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Shoot potassium content provides a physiological marker to screen cotton genotypes for
osmotic and salt tolerance

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Abstract

Drought and salinity are considered two major abiotic stresses that diminish cotton production worldwide. Studying common morphological and physiological responses in cotton cultivars may help plant biologists to develop and apply standard screening criteria for either of these stresses and for their combination. Therefore, this research aimed to assess the suitability of several physiological parameters as diagnostic to report on osmotic and salinity tolerance in six elite cotton genotypes. Data for relative growth rate (RGR), RGR-reduction, potassium (K^+) concentrations in roots, xylem sap and shoots, stomatal conductance (gs) and net photosynthesis rate (Pn) were assessed. Based on RGR and RGR-reduction, we observed an association between osmotic tolerance and salinity tolerance of cotton genotypes. Furthermore, this study found that tolerant cotton genotypes were better able to maintain high RGR, tissue K^+ , and gas exchange under both hyperosmotic and saline conditions. Shoot K^+ levels showed high negative correlations with both osmotic and salinity stress and emerged as a convenient and suitable parameter to assess cotton tolerance to either stress.

Keywords: *Gossypium hirsutum*, relative growth, potassium

Novelty statement

Cotton (*Gossypium hirsutum*) is a leading fibre crop that is cultivated in more than 52 countries. Much of the land where cotton is grown faces co-occurring drought and salinity abiotic stress which negatively impacts cotton yield and fibre quality. In the present study, cotton genotypes were identified with tolerance to both hyperosmolarity and salinity. Furthermore, we show that shoot potassium content is a diagnostic trait that reports on both osmotic and salinity stress and hence a convenient tool for screening cotton germplasm.

Introduction

Cotton (*Gossypium hirsutum*) is the major natural fiber crop across the world. The demand for high production and quality cotton is increasing because of the growing human population and natural fiber preferences for clothing (Townsend, 2020). The United States, China, India, Brazil, and Pakistan are the main producers of cotton. The production of cotton by these countries ranges from 1,350 and 6,423 thousand metric tonnes per year, whereas the cotton yield ranges from 1.7 to 2.2 t ha⁻¹ (Sahay, 2019; Statista, 2020). The yield fluctuation is due to diverse cotton genotypes, different agricultural production systems and variations in climatic conditions. The selection of a cotton variety for a specific geographical region is based on the flexibility in planting time, maturation time, yield, superior fibre quality and tolerance to both biotic and abiotic stress conditions.

Cotton, as do other plants grown in field conditions, copes with a variety of abiotic stresses during its life cycle. Abiotic stresses like nutrient deficiency, extreme temperatures, flooding, drought and salinity diminish cotton growth performance and even cause plant death. Each type of abiotic stress affects cotton growth to a different degree. For example, low temperature and salinity have a higher impact on growth and lint yield in cotton than drought stress (Singh *et al.* 2018).

The detrimental effects of global warming force us to develop new cotton cultivars with resilience to single and combined stresses. Although cotton is broadly adapted to temperate, subtropical and tropical environments, the current increasing temperature and unpredictable rainfall force cotton producers to increasingly rely on cultivars with multiple tolerance to combinations of stress.

An example of frequently co-occurring stress is that of drought and salinity. Drought is often the result of a lack of precipitation especially when cotton cultivation occurs outside the rainy season, whereas salinity is caused by natural geology, seawater ingression, salt mining, poor

irrigation and chemical fertilization management. A key aspect of drought and salinity is the osmotic stress caused by decreases in soil water potential and consequently, the water uptake by roots is restricted (Aroca *et al.* 2011). Furthermore, the cationic (Na^+) and anionic (Cl^-) components reinforce the detrimental effects of salt stress in cotton performance (Munns and Tester 2008).

Relatively little is known about plant responses to multiple stresses and we urgently need robust phenotypic assays to identify germplasm with multiple tolerance.

The first step towards this goal is the identification of suitable traits that report on both drought and salinity with high fidelity. Growth parameters such as final fresh and dry weights or relative growth rate (RGR) are associated with the degree of tolerance to drought, osmotic and salinity stress but are time-consuming to establish and lack specificity (Abdelraheem *et al.* 2018; Patishtan *et al.* 2018). A number of further, easily scorable traits were therefore tested, based on the hypothesis that they are suitable diagnostic markers to report on both salinity and drought tolerance (Ahmad *et al.* 2016; Abdelraheem *et al.* 2018). The data of this study show that in particular shoot K^+ levels provide a convenient parameter for screening cotton genotypes at the seedling stage and that this trait can be used to report on both osmotic and salinity tolerance of elite cotton genotypes.

Materials and Methods

Six elite cotton genotypes were assessed for their responses to osmotic and saline conditions. These genotypes were previously characterized with respect to tolerance to drought stress conditions (Chattha *et al.* 2017; Table 1). Experiments were conducted in hydroponic glasshouse conditions at the Biology Department, University of York, UK. During the experiments, the photoperiod was 12 hours, whereas the diurnal and nocturnal average temperature was 28 °C and 24 °C, respectively.

The cotton seeds were germinated using terragreen substrate for 10 days. Plants were then transferred to a standard hydroponic medium (Yoshida *et al.* 1976) for another 15 days. Subsequently, plant weights were recorded and plants were exposed to hydroponic standard medium (control) or media that either induced osmotic or saline stress for 30 days. For osmotic-stress treatments, media were supplemented with 12% or 14% (w/v) polyethylene glycol (PEG-6000) with an osmolality of 225 and 300 mOsm kg⁻¹, respectively. Salinity treatments consisted of a medium containing 100 mM or 150 mM NaCl (equivalent to the osmolality of 225 and 300 mOsm kg⁻¹, respectively). The hydroponic medium was renewed once per week.

A completely randomized design with a split-plot arrangement was followed for the study. Five treatments (one control, two osmotic stress levels, and two saline conditions) were assigned to the main plot, and genotypes were arranged in sub-plots. Six cotton genotypes were randomized within each main plot with three replications.

Table 1. Elite cotton genotypes used in this study with their reported cross background and drought tolerance.

Cotton genotype	Cross pedigree	Pakistan Institute	Yield potential (t ha ⁻¹)	GOT (%)	Fiber length (mm)	Micronaire (µg/inch)	Fiber strength (g/tex)	Abiotic tolerance
NS-131**	IR-448 × C-2-2	Neelum seeds, Vehari.	3.0-4.9	40.9	29.0	4.6-4.9	29	¹ Drought sensitive
VH-291*	VH-289 × FH-114	Cotton Research Station, Vehari, Pakistan.	5.4	40	29.0	4.7	34.0	¹ Drought tolerant

AA-703**	CIM-482 × Exotic Line	Ali Akbar Seeds (Pvt) Ltd – Lahore.	5.2-5.5	39.5	29.0	4.4	35.0	¹ Drought sensitive
KZ-191**	CIM × Bollguard-I	Kanzo Quality Seeds, Multan.	4.0-5.0	40	27.6	4.9-5.5	31.5	¹ Drought sensitive
MNH-886**	FH - 207 × (MNH- 770) × Bollgard-1)	Cotton Research Station, Multan.	6.0	39-40	28.5-28.9	4.6-4.9	36.0	¹ Drought tolerant
FH-207*	FH-113 × IR-3	Cotton Research Institute, Faisalabad.	5.0-5.4	38.4	28.5	4.7	34.4	¹ Drought tolerant

NOTE: Cotton genotypes developed through bulk pedigree approaches. *Promising and

**released cotton cultivars approved by the Pakistan Federal Seed Certification and

Registration Department. ‘GOT’: Percentage of ginning outturn (%). ¹(Chattha *et al.* 2017).

Cotton growth measurements

To determine the effects of osmotic and saline conditions, relative growth rate (RGR, % day⁻¹) was calculated using the formula of Evans (1972) multiplied by 100. RGR-reduction was calculated using this formula: RGR-reduction (%) = (1-(RGR_{TREATED}/RGR_{CONTROL})) × 100.

Measurements of K⁺ concentrations in cotton tissues

To measure K⁺ cation concentrations, fresh root and shoot tissues were dried at 80 °C for three days, then dry weights of tissues were recorded. The root and shoot tissues were then transferred to falcon tubes (15 ml) and 10 ml of 20 mM CaCl₂ was added to each sample for cation extraction. The samples were kept at room temperature for three days and soluble K⁺ ion concentrations in the extract were measured using a flame photometer (Kenwood, UK).

To assess the K^+ concentrations in xylem sap, the cotton plants were cut 80 mm above the root-shoot junction. The cut root parts were then mounted in a pressure chamber by inserting the stem of the plant through the chamber's head. A pressure of about 20-30 kPa was exerted and around 30 microliters of xylem sap were collected using a micropipette. The collected xylem sap was then diluted 100 times with deionized water and analyzed for K^+ ion concentrations using a flame photometer.

Measurement of leaf gas exchange

To determine gas exchange rates, fully expanded third leaves were selected to measure stomatal conductance (g_s) and net photosynthesis rate (P_n) using an LI-COR 6400. The settings for the LI-COR 6400 were factory default with a leaf temperature of 20 °C and 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ quantum flux light, whereas the light intensity was 640-830 $\mu\text{molm}^{-2}\text{s}^{-1}$. The rates of gas exchange were measured after 15 days of treatments.

Statistical analysis

One-way ANOVA was performed to growth and physiological traits in cotton genotypes exposed to either control or at different strengths of osmotic and saline conditions. Tukey's honest significant test (Tukey HSD; $P < 0.05$) was applied where significant differences were detected by ANOVA to test the mean differences for significance. Regression tests were performed for different data sets to examine the relationships among physiological traits of cotton genotypes grown in either control, osmotic, or saline conditions. To test the differences between osmotic and salinity effects across cotton genotypes, the morphological and physiological traits were tested by the Mann-Whitney U-test (a nonparametric test that compares two independent groups). A two-tailed probability value of less than 0.05 was considered to be significant. All analyses were performed using the statistical analysis system SAS 9.3 and R software packages.

Results

Relative growth of cotton genotypes

A significant difference in RGR was found among cotton genotypes grown in either control, osmotic and saline conditions (Figure 1). In a standard medium, cotton genotypes showed on average 7.6% day⁻¹ for RGR. However, in osmotic and saline conditions the RGR was between 2.8 and 4.2% day⁻¹. The highest RGR was identified in cotton genotype VH-291 when plants were grown in the control medium. During osmotic and saline treatments, the highest RGRs were found in genotypes MNH-886 and FH-207, respectively. In general, greater stress levels led to lower RGR.

To normalize and compare genotype tolerance, RGR-reduction (relative to control conditions) was determined. Figure 2 shows there were significant differences in tolerance to both salinity and osmotic stress between cotton varieties with RGR-reduction ranging from 45 to 63%. The smallest RGR-reduction, and hence greatest tolerance to either stress, was observed for FH-207 whereas genotypes NS-131, NH-291 and AA-703 were clearly more sensitive. The Mann–Whitney U test revealed that for all the genotypes there was a non-significant difference in tolerance to osmotic versus salt stress.

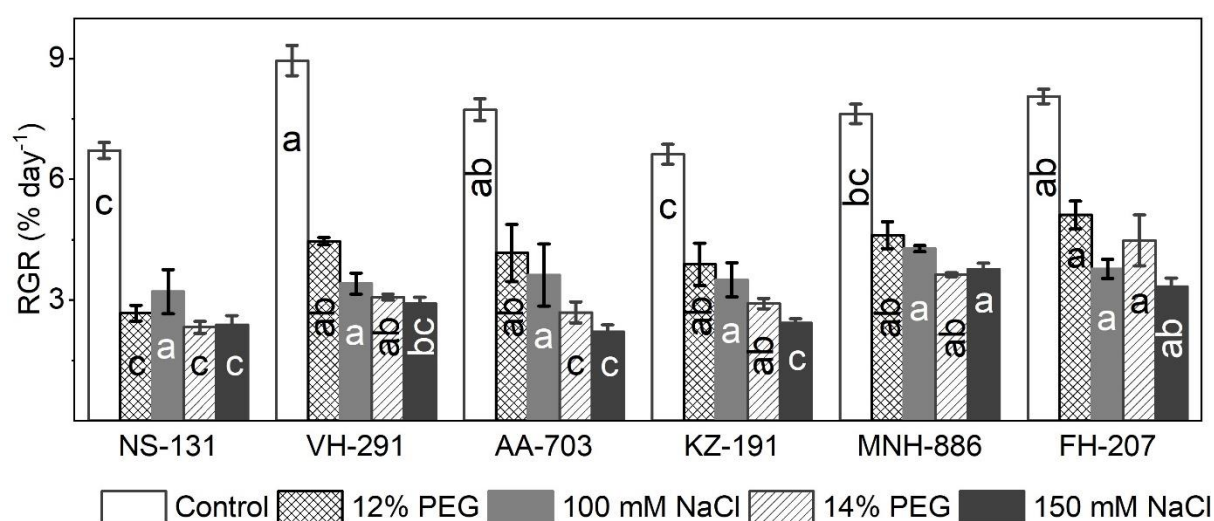


Figure 1. Relative growth rates of cotton genotypes exposed to standard medium and hydroponic medium plus-either PEG or NaCl at 225±5 mOsm kg⁻¹ (for 12% PEG and 100 mM NaCl) and 300±5 mOsm kg⁻¹ (for 14% PEG and 150mM NaCl).

Note: Each bar shows the mean ±SE of three cotton plants. Mean bars with different letters show significant differences among cotton genotypes (Tukey's HSD test, P<0.05). Similarly, the differences between osmotic and saline conditions were tested using the Mann–Whitney U test (n=3, P<0.05).

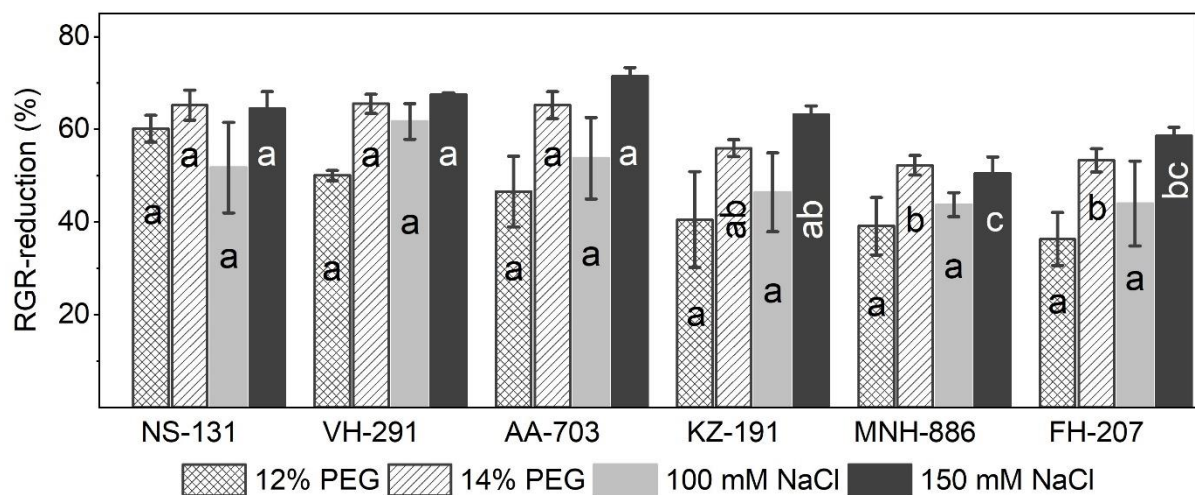


Figure 2. Reduction in the relative growth rate of cotton genotypes exposed to hydroponic medium-plus either PEG or NaCl at 225±5 mOsm kg⁻¹ (for 12% PEG and 100 mM NaCl) and 300±5 mOsm kg⁻¹ (for 14% PEG and 150mM NaCl).

Note: Each bar shows the mean ±SE of three cotton plants. Mean bars with different letters show significant differences among cotton genotypes (Tukey's HSD test, P<0.05). Similarly, the differences between osmotic and saline conditions were tested using the Mann–Whitney U test (n=3, P<0.05).

Concentrations of K⁺ in roots

The concentration of K⁺ in roots varied among cotton genotypes and furthermore depended on treatment (Figure 3). The genotypes NS-131, VH-291 and KZ-191 showed the highest concentration of K⁺ in roots under standard medium. In osmotic and salt-treated plants, the

highest K^+ concentration in roots was found in genotypes VH-291, MNH-886 and FH-207. Higher stress levels generally led to lower K^+ concentration in root tissues with an average decrease of root K^+ between 17% and 53%. The root K^+ concentrations were lower in salt-treated plants in comparison to osmotic-treated ones.

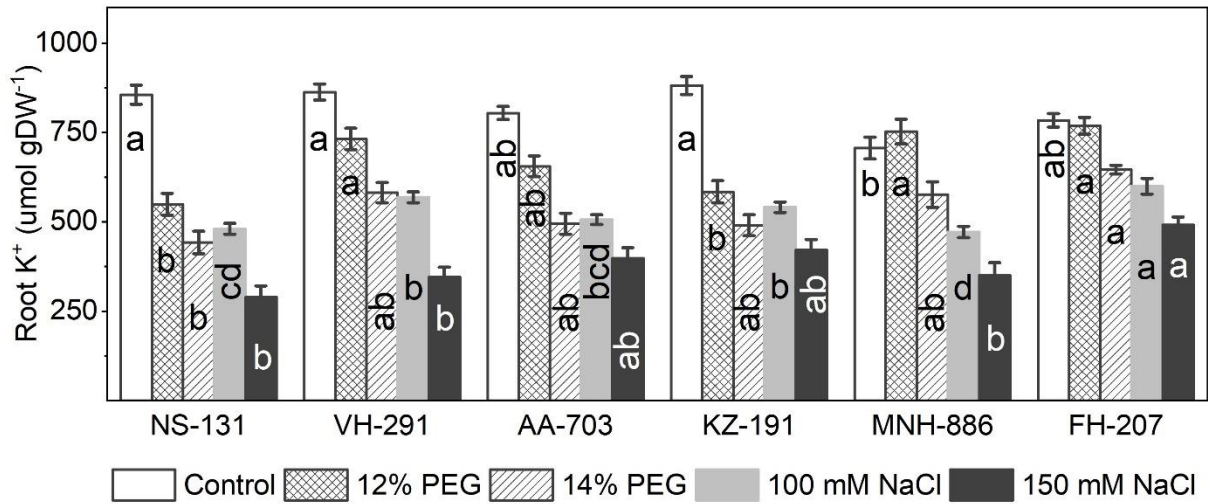


Figure 3. Root K^+ concentrations of cotton genotypes grown in standard medium and hydroponic medium-plus either PEG or NaCl at 225 ± 5 mOsm kg^{-1} (for 12% PEG and 100 mM NaCl) and 300 ± 5 mOsm kg^{-1} (for 14% PEG and 150mM NaCl).

Note: Each bar shows the mean $\pm$ SE of three cotton plants. Mean bars with different letters show significant differences among cotton genotypes (Tukey's HSD test, $P < 0.05$). Similarly, the differences between osmotic and saline conditions were tested using the Mann–Whitney U test ($n=3$, $P < 0.05$).

Concentrations of K^+ in xylem sap

Further analysis showed that xylem sap K^+ concentrations significantly differed among cotton genotypes and treatments (Figure 4). In the standard medium, xylem K^+ values were comparable except in VH-291, which showed a somewhat lower concentration. In all genotypes, there was a clear trend of decline in xylem sap K^+ in response to both types of

stress. The reduction of K^+ in xylem sap ranged from around 20% tolerant to well over 40% in more sensitive genotypes.

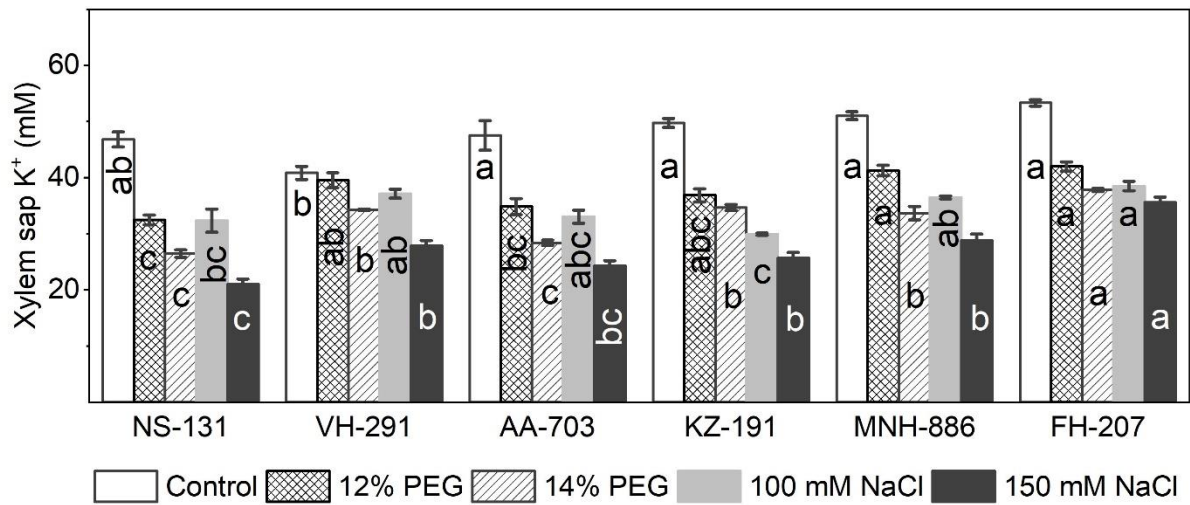


Figure 4. Xylem sap K^+ concentrations of cotton genotypes grown in standard medium and hydroponic medium-plus either PEG or NaCl at 225 ± 5 mOsm kg^{-1} (for 12% PEG and 100 mM NaCl) and 300 ± 5 mOsm kg^{-1} (for 14% PEG and 150mM NaCl).

Note: Each bar shows the mean $\pm$ SE of three cotton plants. Mean bars with different letters show significant differences among cotton genotypes (Tukey's HSD test, $P < 0.05$). Similarly, the differences between osmotic and saline conditions were tested using the Mann–Whitney U test ($n=3$, $P < 0.05$).

Concentrations of K^+ in shoots

In the standard medium, the shoot K^+ concentrations were comparable across genotypes.

Shoot K^+ decreased between 10% and 50% when plants were exposed to osmotic and saline conditions with reductions generally being more substantial after saline treatments compared to osmotic stress. Surprisingly, only non-significant differences were found between cotton genotypes for shoot K^+ when plants were exposed to standard or saline conditions (Figure 5).

However, significant differences were observed when plants were treated with 12% PEG with relatively high shoot K^+ observed in the more tolerant genotypes MNH-886 and FH-207.

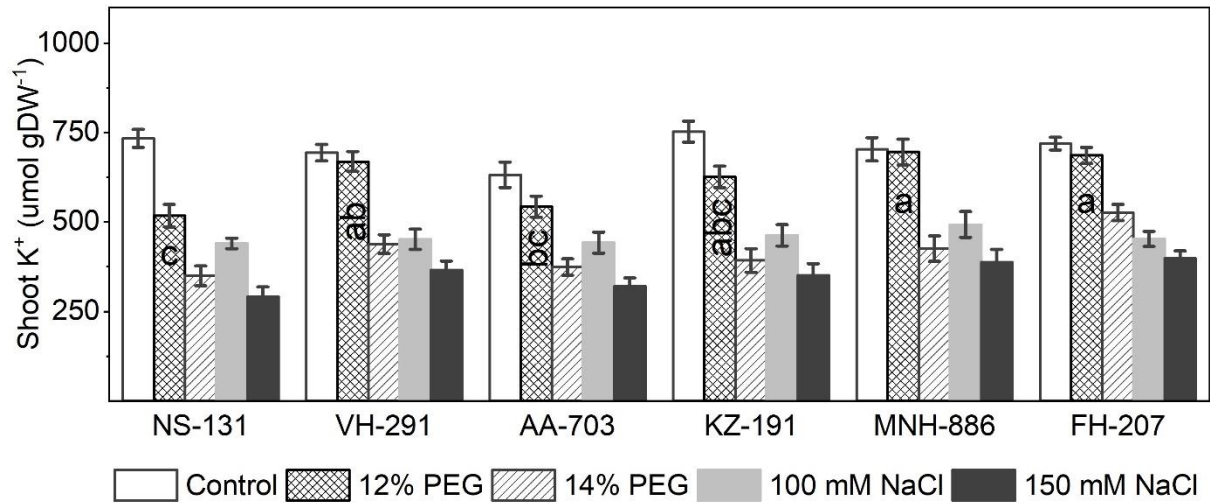


Figure 5. Shoot K⁺ concentrations of cotton genotypes grown in standard medium and hydroponic medium-plus either PEG or NaCl at 225±5 mOsm kg⁻¹ (for 12% PEG and 100 mM NaCl) and 300±5 mOsm kg⁻¹ (for 14% PEG and 150mM NaCl).

Note: Each bar shows the mean ±SE of three cotton plants. Mean bars with different letters show significant differences among cotton genotypes (Tukey's HSD test, $P < 0.05$). Similarly, the differences between osmotic and saline conditions were tested using the Mann–Whitney U test ($n=3$, $P < 0.05$).

Stomatal conductance and net photosynthesis rate

Saline and osmotic conditions led to a significant reduction in stomatal conductance (g_s). The average g_s ranged from 0.2-0.30 mol m⁻² s⁻¹ in standard conditions, and was lowered to 0.13-0.28 for plants exposed to osmotic conditions and to 0.10-0.27 mol m⁻² s⁻¹ when plants were exposed to saline treatment (Figure 6). Furthermore, gas analyzer data showed non-significant differences for P_n (Figure 7) when plants were exposed to standard medium with P_n levels ranging between 15 and 20 μmol m⁻² s⁻¹. In PEG-induced osmotic and salt stress conditions, P_n decreased and significant variability between genotypes became apparent. The higher stress levels induced by either PEG or NaCl led to greater suppression of P_n values.

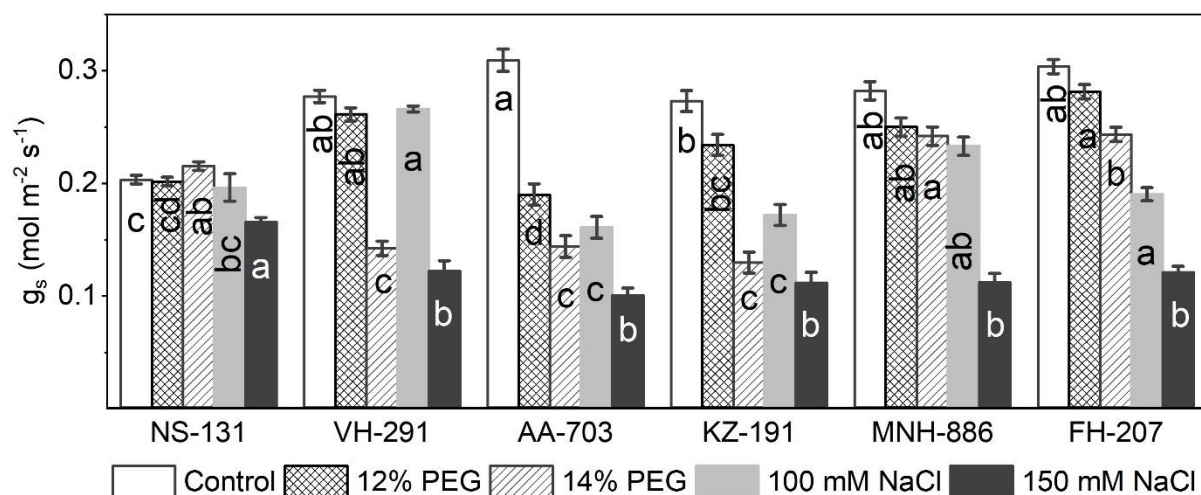


Figure 6. Stomatal conductance of cotton genotypes grown in standard medium or medium-plus either PEG or NaCl at 225 ± 5 mOsm kg⁻¹ (for 12% PEG and 100 mM NaCl) and 300 ± 5 mOsm kg⁻¹ (for 14% PEG and 150mM NaCl).

Note: Each bar shows the mean $\pm$ SE of three cotton plants. Mean bars with different letters show significant differences among cotton genotypes (Tukey's HSD test, $P < 0.05$). Similarly, the differences between osmotic and saline conditions were tested using the Mann–Whitney U test ($n=3$, $P < 0.05$).

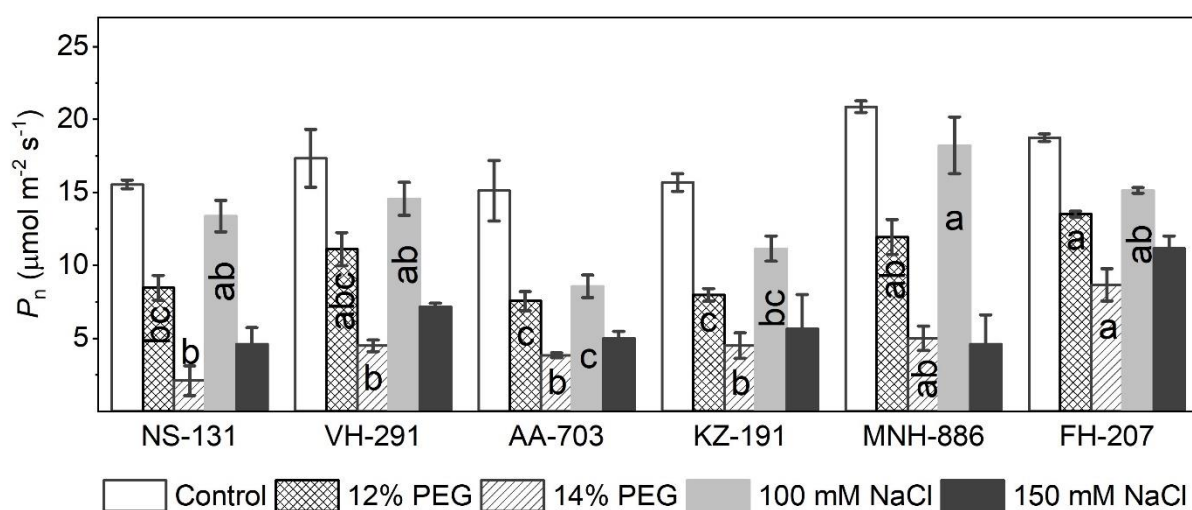


Figure 7. Net photosynthesis rate for cotton genotypes grown in standard medium or medium-plus either PEG or NaCl at 225 ± 5 mOsm kg⁻¹ (for 12% PEG and 100 mM NaCl) and 300 ± 5 mOsm kg⁻¹ (for 14% PEG and 150mM NaCl).

Note: Each bar shows the mean $\pm$ SE of three cotton plants. Mean bars with different letters show significant differences among cotton genotypes (Tukey's HSD test, $P < 0.05$). Similarly, the differences between osmotic and saline conditions were tested using the Mann–Whitney U test ($n=3$, $P < 0.05$).

Correlations between measured parameters and stress

To identify easily scorable traits that reliably report on both osmotic and salinity stress, we carried out correlation analyses between each measured parameter and RGR-reduction.

Correlation coefficients between stomatal conductance and stress were negative (Supplementary Figure S1) showing that in both conditions water preservation is of utmost importance. Although highly significant in the case of either stress, the coefficient for salinity was rather weak ($r^2 = 0.28$; Supplementary Figure S1). Photosynthetic rates correlated reasonably well ($r^2 = 0.61$) with osmotic stress in a negative manner but only poorly correlated with salinity ($r^2 = 0.36$; Supplementary Figure S1). Root K^+ and xylem sap K^+ showed significant negative correlations with osmotic stress ($r^2 = 0.62$, $r^2 = 0.68$, respectively) but only yielded r^2 values of 0.36 and 0.38 for salinity stress (Figure 8). Thus, in all the above cases, the measured physiological parameters showed a considerably stronger link with osmotic stress than with salinity stress. In contrast, shoot K^+ levels not only showed the highest correlation coefficients, but they were also very similar to each other (Figure 8). In both cases, a strong negative interaction was observed with r^2 values of 0.72 and 0.70 for osmotic and salinity stress, respectively.

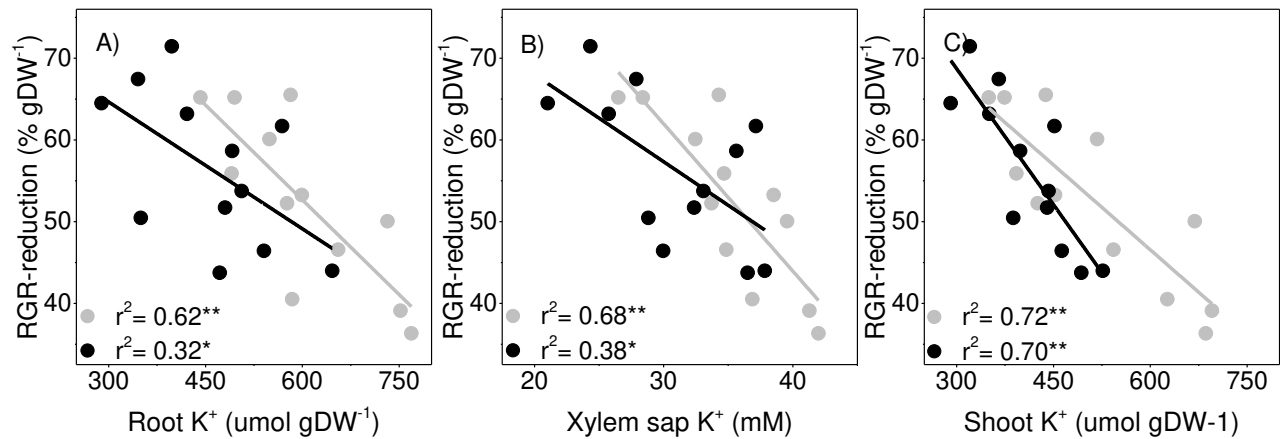


Figure 8. Correlations for root K^+ versus RGR-reduction (A); xylem sap K^+ versus RGR-reduction (B); shoot K^+ versus RGR-reduction (C) of cotton genotypes exposed to osmotic and saline conditions. *Correlation is significant at the $P \leq 0.05$ level. **Correlation is significant at the $P < 0.01$ level.

Discussion

The preference for natural fiber for clothing forces industries to keep producing quality cotton. However, increased demand and effects of global warming increasingly force cotton production to semiarid and saline environments. Especially the combination of stresses found in these areas causes severe damage to cotton yields and the identification and development of new cotton genotypes with resilience to combined stresses are essential. The latter will be greatly facilitated by robust and time-saving assays that reliably report on both types of stress. We, therefore, tested a number of physiological parameters for their suitability in this respect using six elite cotton genotypes.

The growth parameter RGR and tolerance index (RGR-reduction) is relatively easy to determine and quantify. Obtaining such data is facilitated by the fact that both RGR and RGR-reduction show a high correlation between salinity and osmotic stress-treated plants (Supplementary Figure S2), and based on RGR and RGR-reduction the genotypes MNH-886 and FH-207 showed the greatest tolerance. These genotypes were previously identified as

drought-tolerant lines (Table 1; Chattha *et al.* 2017). Our findings are consistent with those of (Abdelraheem *et al.* 2018) who assessed morphological traits of cotton genotypes exposed to soil water deficit, PEG-osmotic and saline conditions.

Drought and salinity stresses have a direct impact on stomatal and mesophyll conductance. It is well recognized that stomatal closure caused by abiotic stress decreases CO₂ intake, which results in decreased photosynthetic rate, ultimately inhibiting cotton growth (Abdelraheem *et al.* 2019).

Although these processes are to a large degree paralleled during both types of stress, we found only a moderate correlation between either stomatal conductance or photosynthetic rate on the one hand, and salinity stress on the other.

The positive role of K⁺ in averting drought and salinity stress has been reported many times in the literature (e.g. Maathuis and Amtmann 1999; Chen *et al.* 2011; Ahmad *et al.* 2016; Patishtan *et al.* 2018; Oosterhuis *et al.* 2013; Zahoor *et al.* 2017; Isayenkov and Maathuis 2019). In the context of salt stress, root K⁺ efflux was found to be a reliable diagnostic for wheat salt tolerance and proposed as a suitable factor for screening germplasm (Cuin *et al.* 2008). Other studies showed that in many plants, salinity tolerance correlates more strongly with tissue K⁺ than with tissue Na⁺ (Tester and Davenport 2003). We, therefore, tested if tissue K⁺ concentrations were suitable factors to report on hyperosmolarity and salinity-induced RGR-reduction in cotton.

Correlations among different traits under abiotic stress conditions can be useful in developing selection criteria. For example, the correlated traits with osmotic and saline conditions can be efficiently used for screening large cotton germplasm for osmotic and salinity stress. In our study, a high correlation was observed between salinity and osmotic stress treated plants for RGR and RGR-reduction (Supplementary Figure S2), although these processes are to a large degree paralleled during both types of stress, we found only a moderate correlation between

either stomatal conductance or photosynthetic rate on the one hand, and salinity stress on the other. Thus, while stomatal conductance and photosynthetic rate were highly correlated to the imposition of hyperosmolarity it appears neither of these traits is an adequate diagnostic to report on combined osmotic-salinity stress. We generally observed moderate to high correlations when studying root K^+ and xylem sap K^+ but coefficients were markedly higher in the case of osmotic stress relative to those obtained for salinity treatment. However, shoot K^+ content was found to be highly correlative with both hyperosmolarity and salinity tolerance with a coefficient value of ~ 0.85 . Thus, shoot K^+ content appears an excellent parameter to use as a screening device to detect either osmotic tolerance or salinity tolerance. Although further study is required, these results also suggest that shoot K^+ may reliably report on tolerance to a combination of the two stresses. Furthermore, K^+ levels can be accurately quantified using very straightforward protocols and relatively inexpensive equipment such as flame photometers. A turnover of several hundred samples per day should be attainable enabling screening of large germplasm collections.

Conclusions

This research found that cotton genotypes have different resilience to abiotic stress and that there is a close relationship between osmotic- and salinity-tolerance with reference to morphological and physiological traits, in particular the shoot K^+ content. Thus, shoot K^+ content may provide an easily scorable trait to not only report on cotton resilience to osmotic and salinity stress in isolation but also to the frequently co-occurring combination of these abiotic stresses.

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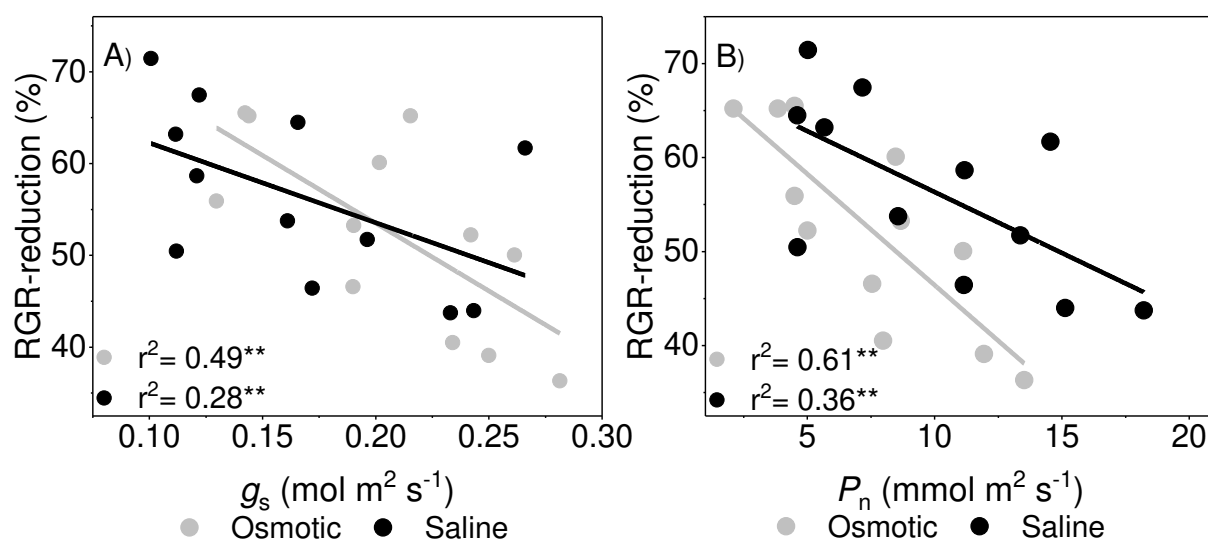
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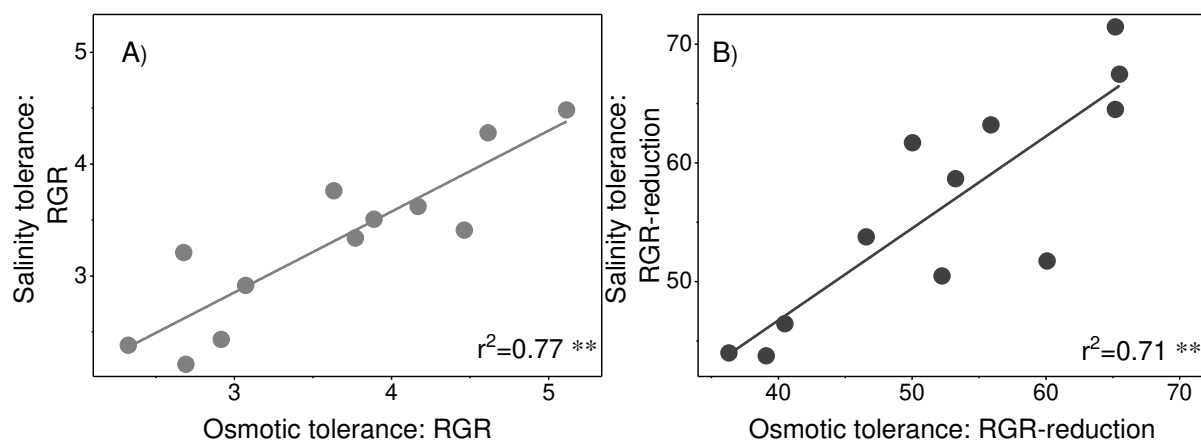
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Supplementary material



Supplementary Fig. S1. Correlations for g_s versus RGR-reduction (A) and P_n versus RGR-reduction (B) of cotton genotypes exposed to osmotic and saline conditions. ******Correlation is significant at the $P < 0.01$ level.



Supplementary Fig. S2. Correlation between osmotic-tolerance and salinity-tolerance based on RGR (A) and RGR-reduction (B) of cotton genotypes exposed to PEG and NaCl treatments. ******Correlation is significant at the $P < 0.01$ level.