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COMPARATIVE ANALYSIS OF LEAF RELATIVE WATER CONTENT AND WATER DEFICIT IN MAIZE (ZEA MAYS L.) GENOTYPES UNDER MODERATELY SALINE SOIL CONDITIONS

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Abstract
The study compared leaf relative water content (RWC) and the calculated water deficit in 24 maize genotypes grown in naturally moderately saline field soil. For each genotype, the mean and standard error (SE) of two replicates are available. RWC ranged from 77.49 to 95.56%, with the highest values observed in Ero 703 (95.56 ± 0.41%) and WA6373 (95.54 ± 0.71%), and the lowest in Teslo (77.49 ± 1.12%). Because values for a nonsaline control and the raw replicate data were not available, no firm conclusions were drawn regarding salinity tolerance or statistical significance. An inconsistency between RWC and water deficit for WA6544 was noted in the source table. The results can serve as a basis for selecting genotypes for further in-depth investigation.

Keywords: Zea mays L.; salinity; leaf relative water content; RWC; water deficit; genotype; osmotic adjustment.

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INTRODUCTION:

Maize (Zea mays L.) is an important crop used for grain, forage and industrial raw material, and its productivity depends on the water and salt regime of the soil. In naturally saline soils, plants may experience osmotic limitation of water uptake; in the long term, ion exchange and cellular metabolism are altered. [3, 4]

The degree of salinity is usually characterized by the electrical conductivity of the soil solution or of the saturated paste extract. The term “moderately saline” is the description of this experiment provided by the user; laboratory EC, pH, the composition of the main salts and the sampling depth were not given. Therefore, the quantitative level of salinity cannot be independently confirmed in this work.

Leaf relative water content (RWC) expresses the hydration level relative to the water-saturated state. This parameter is easy to measure and helps compare genotypes under the same field conditions. However, RWC is not a direct measure of cell turgor pressure; osmotic adjustment, leaf age and anatomy affect its interpretation. [1, 2]

The aim of the study is to characterize the leaf water status parameters of 24 maize genotypes grown in moderately saline soil, to distinguish genotypes with relatively high and low RWC, and to formulate hypotheses for further physiological and biochemical research.

LITERATURE REVIEW:

Barrs and Weatherley (1962) analyzed the errors in RWC measurement arising from loss of dry matter during saturation of leaf discs, infiltration of water into intercellular spaces and excess water uptake. For this reason, methodological conditions must be standardized. [1]

Weatherley and Slatyer (1957) showed the convenience of expressing relative turgidity relative to the water-saturated state of the leaf, but warned that the same relative turgidity does not indicate the same water potential in different species. [2]

To calculate RWC, fresh weight (FW), fully turgid weight (TW) and dry weight (DW) are determined. RWC = (FW − DW)/(TW − DW) × 100. This ratio is the

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proportion of water present in the tissue relative to the potential saturated water content and should not be confused with a simple moisture percentage. [1]

The position of the sampled leaves, developmental stage, time of collection and conditions of transport to the laboratory may significantly change the result. Since water loss may begin once a sample is cut, it is necessary to measure fresh weight quickly, keep the saturation time identical for all samples and check the final weight after drying.

These methodological principles are important for separating differences between replicates from true biological variation at the next stage of the experiment.

Munns and Tester (2008) divided the salinity response into a rapidly developing osmotic phase and a later phase associated with accumulated ions. They distinguished three main directions of adaptation: osmotic stress tolerance, ion exclusion and tissue tolerance to ions. [3]

The initial osmotic component of salinity lowers the water potential outside the root. To retain water, the plant may alter stomatal conductance and restrict leaf growth. Such changes are not always accompanied by an immediate sharp drop in RWC: the balance between water loss and water uptake is important.

The subsequent ionic component is associated with the accumulation of sodium and chloride in tissues. The intracellular distribution of ions, their compartmentation in the vacuole and potassium balance may affect leaf function. [3]

A single time-point RWC measurement does not determine which of these phases predominates. This requires information on growth stage, monitoring over time, ion composition and photosynthesis-related measurements.

Farooq et al. (2015) summarized the effects of salinity on germination, nutrient uptake, photosynthesis and yield in maize. It was noted that increases in sodium and chloride may disrupt the balance of potassium and other mineral elements. [4]

The review by Farooq et al. emphasizes that the response of maize to salinity depends on growth stage. Germination and early development may be more sensitive under certain conditions. In addition, ion exclusion by the plant and ion compartmentation in vacuoles contribute to adaptation. [4]

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Photosynthetic rate, stomatal conductance and leaf water status are interrelated but are not the same parameter. A genotype with high RWC is not necessarily the best in terms of photosynthesis or yield.

Comparative evaluation of cultivars under saline field conditions should rely on multiple traits: RWC, growth rate, biomass, ion ratios, chlorophyll status and yield parameters. Of these, the present work directly measures only the relative water saturation of the leaf.

Blum (2017) emphasized that osmotic adjustment may help maintain cell turgor, but that linking it to yield requires comprehensive field evidence. The article is mainly devoted to drought, so its application to salinity requires caution. [6]

Osmotic adjustment may change the water potential of a cell through regulation of solute concentration. This process may help retain water and maintain metabolic activity. However, high RWC by itself is not sufficient evidence that osmotic adjustment has occurred.

Proline, soluble sugars and other compatible osmolytes may participate in the stress response. Their content may increase or change differently depending on stress intensity, developmental stage and genotype. Therefore, the one-sided conclusion "more proline means higher tolerance" is not scientifically correct.

In future research, the combined determination of proline, soluble sugars, leaf water potential and osmotic potential will make it possible to investigate the physiological causes of the differences in RWC.

Gill and Tuteja (2010) reviewed the formation of reactive oxygen species under abiotic stress and the protective role of the antioxidant system. This line of research justifies the need to study biochemical markers together with RWC. [5]

Reactive oxygen species are naturally formed during photosynthetic electron transport and cellular respiration. Under unfavorable conditions, the balance between their production and detoxification may be disturbed. [5]

Superoxide dismutase (SOD), catalase (CAT), peroxidases and the ascorbate–glutathione system participate in protection against oxidative stress. Increased enzyme activity may indicate a stress response, but it is not always a sign of high tolerance.

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To examine the relationship between a decline in RWC and membrane damage or oxidative stress, it is proposed to measure electrolyte leakage, malondialdehyde (MDA), hydrogen peroxide and antioxidant enzyme activity. These were not measured in this article; they are prospective, literature-based indicators.

Although water status, ion homeostasis, photosynthesis and antioxidant defense are interconnected, they are distinct mechanisms. When the model of Munns and Tester (2008) on the osmotic and ionic components, the review of maize by Farooq et al. (2015) and the conclusions of Gill and Tuteja (2010) on oxidative stress are interpreted together, it becomes clear that overall tolerance cannot be assessed with a single physiological parameter. [3–5]

Barrs and Weatherley (1962) raised the issue of methodological accuracy: procedural differences in measuring the water-saturated weight of the leaf lead to errors. Therefore, the reproducibility of measurements should be checked before assessing the biological meaning of RWC differences between genotypes. [1]

The research gap is that RWC values of these 24 genotypes, measured by the same method under naturally saline field conditions, are presented comparatively. These data are useful as a preliminary screening, but they should be supplemented with a nonsaline control and a quantitative salinity profile.

Hypothesis of the article: some genotypes maintain higher leaf relative water content than others in a naturally saline field. This hypothesis is assessed descriptively using the available table; the mechanism and causal effect require a separate experiment.

MATERIALS AND METHODS

Twenty-four maize genotypes were selected as the object of the study. The experiment was conducted in naturally moderately saline field soil. The field experiment was arranged in four replicates; for RWC, samples were taken from two replicates of each genotype (n = 2). Exact information on the experimental site, year, plant developmental stage, sampling time, irrigation regime, EC and soil chemical composition has not yet been provided.

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Based on leaf fresh weight (FW), water-saturated weight (TW) and dry weight (DW), RWC (%) = [(FW − DW)/(TW − DW)] × 100 was calculated. Water deficit is expressed by the formula WD (%) = 100 − RWC. The methodological basis follows the work of Barrs and Weatherley (1962). [1]

Results are presented as mean ± standard error (SE). For two independent observations, SE = SD/√2. Since the raw values of the two replicates were not provided, the possibility of testing normality, residual variance and pairwise comparisons is limited. P-values and statistical significance letters were not calculated.

All figures in the table were transcribed from a photograph provided by the user. For WA6544, if RWC is 94.13%, WD should be 5.87% according to the formula, but 2.91% is given in the source. This value needs to be checked against the author's original notebook.

RESULTS

Table 1. Leaf relative water content in naturally moderately saline soil (mean ± SE; n = 2). The water deficit column gives the values from the photograph; the WA6544 discrepancy is noted below the table.

№	Genotype	RWC, % (M ± SE)	WD, % (source)
1	WA6544	94.13 ± 2.96	2.91
2	WA6373	95.54 ± 0.71	4.46
3	Maxsima	91.83 ± 6.47	8.17
4	Yulduz	84.73 ± 3.09	15.26
5	WA 6810	90.88 ± 2.30	9.12
6	WA6652	91.95 ± 5.36	8.05
7	Ivola	89.50 ± 0.45	10.49
8	O‘zbek 300	88.32 ± 3.76	11.68

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9	WA9853	88.78 ± 3.03	11.21
10	Mashuk 250	91.37 ± 3.32	8.63
11	Hacibey	92.76 ± 0.82	7.24
12	Estafik 80	90.88 ± 2.82	9.12
13	WA6830	91.48 ± 0.70	8.52
14	Mashuk 170	85.61 ± 3.38	14.39
15	Ero 703	95.56 ± 0.41	4.44
16	Mongoliski	89.81 ± 0.52	10.14
17	WA6900	92.63 ± 0.79	7.34
18	Teslo	77.49 ± 1.12	22.51
19	Erkin	91.31 ± 4.85	8.69
20	Xorazm 60	92.21 ± 0.38	8.69
21	WA6610	88.93 ± 0.54	11.07
22	Gran	92.54 ± 1.83	7.46
23	Kelajak	91.53 ± 3.13	8.47
24	Mahalliy	92.48 ± 1.08	7.52

Note: for WA6544 the source gives WD = 2.91% and RWC = 94.13%; these two numbers do not add up to 100%. If WD = 100 − RWC, the value is 5.87%. Some other values show a rounding discrepancy of 0.01%.

ANALYSIS OF RESULTS

Across the 24 genotypes, the arithmetic mean of the mean RWC values was 90.51% and the median was 91.43%. The minimum value was recorded in Teslo (77.49%) and the maximum in Ero 703 (95.56%). The range of genotype means was 18.07

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percentage points. This overall mean is not based on individual leaf measurements but is the equally weighted mean of the 24 genotype means.

Genotypes with high RWC: Ero 703 (95.56 ± 0.41%), WA6373 (95.54 ± 0.71%), WA6544 (94.13 ± 2.96%), Hacibey (92.76 ± 0.82%) and WA6900 (92.63 ± 0.79%). These indicate high relative water saturation under the existing field conditions.

Genotypes with low RWC: Teslo (77.49 ± 1.12%), Yulduz (84.73 ± 3.09%), Mashuk 170 (85.61 ± 3.38%), O‘zbek 300 (88.32 ± 3.76%) and WA9853 (88.78 ± 3.03%). Such differences may also be related to leaf age, growth stage, root system or local soil heterogeneity.

The difference between the highest and lowest genotypes is 18.07 percentage points. This is only a descriptive difference and does not imply statistical reliability. For each genotype, M ± SE is given in the table. SE provides information on the precision of the mean, but with two replicates it can be highly unstable. It is incorrect to automatically call a genotype with a small SE biologically stable.

RWC and WD are mathematical complements of each other: WD = 100 − RWC. Therefore, they are not the results of two independent physiological experiments. A change in one exactly mirrors a change in the other in the opposite direction.

In Teslo, RWC is 77.49% and WD is 22.51%; in Ero 703, RWC is 95.56% and WD is 4.44%. It may be advisable to use these two genotypes as contrasting phenotypic groups in further studies, but there is not yet sufficient basis to formalize this as a tolerance category.

Independent analysis of the WD value for WA6544 is not recommended until the source error is corrected. The value in the RWC column should also be checked against the original records.

DISCUSSION:

Munns and Tester (2008) explained that the osmotic component of salinity affects water uptake and growth processes. In this study, the variation in RWC values among the 24 genotypes is an observation consistent with this theoretical framework; however, in the absence of a control, it cannot be stated that salt stress specifically caused the differences. [3]

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Farooq et al. (2015) emphasize assessing adaptation to salinity in maize together with ion balance, photosynthesis and growth parameters. The high RWC of Ero 703 and WA6373 identifies these genotypes as promising candidates for further multifactorial evaluation but does not provide a final breeding conclusion. [4]

Blum (2017) showed that osmotic adjustment helps maintain turgor. Nevertheless, since osmotic potential and osmolytes were not measured in our study, explaining the differences between genotypes specifically by osmotic adjustment remains a hypothesis for now. [6]

The measurement errors highlighted by Barrs and Weatherley (1962) and the n = 2 limitation require cautious interpretation of the results. With two replicates, the power of statistical comparison is low; additional replicates would increase the reliability of the scientific conclusions. [1]

In saline soil, relatively high RWC may be associated with water retention, stomatal regulation or osmotic balance. However, distinguishing among these mechanisms requires measuring leaf water potential, proline, soluble carbohydrates and the Na^+/K^+ ratio. [3, 4, 6]

Gill and Tuteja (2010) summarized the effects of oxidative stress on cellular biomolecules. From this perspective, examining membrane stability and antioxidant defense parameters in genotypes with low RWC is of scientific interest. However, this study provides no data on whether oxidative damage occurred in these genotypes. [5]

Main limitations: (1) there is no nonsaline control; (2) the EC and ion composition of the salinity are not reported; (3) RWC was determined in two replicates; (4) raw measurements are not available; (5) developmental stage and sampling time are not specified; (6) there is a computational inconsistency in the WA6544 row.

Plan for future experiments: establishing nonsaline control and saline treatments for each genotype under identical conditions, recording EC, increasing independent biological replication, and evaluating ions, pigments, proline and yield together with RWC. These recommendations are given for the future and are not presented as work performed in the current experiment.

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CONCLUSIONS AND PRACTICAL RECOMMENDATIONS

1. Mean leaf RWC values of 24 maize genotypes grown under naturally moderately saline soil conditions ranged from 77.49 to 95.56%.
2. Leaf relative water content was highest in Ero 703 (95.56 ± 0.41%) and WA6373 (95.54 ± 0.71%) and lowest in Teslo (77.49 ± 1.12%).
3. The maximum difference of 18.07 percentage points between genotypes is descriptive in nature. Two biological replicates and the absence of raw data do not allow a firm judgment on statistical significance.
4. Since a nonsaline control was not available, the level of salinity tolerance of the genotypes is not considered established. Genotypes with high RWC are selected as candidates for further experiments.
5. The inconsistency between RWC and WD values for genotype WA6544 must be verified against the original field and laboratory records.
6. Before submitting the article to a journal, the experimental site, year, soil EC, developmental stage, sampling protocol and initial weights for the two replicates should be included.

CONCLUSION

The available material shows that leaf water supply of maize genotypes varies in a naturally saline field. It is not a final experiment proving salinity tolerance but a preliminary comparative study that provides a basis for further breeding and physiological investigation.

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The literature review is based only on the scientific works whose bibliographic data have been verified above. Artificially increasing the number of unrelated or unverified sources was avoided.

DATA SOURCE. The RWC and WD values of the 24 genotypes were transcribed from a photograph of a table provided by the user; they are not presented as figures taken from literature sources.

ADDITIONAL INFORMATION REQUIRED. For final submission to a journal, the institution where the experiment was conducted, the authors, the year of the study, laboratory parameters of the field soil and the raw measurements should be included.