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Anatomical Analysis of Bread Wheat (*Triticum aestivum* L.) in Relation to K^+ : Na^+ Ions Antagonism

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Abstract

An experiment was conducted in Najaf province during the 2023-2024 growing season to study the effect of potassium:sodium ion antagonism on the stomatal performance of four wheat Cultivars (Tamooz, Rasheed, Bohooth, and Iraq), Treatments of 3 dSm-1 EC, 3 dSm-1 EC + 400 ppm K, 6 dSm-1 EC, 6 dSm-1 EC + 400 ppm, K which are symbolized as S1, S1+ K, S2, S2+ K respectively were used. Supplementation of irrigation water with potassium (S1+ K) significantly improved the upper surface of leaf stomatal length (55.96 μ m), compared to treatment (S2+ K) (54.88 μ m). Supplements of potassium to treatment S1 increased the width of stomata pores at the lower and upper surface of leaf by (12.01 and 9.83 μ m respectively). The results showed also that the Rasheed cultivar at the upper surfaces of the leaves recorded the highest length of the stomatal apperture (48.95 μ m), width (34.99 μ m) and length (55.85 μ m). In addition to that, at lower leaf surface, Rasheed also gave the highest width and length (48.72 and 56.03 μ m, respectively). Tamooz cultivar came after Rasheed, as it recorded the longest length and width of the stomatal pores and the highest width, which reached (47.83, 9.87 and 11.48 μ m, respectively) on the upper surface of the leaf.

Rasheed cultivar irrigated with (S2+K) recorded superiority over the rest of the cultivars in the upper and lower leaf surface, in the length of the stomatal apperture (54.55 and 56.11 μ m, respectively), the width of the stomatal apperture (18.02 μ m), the width of the stomatal (45.97 and 35.91 μ m, respectively) and the length of the stomatal (61.87 and 62.68 μ m, respectively).

Keywords: Wheat, salinity, Anatomy, Stomata, Ion antagonism, K, Na.

Introduction

Stomata highly influenced by salt and drought stresses. It was found that H_2O_2 create a signal that modified stomatal opening through Zinc finger transcription factor, which negatively affects closure of stomata by genes

directly related to hydrogen peroxides homeostasis, which creates a novel synthesis pathway for the transduction of drought and salt signal aid by H_2O_2 , and then the closure induction of stomatal. Increased stomatal closure and reductions in stomatal population, tended to improve drought and salt resistance

in rice (Al-Khafaji and Al-Burki, 2021; Huang, *et al.*, 2009). Well-established guard cells of stoma, and epidermal cells coincided by lower stomata densities, especially at upper surface was approved to be criterion for higher tolerance of salt, drought, temperature besides, other abiotic stresses (Abdel and Stutzel, 2016). Abdel, (2016) observed no epidermal cell underwent divisions to form two guard cells throughout leaf expansions during the episodes of both stressed and unstressed. It can be inferred that all stomata had been performed during dividing stage of leaf primordia, subsequently, expanding of stomata guard are cells occurred after completion of division stage, however, cell expansion is an environment dependent, which reflected on the final sizes of stomata dimensions at full leaf expansions. Yet, the highest stress resistant cultivar is confined to stomata of the highest size lower stomata density, particularly at the upper leaf size, where this leaf surface is directly exposes to environment, especially sunlight, as compared to sensitive varieties.

(Yang *et al.*, 2008) observed that wheat plants exposed to sodium chloride, sodium sulfate, sodium carbonate, and sodium bicarbonate in equal proportions decreased the relative growth rate, leaf area, and water content with increasing salt. Moreover, the slope was steeper with alkali stress than with salt stress. Foliar sprayed on bottle gourd plants grown on saline soil by 250 ppm KNO resulted in toxic effect reductions on fruit performances, and yield per individual plant by (76.91 %), but, insignificant impacts were observed at higher rates (Ahmad, and Jabeen, 2005). 50 mM, 150 mM NaCl manifested substantial variations among wheat investigated cultivars, where, cultivar responses appeared to be resulted from sodium chloride osmotic rather than the specific of sodium ion (Rahnama *et al.*, 2010). Yang *et al.*, (2008) found significant increases in the ratio of Na^+ and $\text{Na}^+ : \text{K}^+$ in shoots with significant decreases in potassium when exposed to both stresses. Moreover,

mineral accumulation and imbalance of $\text{Na}^+ : \text{K}^+$ ratio in the intercellular spaces led to high pH to alkalinity which may tend to reduce the photosynthetic network, stomatal conduction and disintegration of photosynthetic pigments (Sarkar *et al.*, 2023).

Rate of photosynthesis net (P_N), conductance of stomata (g_s), and stomatal water loss (E) were the same at all at salinity over 150 Mm. However, variations occurred in a single-peak curve, as salinity increased over 150 mM (Yang *et al.*, 2008). The results obtained by (Rahnama *et al.*, 2010) indicate that the relative size of the stomata can be a criterion for selecting salt-tolerant cultivars in wheat. Yang *et al.*, 2008 also observed that there were significant negative effects of alkalinity on photosynthesis and growth traits in wheat when compared to the effects of salt stress. The same stomatal conductance values were found in potassium chloride and sodium chloride treatments, as the conductance response to 50 mM sodium chloride or potassium chloride occurred within 45 minutes, which is independent of sodium in the leaves. Applying K profoundly activated photosynthetic rate, filled spikelet (%), grain yield and potassium in rice straw. On the other hand, it significantly decreased potassium and magnesium content, which showed improvement of potassium: Sodium, potassium: magnesium and potassium calcium ratios (Al-Burki *et al.*, 2018; Pardo, and Quintero, 2002).

This study aimed to confirm what was reached by Abdul (2016) and Al-Baraki *et al.* (2019) regarding the adoption of the largest stomata dimensions and lowest stomata population produced on varying cultivar leaves to be the most stress tolerant Cultivars, when compared to susceptible cultivars, as it was previously confirmed in crops production onion, faba bean and barley, but not in plant survival, where no yield required, because the survival of the plant depends on the presence of the largest number of small guard cells, which is a sign that most cultivars are drought resistant. This study was also conducted to determine the

ability of potassium to reduce the negative effects of salt stress on the cells of the stomata and epidermis.

Materials and methods

A field experiment was conducted in the fields of a farmer in the south of Najaf Governorate during the agricultural season 2022-2023 with the aim of studying the effect of potassium ions conflict with sodium on the stomatal performance of four wheat cultivars (Tamooz, Rasheed, Bohooth, and Iraq). The treatments 3 dSm⁻¹ EC (the average concentration of irrigation water in the Euphrates River during the growing season as a comparison treatment), 3 dSm⁻¹ EC + 400 ppm K, 6 dSm⁻¹ EC, and 6 dSm⁻¹ EC + 400 ppm K were used, which were denoted S1, S1+ K, S2, and S2+ K, respectively. A F-RCBD design with three replications was used.

Leaf samples were kept in polyethylene bags, then transferred to the laboratory for slide preparations. Slides were examined under light microscope with lenses of 40X and graded eyepiece of 7X.

Results and discussion

Responses to varying water qualities

Supplementation of Euphrates water with K (table, 1) profoundly increased leaf stomata length (55.96 µm) at the upper surface of leaf, in comparison to Euphrates water (49.38 µm). Resemble results were observed when K was added to salty water (especially at S1+ K), where addition of Potassium increased the aperture width of stomata (stoma Ap W) of both upper and lower leaf (9.99 µm, and 12.01, respectively). This was confirmed by Al-Rawi and Abdel (2011), as the results of their study were very close. The ultimate size of full expanded stomata is dependent on cells turgor pressure, which is actually decreased under situation of salt stress, and subsequently impacts the growth rates of guard cells (Karimi *et al.*, 2021). These results manifested the potency of Potassium ions in antagonizing the negative action of Sodium ions (Hedrich, and Shabala, 2018; Pardo, and Quintero, 2002).

Table No.1. Response of stomatal dimensions to NaCl and K treatments (*)

Treatment	Dimensions of the upper surface of the stomatal leaves (micron)				
	Lines between the stoma	Up stoma Ap L	Up stoma Ap W	Up stoma W	Up stoma L
S1	A3.4823	B43.99	6.87 B	31.16 A	49.38 B
S2	A3.69	A48.68	7.14 B	32.34 A	51.43B
S1+ K	A3.8567	46.31 AB	9.99 A	33.00 A	55.96 A
S2 + K	A4.27	49.19 AB	9.72 A	35.46 A	54.88 A
Treatment	Dimensions of the lower surface of the stomatal leaves (micron)				
	Lines between the stoma	Lo St Ap L	Lo St Ap W	Lo St W	Lo St L
S1	4.11 AB	A44.22	6.55 C	33.41 A	53.04 A
S2	4.11AB	46.41 A	2.10C	33.52 A	49.37 A
S1+ K	2.07B	46.33 A	12.01 A	31.93 A	51.11 A
S2 + K	3.99A	46.9 A	9.72 B	31.52 A	53.98 A

(*). Figure of significantly different non-shared traits, at 0.05 level, Duncan's test

Wheat Cultivar responses

Results in (table, 2) showed that Rasheed was the most potent wheat cultivar, as it possesses the longest aperture of stomata (48.95 μm), stomata aperture width (34.99 μm), and longest stomata (55.85 μm) on the upper surface of the leaf. Moreover, Rasheed also revealed the widest stomata (48.72 μm), and stomata longest (56.03 μm), at lower leaf surface. Tamooz take the next in the order, since it gave the longest stomata opening (45.81 μm), and the widest stomata (33.02 μm) on the upper surface of the leaf, moreover its it gave the widest aperture (11.31 μm) and it also revealed insubstantial differences with Rasheed in length of stomata pore (46.72 μm), and length of stomata (56.03 μm) on lower surface. Bohooth was categorized as third in

the order of superiority. It revealed the highest number of of lines on separated stomata lines on epidermis (4.58), Additionally, insubstantial variations were observed between Bohooth and Rasheed in length of stomata on upper and lower leaf surfaces and also in stomata aperture length. The lowest stomata dimensions were confined to Iraq wheat cultivar, as compared to others. However, the results also showed no significant differences between this cultivar and Bohooth in length of stomata at upper and lower surfaces of the leaf. Differences between varying cultivars are obvious in any investigation of cultivar evaluation and this is usually due to their genome diversity and their ability to switch ON/OFF gene expression (Majeed *et al.*, 2022).

Table No.2. Stomata dimensions of different wheat cultivars (*)

Cultivars	Dimensions of the upper surface of the stomatal leaves (micron)				
	Lines between the stoma	Up st Ap L	Up St Ap W	Up St W	Up St L
Tamooz	3.40 B	43.44 B	9.12 A	33.02 A	49.67 B
Bohooth	4.58 A	47.83 A	9.87 A	31.24 A	52.94 A
Rasheed	3.56 B	48.95 A	7.18 B	34.99 A	55.85 A
Iraq	3.82 B	47.19 A	5.01 B	32.73 A	54.22 A
Cultivars	Dimensions of the lower surface of the stomatal leaves (micron)				
	Lines between the stoma	Lo St Ap L	Lo St Ap W	Lo St W	Lo St L
Tamooz	3.3994A	43.83 B	11.21 A	34.21 A	52.97 AB
Bohooth	3.4739A	41.32 AB	11.48 A	35.4 A	47 B
Rasheed	3.6012A	46.72 A	7.23 B	33.31 A	56.03 A
Iraq	3.5111A	43.81 B	8.19 B	32.96 A	50.51 AB

(*). Figure of significantly different non-shared traits, at 0.05 level, Duncan's test

Cultivars responses to K^+ : Na^+ antagonisms and water qualities

The results of table (3) indicated that the Rasheed irrigated with Euphrates water supplemented with K (S1+K) was the most

potent interaction treatment, which it exceeded other dual treatments at upper and lower surfaces of leaf, in stomata aperture length (54.55 and 56.11 μm , respectively), width of stomata (45.97 μm), and stomata length (61.69 and 61.68 μm , respectively).

Table No.3. Response of stomatal dimensions to NaCl and K treatments (*)

Treatment	Cultivars	Dimensions of the upper surface of the stomatal leaves (micron)				
		Lines between the stoma	Aperture length	Aperture width	Stomata Width	Stomata Length
S1	Tamooz	4CB	38.89 D	3.99 C	33.00 B	45.90 F
	Bohooth	3.39CB	42.15 CD	4.11 C	32.47 B	49.01 EDF
	Rasheed	3.43CB	47.87 AB	15.47 AB	32.31 B	55.11 CADB
	Iraq	3.56CB	47.87AB	3.94 C	30.81 B	48.95 CEDF
S2	Tamooz	3.516B	44.88 CDB	3.87 C	31.29 B	53.33CEDBF
	Bohooth	4.33B	50.96 AB	4.92 C	30.94 B	57.31 AB
	Rasheed	3.99CB	47.80CAB	3.65 C	30.94 B	55.93 CAB
	Iraq	3.51CB	51.17 AB	16.66 AB	31.11 B	61.58 A
S1+K	Tamooz	2.67C	45.13 CDB	16.66 AB	35.59 B	47.51 EF
	Bohooth	5.67A	49.00 AB	15.47 AB	33.00 B	55.00 CADB
	Rasheed	3.39CB	54.55 A	18.02 A	45.97A	61.69 A
	Iraq	3.61CB	45.28 CDB	4.12 C	33.06 B	52.99 CEDF
S2 + K	Tamooz	3.79CB	44.41CDB	4.47 C	29.99 B	53.98CEDBF
	Bohooth	4.67AB	46.41 A-D	13.86 B	29.21 B	53.50 CEDB
	Rasheed	4.33B	46.99 CAB	4.11 C	30.53 B	56.04 CAB
	Iraq	4.33B	48.79 CAB	3.49 C	35.7 B	54.77 CAB
Treatment	Cultivars	Dimensions of the lower surface of the stomatal leaves (micron)				
		Lines between the stoma	Aperture length	Aperture width	Stomata Width	Stomata Length
S1	Tamooz	3.43AB	49.11 AB	18.26 A	33.00 AB	56.78 AB
	Bohooth	3.14AB	43.99 CDB	3.73 C	34.45 AB	49.84 ABC
	Rasheed	3.43AB	44.78 CDB	3.93 C	33.99 AB	52.35AB
	Iraq	3.16AB	42.23 CDB	3.77 C	33.42 AB	50.87 AB
S2	Tamooz	3.43AB	46.91A-D	3.54 C	31.17 AB	55.12 AB
	Bohooth	3.43AB	42.66 CDB	3.55 C	34.69 AB	33.98 C
	Rasheed	2.67AB	46.75 A-D	3.55 C	33.18 AB	61.68 A
	Iraq	3.51AB	45.99 A-D	19.13 A	34.87 AB	52.63 AB
S1+K	Tamooz	2.02B	39.14 D	19.89 A	27.32 B	46.32 ABC
	Bohooth	3.00AB	45.87 A-D	19.99 A	41.51 A	48.79 ABC
	Rasheed	3.49AB	56.11 7A	3.81 C	30.62 AB	54.16 AB
	Iraq	3.49AB	46.81 A-D	3.79 C	35.69 AB	55.11 AB
S2 + K	Tamooz	4.43A	46.86A-D	3.88 C	32.54 AB	56.01 AB
	Bohooth	3.13AB	46.82 A-D	14.27 B	33.49 AB	55.34 AB
	Rasheed	4.22A	47.12 CAB	18.23 A	35.91 AB	53.14 AB
	Iraq	3.4823AB	40.24 CD	4.11 C	30.28 AB	44.17 BC

(*). Figure of significantly different non-shared traits, at 0.05 level, Duncan's test

Euphrates water supplements with Potassium and K^+ impacts on stomata growth

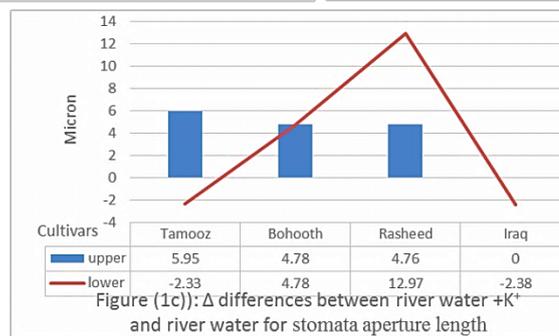
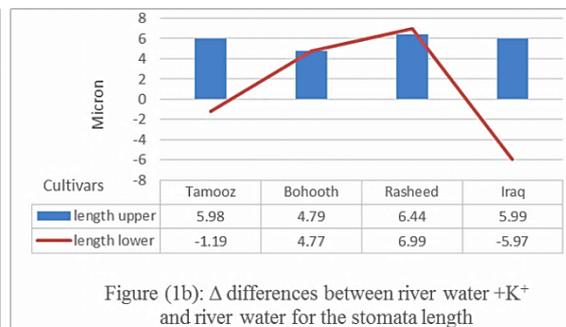
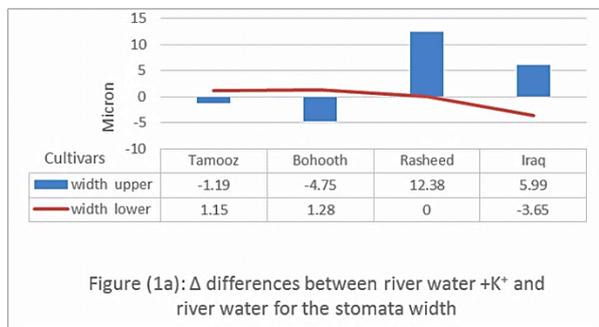
The ability of potassium to stimulate stomatal growth was confirmed by the differences in Δ between Euphrates water supplemented with potassium (3 dSm^{-1} EC + 400 ppm K) and Euphrates water alone (3 dSm^{-1} EC) by results

in (figure 1a, b, c). Rasheed cultivar showed better response when irrigated with river water with K⁺, as it achieved the highest difference in the size of the stomata width ($\Delta = 12.38 \mu\text{m}$), length ($\Delta = 6.44 \mu\text{m}$), and pore length ($\Delta = 4.76 \mu\text{m}$) on the upper leaf surface, and this cultivar also recorded a positive response to K⁺ addition in stomatal length ($\Delta = 6.99 \mu\text{m}$) and pore length ($\Delta = 12.97 \mu\text{m}$) on the lower surface of the leaf.

A similar response to potassium addition was observed for the cultivar Bohooth in both stomatal width ($\Delta = 1.28 \mu\text{m}$), length ($\Delta = 4.79 \mu\text{m}$), and aperture length ($\Delta = 4.77 \mu\text{m}$), on the

lower leaf surfaces, as well as increases recorded on the upper surface in stomatal pore length and diameter ($\Delta = 4.78$ and $4.78 \mu\text{m}$).

The Tamooz cultivar recorded positive responses to potassium in terms of stomata length ($\Delta = 5.98 \mu\text{m}$) on the upper and lower surfaces and width on the lower surface of leaves ($\Delta = 1.15 \mu\text{m}$), while it is noted that the Iraq cultivar achieved an average width and length on the upper surface of the leaf of ($\Delta = 5.99 \mu\text{m}$), but there were no benefits from adding potassium with sodium chloride in the other studied traits.



Salted water supplemented with Potassium and its effects on stomata growth

The results of (Figure 2a, b, and c) indicated that the Bohooth cultivar responded best to potassium-amended salt water, recording the highest Δ value in length ($\Delta = 14.17 \mu\text{m}$), pore length ($\Delta = 7.14 \mu\text{m}$) and width ($\Delta = 7.66 \mu\text{m}$)

on the lower surface of the leaf, and it gave width differences of ($\Delta = 1.19 \mu\text{m}$) at upper surface of leaf. Similar results were detected in Iraq cultivar, it increased length ($\Delta = 7.12 \mu\text{m}$), and pore length ($\Delta = 3.53 \mu\text{m}$), at lower surface, additionally, width at upper surface of the leaf ($\Delta = 4.44 \mu\text{m}$). Tamooz showed width increases of ($\Delta = 3.69 \mu\text{m}$), and pore length ($\Delta = 5.28 \mu\text{m}$) at upper surface of leaf.

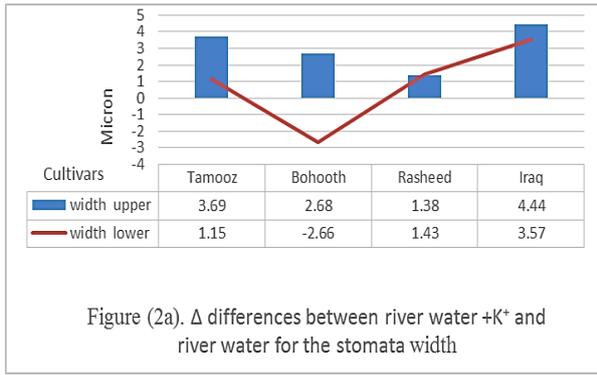


Figure (2a). Δ differences between river water +K⁺ and river water for the stomata width

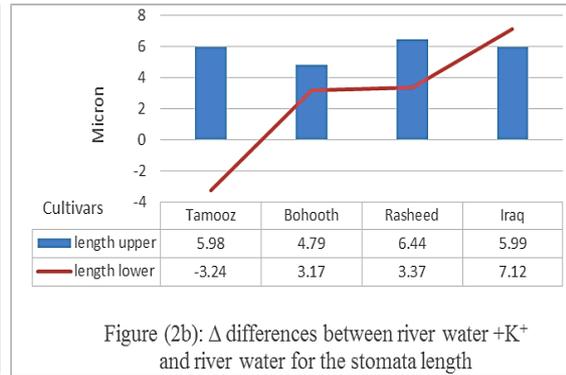


Figure (2b): Δ differences between river water +K⁺ and river water for the stomata length

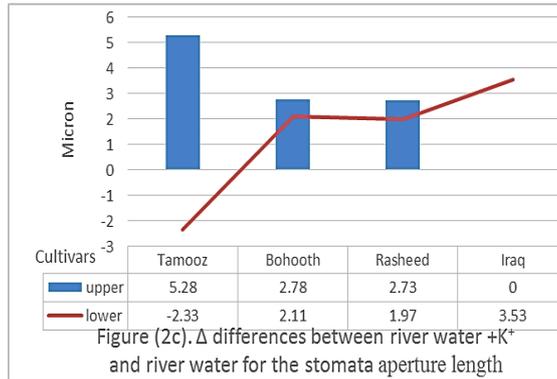


Figure (2c). Δ differences between river water +K⁺ and river water for the stomata aperture length

Salty water adversity on growth of stomata

It is noted from (Figure 3a, b, c) that the Δ differences between irrigation water from the river with potassium added to it and water without it are the effectiveness of potassium in resisting the effect of sodium on the growth of the stomata. Tamooz gave the widest stomata

($\Delta = 5.12 \mu\text{m}$), length ($\Delta = 5.53 \mu\text{m}$), and length of pore ($\Delta = 3.53 \mu\text{m}$) at lower surface of leaf. Resemble aspects were detected in Bohooth, since it gave length ($\Delta = 15.18 \mu\text{m}$), aperture length ($\Delta = 4.11 \mu\text{m}$), at lower surface and width at upper surface ($\Delta = 2.37 \mu\text{m}$). Finally, Rasheed showed increases of ($\Delta = 2.38 \mu\text{m}$) in width of upper surface.

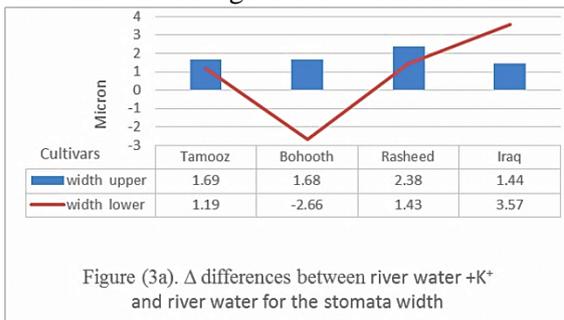


Figure (3a). Δ differences between river water +K⁺ and river water for the stomata width

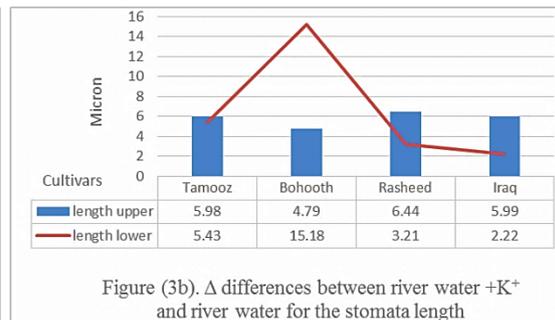


Figure (3b). Δ differences between river water +K⁺ and river water for the stomata length

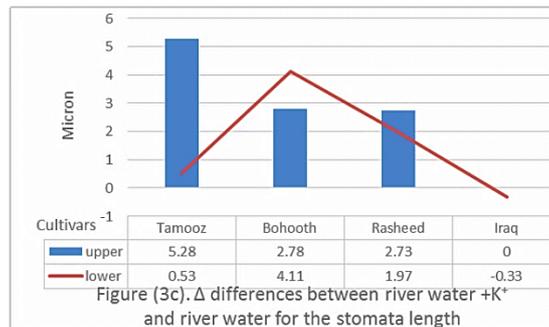


Figure (3c). Δ differences between river water +K⁺ and river water for the stomata length

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