic potentials were found to have an additive effect on yield of corn (Hanks et al. 1976; Stark and Jarrell 1980; Frenkel et al. 1990), on wheat (Broadbent et al. 1988) and on cotton and pepper (Shalhevet and Hsiao 1986). In contrast, Hao and de Jong (1988) found that

shoot growth rates of wheat and barley were not affected by osmotic stress. Non-additive effect of soil matric and osmotic potentials on growth of plants was also reported for carrot (Schmidhalter and Oertli 1991), for tall fescue and bermudagrass (Dean-Knox et al. 1998) and for corn, melon and alfalfa (Shani and Dudley 2001).

To explain the additive effect of osmotic and matric potential in soil, Wadleigh and Ayers (1945) postulated that yield is related to the total soil water potential, regardless of which component or combination of components contributes to the total potential. The total water potential of a soil may include soil matric potential, soil osmotic potential and soil gravitational potential. The gravitational component of soil water potential is a function of position and therefore generally neglected. The osmotic component has little effect on the movement of liquid water within soils because there is no membrane barrier and usually no change of phase in the flow path. The theoretically expected osmotic potentials will only be realized, if plants behave as ideal osmometers. Consequently soil matric potential remains the most significant component to be taken into account when considering water movement within soils. The non-additive effect may have been due to both tissue dehydration and increased concentration of ions occurring simultaneously under saline and water deficit conditions. Osmotic adjustment in a saline medium is generally favoured by the presence of solutes, whereas in the case of matric stress the plant is much more dependent on internally generated osmotica, which is probably associated with a higher metabolic energy requirement (Hu and Schmidhalter 1998).

Our results showed that for the treatment of salinity combined with drought at the end of the second drought cycle, soil matric and osmotic potential in containers at a soil depth of 20 cm were approximately -0.08 MPa and -0.80 MPa, respectively. However, only a similar effect of both combined stress and drought alone on grain yield was found regardless of cultivars. This may suggest that the soil osmotic potential from salinity does not contribute significantly to the additive effect of the total soil water potential in our study. If this is true, the two-phase model that salinity stress causes the first phase (water deficit effect) and the second phase (ionic effect) needs to be further examined. In fact, a number of studies have compared the relative effect of soil osmotic and matric potential. For example, Dean-Knox et al. (1998) reported that, in comparing the effect of a unit change (1 MPa in matric vs. osmotic potential), there was a 20 to 35% greater decrease in yield of both tall fescue and bermudagrass under the unit decrease in matric potential. A generalized relationship of relative plant yield to decreasing potentials and possible interactive effects of matric and osmotic potential was suggested by Schmidhalter and Oertli (1986). Seedling growth of carrot was not affected by

osmotic potentials as low as -0.5 MPa. Shoot growth was optimum at matric potentials of -0.05 to -0.1 MPa, whereas lower matric potentials caused marked reductions. Therefore, drought seems to be much more dominant than salinity stress and the osmotic stress induced by salinity affects plant growth less than soil matric potentials induced by drought (Shalhevet and Hsiao 1986; Schmidhalter and Oertli 1991; Singh Grewal 2010). However, the above studies cannot still distinguish whether the negative effect of salinity on plant growth is from the contribution of salinity to soil water potential or also due to ionic effects of salinity on plant growth under combined stress conditions. Since the degree of the relative interactive effect of osmotic and matric potential may depend on the level of stress, we may expect that differences between matric and osmotic potentials are mainly found under mild to moderate stress conditions, whereas more similar effects may be found under more severe stress conditions due to ionic effects influencing growth of plants more negatively than negative effects of a decreasing water potential. However, with regard to the ionic effects, Na^+ and Cl^- concentrations in plants in the combined treatment were much higher than those in the treatment drought alone, which did not result in a greater decrease in grain yield under salinity+drought conditions regardless of cultivars. This may suggest that there was no ionic effect due to salinity in the combined stress treatment. Higher Ca^{2+} concentrations in plants are known to be able to increase salinity tolerance (Cramer 2002). Furthermore, the addition of Ca^{2+} can lead to improved plant growth compared to salinity stress, caused by NaCl solely (Ehret et al. 1990). Compared with the treatment drought, higher Ca^{2+} in plants of the combined treatment may contribute to non-additive effects of salinity combined with drought on grain yield of wheat in both platform experiments. Although El-Hendawy et al. (2005b) found differences in the Na^+ and Cl^- concentration in leaves between these two cultivars, Na^+ and Cl^- concentration in plants of this study were similar for both cultivars regardless of the growth platform, suggesting that Na^+ and Cl^- exclusion may not always be the trait for screening plant salt tolerance, which is supported by Genc et al. (2007).

For the comparison of containers as a simulated field platform compared to pots, our results showed an interactive effect of treatments and different platforms on growth and grain yield regardless of cultivars. Under control and pure saline conditions, grain yield was higher in pots than in containers regardless of cultivars. In contrast, grain yield was lower in pots under salinity combined with drought and drought alone than in containers. These may suggest that pot experiments can lead to different conclusions for screening salt tolerant wheat genotypes compared to experiments in containers. A well-recognized problem with growing plants in pots under limited soil volume conditions is the possibility that plants may become “root

bound" (Townend and Dickinson 1995; Whitfield et al. 1996; Ray and Sinclair 1998). Therefore, the rooting environment in pots may cause the artefacts due to differences in: i) rates of soil drying; ii) uniformity of moisture content throughout the rooting horizon; iii) soil temperature, and iv) different availability of nutrients. An increase in grain yield in the control and salinity treatments from our study is inconsistent with numerous studies which demonstrated a general reduction in growth associated with smaller pot sizes (Peterson et al. 1984; Robbins and Pharr 1988; Townend and Dickinson 1995; Whitfield et al. 1996; Ray and Sinclair 1998; Passioura 2006; Wu et al. 2011; Poorter et al. 2012), caused by the restricted rooting environment. Under control and saline conditions, the grain number per ear was higher in pots than in containers, whereas a slight decrease in TGW in pots was observed compared with that in containers. Grain number of wheat is established in earlier growth stages, while TGW is determined during later stages, i.e. grain filling after anthesis (Grieve et al. 1994; Rajala et al. 2011). The difference in grain yield of wheat between pots and containers may be due to the different soil temperature in both growth platforms. Small pots can be much warmer than the air in a glasshouse, while larger pots are better buffered against changes in the air temperature (Townend and Dickinson 1995). Our plants were sown in early spring and the air temperature was still relatively low (18.7 °C at the beginning of drought cycle 1 versus ~ 30 °C at the end of both drought cycles). If the soil in pots is warmer than in containers, higher temperature may stimulate the plant development in spring time, which results in higher grain numbers. In later growth stages, however, hot weather in summer may heat up the soil and cause heat stress in pots, which may lead to a lower TGW in pots. More importantly, soil in pots may dry out more quickly under the combined stress and drought alone which may result in lower grain numbers per ear and TGW in contrast to control and salinity alone (Townend and Dickinson 1995; Ray and Sinclair 1998; Passioura 2006; Singh Grewal 2010; Poorter et al. 2012). In a study by Katerji et al. (2009), drought stress reduced the grain and straw weight of wheat and barley by reducing the number of ears per plant independently from the level of soil salinity. While there seems to be no short term adjustment for rapid soil drying, leading to unavoidable water stress to the plants and possibly to the death of a considerable proportion of the roots, roots may adjust to salinity within a short period of time (Homaee and Schmidhalter 2008).

4.2 Section II: Spectral assessment of wheat plants grown in pots and containers under saline conditions

This section shows the potential to spectrally assess agronomic and physiological traits of salt-stressed wheat plants by relating spectral indices to the fresh weight and water content of the above-ground biomass as well as the water potential of the youngest fully developed leaf of container- and pot-grown plants. Although spectral measurements have been previously applied in pot experiments under saline conditions (Turhan et al. 2008; Rud et al. 2011; Elmetwalli et al. 2012), no direct comparison to a close-to-field container platform has been previously reported. Results of the current experiments suggested that the selected spectral indices were generally better related to traits of plants grown in containers than in pots. Further analysis indicated the potential to differentiate between relevant traits of the two investigated cultivars destructively as well as by non-destructive spectral assessments for both growth platforms.

4.2.1 Characteristics of spectral measurements in close-to-field containers and pots

The water potential of the youngest fully developed leaf, the fresh weight of the above-ground biomass and the water content of the above-ground biomass of pot-grown plants showed at least similar to significantly larger ranges than in container-grown plants. Nevertheless, the relationships to the spectral indices in the pots were less robust at the end of both drought cycles for both years studied compared to the results in the containers. These results contrasted with those from a study by Peñuelas et al. (1997a), who found improved relationships with increasing ranges of the plant water concentration to the WBI in pots. Furthermore, the slopes of the linear regression lines obtained for the container-grown plants were significantly steeper compared to those of pot-grown plants, except for the NDVI. These results implied that the investigated agronomic parameters cannot be detected as precisely in pots as they can be in containers, thereby lowering the coefficients of determination. Visual information clearly showed a greater number of senescent leaves for the plants grown in pots compared to the plants grown in containers, as were first observed at the end of drought cycle 1. Although the same amount of NPK fertilizer per area was applied in both growth platforms, the increased senescence may have been caused by the restricted rooting volume, thus resulting in a decreased soil nitrogen supply that is corresponding to Poorter et al. (2012).

Additionally, the areal canopy differences of the crop stands of pot- and container- grown plants may impact the quality of the relationships to the spectral indices. Nadir downward-oriented spectral measurements of pot-grown plants are confronted with increased background noises compared to container or field measurements due to the device-specific footprint at certain distances above the plants. The pots were placed 1 m above concrete ground on a steel carrier to reduce background noise with respect to the inverse square law, which states that the light intensity decreases four times by duplicating the measuring distance (Kipp, et al. 2012, unpublished). All of the above mentioned factors, measurements and observations collected throughout the two year duration of this study may indicate that the spectral measurements from the nadir position were better suited to a saline container platform with a larger canopy area compared to screening only a few plants in pots. Whereas many spectral measurements are performed from nadir (Elsayed et al. 2011), the angle at which spectral measurements are taken has a tremendous influence on spectral indices in a pot experiment under salt and water stress (Poss et al. 2006). The combined analyses from the top and side of single plants or plant canopies reported by Golzarian et al. (2011) and Rajendran et al. (2009) have contributed to improved results in plant phenotyping under salt stress and may lead to closer relationships between spectral or image data and plant stress or biomass data. While close-to-field container measurements delivered better results in the present experiment, phenotyping tools in dedicated high-throughput and controlled platform facilities may have the potential to improve precision and reduce the need for replication in the field, although traits phenotyped in pots may not always be replicated in field-scale experiments (Furbank and Tester 2011).

4.2.2 Influence of seasons on spectral measurements

A notable difference in the destructively assessed biomass parameters was found between the two years (seasons) even at comparable growth stages, thus requiring a separate analysis. The different growth conditions most likely resulted in morphological and biomass differences. Such differences are not uncommon and were also reflected in the spectral records. However, the same spectral indices performed reliably in both years and allowed to differentiate stress related traits and both cultivars consistently in both years. By combining data over time, even though good relationships occasionally may be obtained, a non-continuous behaviour frequently becomes obvious by carefully inspecting combined time- or growth stage-specific datasets (Elsayed et al. 2011). A lack of continuous associations over time indicates a need to

establish time-specific or growth stage-specific relationships (Mistele and Schmidhalter 2008, 2010; Winterhalter et al. 2011a, b). Still a relative differentiation of cultivars at given times is highly useful being congruent with the relative scoring as adopted by breeders.

Spectral measurements during seeding growth and tillering were not sufficient for recognizing the fresh weight and the water content of the above-ground biomass in both growth platforms, primarily due to disturbance from the soil background and only slight differences between the stress treatments. At later growth stages, the fresh weight and water content of the above-ground biomass were better differentiated due to distinct differences in the agronomic parameters between the stress treatments with similar findings in a study based on thermal measurements (Hackl et al. 2012). Liebler et al. (2001) and Sembiring et al. (1998) reported weak relationships between biomass and nadir spectral measurements up to ZS 30 and 31, respectively. Mistele and Schmidhalter (2010) observed a positive effect of an oblique view for measurements at early growth stages, with the best description of dry matter yield and N status by the NDVI, R_{780}/R_{550}, R_{780}/R_{740} and REIP at ZS as low as 15 (Mistele and Schmidhalter 2008).

4.2.3 Suitability of spectral indices for recognizing agronomic and plant stress parameters

The R_{760}/R_{670}, NDVI and R_{780}/R_{550} spectral indices were significantly related to the water potential of the youngest fully developed leaf, the fresh weight of the above-ground biomass and the water content of the above-ground biomass from the beginning of booting for both years, with the latter result concurring with the results from a study by Winterhalter et al. (2011b). Wavelength ranges of 510-780 nm and of 540-780 nm are most suitable to describe the water content in wheat due to an increase in reflectance (Graeff and Claupein 2007), which may be attributed to a compound effect of a change in the internal leaf structure and to a change in light absorption by photosynthetic pigments. Both near-infrared-based indices, R_{760}/R_{730} and R_{780}/R_{740}, presumed to identify green vegetation over soil (Peñuelas et al. 1995), had been proven to be powerful indices to indicate differences in biomass of wheat (Mistele and Schmidhalter 2010; Erdle et al. 2011). Additionally the index R_{780}/R_{550} was also shown to significantly assess the above-ground biomass dry weight of wheat plants (Mistele and Schmidhalter 2008). In other studies, the NDVI has also been shown to be a powerful and frequently used reflectance index to describe the leaf water potential and green biomass (Elsayed et al. 2011). It has revealed to be useful in differentiating the biomasses of barley and pepper plants at salinity levels up to 15 dS m-1 (Peñuelas et al. 1997b) and 7.8 dS m-1

NaCl (Leone et al. 2001), respectively. However, Wang et al. (2002) did not fully confirm this result for soybeans grown under low salinity levels. Based on high autocorrelations between the above mentioned agronomic parameters, the R_{760}/R_{670}, NDVI, R_{780}/R_{550}, R_{780}/R_{730} and R_{780}/R_{740} were similarly well related to these parameters.

It seems that the visible/NIR based indices were more stable in salinity experiments, whereas the NIR/NIR based indices were more stable under non saline conditions. Hypothetically the NIR reflectance sensitive intercellular water content could be seen as the reason for the different behaviour of the indices (Heege et al. 2008).

The WBI as well as the WBI/NDVI index were not as stable and closely related to the fresh weight, the water content of the above-ground biomass or the water potential of the youngest fully developed leaf, although these indices had been successful in expressing structural changes in the plant due to varying cell water content (Peñuelas et al. 1993). In contrast, Peñuelas et al. (1997a) and Leone et al. (2001) generally found better relationships in their experiments with different soil salinities or water regimes. A drought stress based field study done by Gutierrez et al. (2010) highlighted significant correlations of wheat genotypes between certain water band indices and the leaf water potential and the relative water content of flag leaves across booting, anthesis and grain filling stages, but not at individual growth stages. Furthermore, Peñuelas et al. (1993) and Peñuelas and Inoue (1999) found good relationships between the relative leaf water content of peanut, wheat and gerbera plants and the WBI and WBI/NDVI, which however could not be supported by the present results.

The REIP was significantly related to the fresh weight of the above-ground biomass and to the water potential of the youngest fully developed leaf only in the container-grown plants at the end of both drought cycles. Mistele and Schmidhalter (2008, 2010) found good and stable relationships between the REIP and the above-ground biomass dry weight from the end of tillering to flowering in the field, but these results were not confirmed under the conditions of the present experiment.

4.2.4 Evaluation of agronomic parameters with active and passive spectral sensors

The assumption that active spectral sensing may be more robust compared to passive sensing due to the variable illumination conditions in a greenhouse could not be supported by the present study. The R_{760}/R_{670} and NDVI spectral indices, as calculated for active and passive sensing, as well as all the other indices calculated for passive sensing, indicated comparable relationships to the agronomic parameters and similarly differentiated the cultivars in both

growth platforms during both years of this study. Even though differences in radiation occurred within the season and between the two seasons, they did not affect the stability and suitability of either the passive or active sensors. Whereas active sensors can work independent of varying illumination conditions (Erdle et al. 2011), the bi-directional passive radiometer used in this study could compensate for any changes of light and was not influenced by sunny or cloudy conditions in agreement with previous reports detailing this issue (Mistele and Schmidhalter, 2008, 2010; Erdle et al. 2011). The spectral indices allowed successful and consistent differentiation between the two wheat cultivars varying with respect to their salt tolerance. Gutierrez et al. (2010) described the sensitivity to genotypic differences of the water spectral index NWI-3 related to the leaf water potential across selected growth stages in a drought stress experiment. Similar to a work by Erdle et al. (2011), the selected VIs in our study could successfully and consistently differentiate two wheat cultivars differing in their salt tolerance feature (El-Hendawy et al. 2005) in a saline pot as well as container platform similarly well as did the agronomic parameters over both years.

4.3 Section III: A comparison of plant temperatures as measured by thermal imaging and infrared thermometry

4.3.1 A comparison of methods to measure plant temperatures

The efficacy of leaf surface and canopy temperatures to screen for stress detection and cultivar differentiation has long been recognized and it is becoming common in breeding programs worldwide for screening and comparing genotypes under various conditions. However, in aiming to distinguish three stress scenarios vs. a control for two wheat cultivars to relate plant temperatures to the leaf water potential and to biomass parameters, this section points out important differences in the quality of the results depending on the measurement method (i.e., thermography, IR thermometry or thermistor measurements).

In agreement with previous findings, whole-canopy temperature measurements seem to be preferable to point measurements of selected leaves within the plant stand (Grant et al. 2007). Our results demonstrate that both thermography and IR thermometry are quick and easy to apply under either close to field or greenhouse conditions. Both methods deliver comparable results in dense crop stands, but an advantage to thermography over IR thermometry is that it offers opportunities for further processing and automation that the latter does not because it depicts the temperature distribution over all objects within the entire image area. Thus, through the post-processing of the thermal images, the exclusion of non-plant material as well as the consideration of only those areas of interest are possible. A critical step in this procedure, however, is to separate the soil from plants by assuming threshold values based on the observation that bare and especially dry soils tend to be hotter than the vegetation itself (Luquet et al. 2003). However, the setting of thresholds is ultimately subjective and the resolution of the thermal camera often does not allow perfect separation of the plant from the soil, leading to the problem of mixed soil-plant pixels, especially on the leaf and shoot boundaries. The extent of the problem, however, depends on the device used and the overall subjectivity of the threshold method can be reduced by combining digital colour photos with thermal images, as has been shown by Möller et al. (2007) and Wang et al. (2010). Even though first successful attempts to solve these problems have already been published in the literature, the further transfer to high-throughput phenotyping remains to be shown.

The methods also facilitated the differentiation among the stress treatments at the end of both drought periods in 2010, although it was not as distinct during the first drought period. Our

results agree with those of Fuchs (1990), who found that the variability of the leaf temperature increased as stress increased, as well as with those by Inagaki and Nachit (2008) indicating that screening and differentiation of cultivars under increasing stress was facilitated.

The slight differences between thermography and thermometry probably derive from the different viewing angles and soil influences. Whereas thermometry primarily measured the upper canopy layers as a consequence of the 30° angle of view in our experiment, the nadir measurements with thermography could capture the overall temperature distribution of the entire canopy, including the lower layers. Our results show the potential influence of the soil on the temperatures of the lower canopy layers (also Rodriguez et al. 2005; Möller et al. 2007; Jones et al. 2009; Blum 2011). Ears, as morphological structures with little transpiration, can influence the canopy temperature to a certain extent dependent on the stress treatment especially when measurements are done from the nadir position with thermography and thermometry. Similar results as already shown by Hatfield et al. (1984) were found at the end of drought cycle 2 with decreased ear temperatures compared to the other plant parts under stress treatments.

During the first drought stress period in 2010, the virtual lack of difference between the results from thermometry and thermography was likely a consequence of the milder drought stress (see above), a moister top-soil layer and the reduced canopy height. Again, these results indicate the potential influence of the soil to distort the canopy temperature measurements depending primarily on the degree of soil coverage (Rodriguez et al. 2005; Möller et al. 2007; Blum 2011). To avoid this potential for error when there was no opportunity to post-processing the thermal images, measurements were generally performed using angles varying between 25-35° from the horizontal (Blum et al. 1982; Rashid et al. 1999). Our experiment supports the contention that the degree of soil influence depends on the amount of soil coverage and furthermore indicates that it is most pronounced in early growth stages when soil coverage is low.

A related error revolves around boundary effects in the thermal images, with Möller et al. (2007) finding that the quality of the results depends strongly on the canopy section in the thermal image, with obvious differences between the centre and the boundary areas. Our results support these observations, with the boundary influence on the exposed container side was being clearly visualized in the thermal images.

Although thermistor measurements with a porometer represent the current method of choice to measure stomatal conductance in the field or greenhouse (Grant et al. 2006), they can perform poorly in regard to leaf temperature measurements with a thermistor due to the

modified leaf environment in the cuvette (Camp 1996; McDermitt 1990; Idso and Allen 1988; Meyer et al. 1985) and are comparatively time-consuming and labour-intensive. Although the thermistor may be directly attached to the leaf, the leaf temperature can be significantly influenced by the ambient temperature and the heating of the porometer chamber itself, especially during continuous use in full sun (McDermitt 1990). Thus, thermistor readings can differ greatly from the actual leaf temperature as measured with an infrared thermometer. Previous experiments indicate an overestimation of the leaf temperature by up to approximately 6 °C (Meyer et al. 1985), with our results reinforcing these errors depending on the air temperature and treatment.

4.3.2 Discrimination ability among stress conditions and plant cultivars through temperature measurements

The strongest and most highly significant relationships with the leaf water potential were found with canopy temperatures measured by thermometry or thermography, with the results from thermography being nearly always slightly better. For the most part, the processed thermal images did not result in better relationships compared to the unprocessed ones, probably because the upper canopy layers represent the main source of temperature information. However, the inclusion of the slightly soil-influenced lower canopy layers may also have contributed to stronger correlations.

Likewise, the four experimental treatments examined here (control, drought, salinity and salinity+drought) were differentiated best using thermometry and thermography (and with either the processed and unprocessed thermal information for the latter). The successful differentiation of the treatments, however, did not require exclusion of the soil and boundary effects from the thermal images, especially in plant stands with high soil coverage, because of the large differences between the treatments depending on the stress intensity. Altogether, the canopy temperature reflected the prevailing stress as well as leaf water potential did; similar observations have been obtained with thermometry (Blum et al. 1982) or thermography (Cohen et al. 2005). The strength of the relationships also increased with increasing stress intensity, especially in the drought stress treatment, supporting the findings of Meron et al. (2010). Blum et al. (1982) found that not only the associations of leaf and canopy temperatures with leaf water potential but also their ability to separate genotypes was enhanced with stress intensity. However, with progressing senescence, especially for the

drought and salinity+drought treatments, weaker correlations with leaf water potential were obtained despite using only green leaves for all measurements.

Conflicting information exists with regard to the relationships between biomass parameters and canopy temperatures as measured by thermography and thermometry. Whereas good relationships have been found distinguishing water-stressed from non-stressed plants by either thermometry (Rashid et al. 1999; Selige and Schmidhalter 2001; Babar et al. 2006; Romano et al. 2011; Winterhalter et al. 2011b) or thermography (Inagaki and Nachit 2008), others could not fully confirm these results (Blum et al. 1989). Our experiment showed that the quality of the relationship between canopy surface temperatures and destructively assessed biomass and final harvest parameters depended strongly on the growth stage of the plant, the stress intensity and the parameters being assessed. In general, canopy temperature measurements at later growth stages, were better related to the above-ground dry weight and grain dry weight at the final harvest.

Differentiating between the cultivars was more difficult and presented higher demands on the methods used because the results can be influenced and distorted by soil and boundary influences, even if they are small. Our experiment showed that differentiating between the cultivars, as for the stress treatments, was more successful using thermometry and thermography than with thermistor measurements. Similar results from the literature are mixed. In experiments aimed at analyzing the robustness and sensitivity of thermal imaging for grapevines, beans and lupins, Grant et al. (2006) showed that both the absolute canopy or leaf surface temperatures assessed by thermography and the stomatal conductance assessed by porometry could successfully distinguish between irrigated and non-irrigated plants under greenhouse conditions. Using a low-cost microcontroller-based monitoring system together with an infrared thermometer for measuring the plant canopy temperature under moisture deficit and heat stress conditions, Kebede et al. (2011) could successfully distinguish between several corn genotypes with the most tolerant genotype having the lowest canopy temperature. By contrast, Inoue (1990) pointed out that the canopy temperature alone cannot be the absolute estimator of physiological status of crop plants, instead providing more quantitative and reliable information when it is used as an input into biophysical models or stress indices.

Processing of thermal images with its huge time effort barely led to improved results in dense crop stands compared to unprocessed thermal images and thermometry. Whereas the use of thermometry in high-throughput phenotyping has already successfully been demonstrated elsewhere (Winterhalter et al. 2011b), an outstanding challenge in thermal sensing still

remains the development of fast, automated methods to analyze thermal images, thereby enabling high-throughput phenotyping of genotypes.

4.4 Suitable high-throughput screening methods for simulated field environments with combined salt and drought stresses to phenotype plant tolerance to salinity

This study was based on container- and pot-grown spring wheat cultivars and highlights the impacts of salinity and drought alone and combined, on selected physiological and agronomic plant parameters in both screening platforms. These parameters were further related to active and passive spectral and temperature measurements.

Salinity, drought and the combination of both had different effects on above-ground biomass parameters in both growth platforms. Salinity combined with drought did not show any additive effect on grain yield for both cultivars compared to that under drought stress alone, suggesting that the soil osmotic potential from salinity did not account for a significant contribution to the additive effect of the total soil water potential in our study. This may question the two-phase model (Munns 1993) that salinity causes the first (water deficit effect) and the second phase (ionic effect). Drought seems to be much more dominant than salt stress with the opportunity of roots to adjust to salinity within a short period of time, but not to drought (Homaee and Schmidhalter 2008). Nevertheless, none of those studies (Shalhevet and Hsiao 1986; Schmidhalter and Oertli 1991; Singh Grewal 2010) can distinguish whether the negative effect of salinity on plant growth is from the contribution of salinity to the soil water potential or due to ionic effects of salinity on plant growth under combined stress conditions. While drought and its combination with salinity had a more severe impact on grain yield in pots than in containers, salinity alone reduced grain yield in containers to a higher degree. The above contrasting studies showed a greater reduction in growth associated with smaller pot sizes (Peterson et al. 1984; Robbins and Pharr 1988; Townend and Dickinson 1995; Whitfield et al. 1996; Ray and Sinclair 1998; Passioura 2006; Wu et al. 2011; Poorter et al. 2012) due to the restricted rooting environment. Similarly, our study suggests that pot experiments can lead to different conclusions for screening salt tolerant wheat genotypes from experiments in containers.

The quality of the spectral assessments of biomass and plant stress parameters varied within both growth platforms. While the close-to-field container platform seemed to be better suited to screen for the above-ground biomass, for the water content of the above-ground biomass and the water potential of the youngest, fully developed leaf, the spectral assessments in pots were clearly restricted. The decreased match between the sensors' footprint and the plants´

area of the pot-grown plants, and the differences in senescent leaves due to the decreased soil nitrogen supply in the restricted rooting volume in pots (Poorter et al. 2012) led to different qualities in the spectral assessment of the chosen agronomic parameters in a container or pot platform. While the R_{760}/R_{670}, NDVI, R_{780}/R_{740}, R_{760}/R_{730} and R_{780}/R_{550} indices were best and significantly related to the water potential of the youngest fully developed leaf, the fresh weight of the above-ground biomass and the water content of the above-ground biomass in the containers from the beginning of booting for both years, the indices REIP, WBI and WBI/NDVI were mostly inferiorly suited. In spite of that, all used spectral indices allowed successful and consistent differentiation between the two wheat cultivars varying in their salt tolerance among the treatments of control, salinity and combined salinity+drought. Active and passive sensors seemed to be equally suited to assess the above mentioned parameters of plants exposed to salinity and salinity+drought independently from the intensity of light in the winter experiment under mostly cloudy and low light conditions and in the summer experiment under full sun. Nevertheless, active sensors have the advantage over passive sensors, because they can be used at any time of the day or night without affecting the measurements. However, active spectral sensing is mostly restricted to a limited number of wavelengths and indices (Erdle et al. 2011).

Nevertheless, new screening technologies with different setups and various sensors have already shown opportunities in small pot platforms for precisely investigating single or a few plants (Berger et al. 2010; Furbank and Tester 2011; Golzarian et al. 2011; Arvidsson et al. 2011). These techniques offer new chances for scientists to more precisely investigate plant morphology and physiology, even though they are still restricted to precisely screen one or only a few plants in small pots. Plant breeders may profit from this phenotyping technology as an additional tool to quickly and precisely screen for certain plant traits and for tolerant cultivars to various abiotic and biotic stresses. However, precise phenotyping devices and setups for plants in field plots or close-to-field container platforms would provide even more insight. In addition to the available technology, a precise knowledge of spectral indices and their opportunities for detecting certain plant traits is inevitable.

Similar to spectral measurements, temperature measurements of plants aim to accurately and quickly assess the plant´s stress situation with differences found in the accuracy of the used devices, time duration for the measurements and opportunities for further processing the measured data. Thermography and IR thermometry are quick and easy to apply and deliver comparable and qualitatively good results in dense crop stands in regard to recognition of stress intensity, treatment and cultivar differentiations. Temperatures of single plant leaves

measured with the thermistor of the porometer led to worse stress recognition by relating to the leaf water potential, treatment and cultivar differentiations compared to whole-canopy temperature measurements (Grant et al. 2007). Thermal imaging seems to provide great chances for future plant stress measurements due to their high accuracy, the opportunity of further processing and combination with visible imagery (Leinonen and Jones 2004; Stoll and Jones 2007; Möller et al. 2007; Wang et al. 2010). As soon as new techniques provide an automatic analysis of thermal images, thermal sensing has huge chances in the speed of recognizing plant water stress and will help breeders to identify more tolerant genotypes to drought or salt stress.

Despite these advantages, plant temperature measurements deliver relative parameters similar to spectral measurements. The extent of plant stress can only exactly be identified by comparing the temperature of stressed plants with non-stressed plants, with specific reference surfaces (Jones 1999b; Grant et al. 2006; Stoll and Jones 2007; Jones et al. 2009) or by relating to well-known plant stress parameters (Blum et al. 1982). While the leaf water potential is a reliable parameter for reflecting the stress intensity similarly well as the plant temperature (Blum et al. 1982; Cohen et al. 2005) with increasing associations with increasing stress intensity (Meron et al. 2010), biomass parameters seem to be less well suited (Blum et al. 1989). Furthermore, bare soil can have influences on plant temperatures at early growth stages and because of the lower canopy layer in light canopies (Rodriguez et al. 2005; Möller et al. 2007; Jones et al. 2009; Blum 2011).

Spectral and thermal assessments of biomass and plants´ stress traits under salinity alone and combined with drought, proved to be a promising tool for future stress recognition being useful also for plant breeding. Since pot experiments can lead to different conclusions for screening and phenotyping salt tolerant wheat genotypes from experiments in simulated field platforms, the right choice of the growth platform and environment supports recognizing plant stress and salt tolerant cultivars more effectively.

5 Conclusions

Overall, our results show different effects of salinity alone and combined with drought on the growth and yield parameters of wheat depending on the growth platform, climatic conditions and wheat cultivars. The yield tolerance of two contrasting wheat cultivars to salinity combined with drought was similar regardless of the growth platform, which did not demonstrate any additive effect on grain yield compared to that under drought stress alone.
In the pot experiment, the grain yield under salinity alone was significantly higher than that under salinity combined with drought, whereas drought alone caused the significantly lowest grain yields among stress treatments. Nevertheless, our results suggest that pot experiments may lead to different conclusions for screening salt tolerant wheat genotypes from experiments in simulated field platforms. Spectral sensor technology could be used as a high-throughput method for phenotyping plant traits of salt and drought stressed wheat cultivars in a close-to-field container or pot platform. Agronomic parameters were generally more closely related to the spectral indices of plants grown in containers than in pots. In contrast, canopy temperature as an indicator of plant water stress could easily be measured by thermography and IR thermometry with both leading to similar results in treatment and cultivar differentiation at different growth stages of wheat in the simulated close-to-field container platform.

To ensure grain yield stability and increase the yield potential of cereals, it will be crucial to meet the demand for food with increasing global population and changing climatic trends. Traditional methods in cross-breed phenotyping and in recognizing tolerant cultivars to abiotic stresses will not be able to meet the future challenges. Therefore, for future studies one should focus on the combination of salinity with drought stress in well suited platforms, because salt with drought stress represents the natural occurrence in many areas of the world.
Further research using high-throughput sensing systems will allow a better understanding of the potential of various growth platforms and environments, which could allow them to be brought to a scale comparable to that of conventional field conditions. Therefore, these promising systems have great potential to speed up phenotyping plant traits reflecting tolerance to salt stress combined with drought.

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