Morphological and molecular assessment of several cultivars of bread wheat *Triticum aestivum* L. under different types of irrigation water

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Abstract. The experiment was carried out at the second research station, College of Agriculture, Al-Muthanna University during the winter season 2018-2019, to investigate the performance of four wheat cultivars namely Rasheed, IBA99, Bhooth and Abu Graib subjected K+ supplants to antagonize Na+ effect on growth, yield and genome of four wheat cultivars. Subsequently, varying irrigation water qualities were applied, namely Euphrates river water of 2.4 ds.m-1 electrical conductivity (EC), river water + 400 ppm of K+, saline water of 5 ds.m-1EC, and saline water+ 400 ppm of K+. The results revealed the superiority of K+ addition to river and salty water through irrigation substantially antagonized Na+ adversity. Where K+ improved the performance of river water and salty water in plant height (70.71 and 72.25 cm, respectively) and leaf area (28.29, and 24.41cm2, respectively) as they compared to river water without K+ (63.83 cm and 21.87 cm2, respectively). IBA99 wheat cultivars showed superiority over others, since it gave the highest plant height (70.375 cm), 1000 seed weights (32.583 g) and grain number per spike (55.475), then comes in the second order Rasheed, as it gave the higher values in plant height (74.41 cm), 1000 seed weight (33.33 g), and spike length (14.816 cm), as compared to Bhooth and Abu Graib cultivars. Then Abu Graib comes in the third order, followed by the worst Bhooth cultivar. Rasheed X + river water + k+ was the most potent dual combination, since it showed the highest plant height (78.66 cm), leaf area (35.05 cm2, respectively), and weight of 1000 grain reached (37.33 g). IBA99 + salt water + k+ combination manifested superiority in the grains number of spike which gave 61.36 g. Molecular analysis results in agreement with those of the morphological analysis by the appearance of TaHKT2;2 gene bands very clear in the Rasheed cultivar, the DNA size of the gene reached 500 nucleotides. Indicating the clear genetic expression of the gene in the varieties (except Abu Graib), especially for the Rasheed cultivar with a combination (Rasheed with salt water + K+), and the positive role played by potassium ion in reducing the harmful effect of sodium ions, Rasheed in the breeding programs of wheat crop to the salt stress tolerance through the hybridization because of its superiority over the rest of the local varieties.

1. Introduction

Cereal crops are the main part of human food; it provides 75% of the energy it needs. The bread wheat (*Triticum aestivum* L.) is the most important crop among other cereal crops, being a source of food for
more than 2 billion people worldwide because of its importance in the good balance between proteins and carbohydrates [1]. Owing to over-accumulation of NaCl, salinity is experiencing great impact on crop productions and the most injurious factor in arid and semi-arid regions (29 to 40° Latitudes). Saline soils genesis may be natural or accelerated by the bad water quality utilized for irrigation in agriculture, the intensive use of alkaline water combined with high evaporation rates and irresponsible human practices [2]. Shoot and root growth of the maize plants are inhibited by salinity and NaCl significantly decreased shoot and root dry mass of maize plants. Sodium chloride increased Na, Cl, P, Zn and Mn concentrations in the shoot and root. Applied NaCl decreased Na, Ca and Fe concentrations in the shoot, increased Na, Ca and Fe concentrations in the root. Sodium, Cl, N, P, Ca, Fe, Zn and Mn accumulated in the root in according to applied NaCl [3]. Soil salinity tends to accumulate P, Mn and Zn and decrease K and Fe Content of plants [4]. In general shoots are more susceptible to cation allocations than roots, however, it depends upon plant species and their ability to tolerate the excess salt rates [5].

[6] found that K+ application profoundly increased photosynthetic rate, percentage of filled spikelet, yield and K content in rice straw. It significantly reduced Na and Mg content and improved the K/Na, K/Mg and K/Ca ratios. IR28 cultivar is better to K+ than Pokkali. The mineral content was estimated in grain, straw and roots of rice for Na, K, Ca and Mg at harvest. Results revealed significant differences in mineral composition of straw with K and Na under salinity stress, Na content was found to increase, while K declined. Lagenariasiceraria plants sprayed by 250 ppm KNO under saline conditions not only inhibited toxic effects of salt on fruit formation, but also increased the productivity up to 76.91 % by weight per plant, spray with higher concentrations of KNO3 did not show much benefit [7].

Sodium is an ion that is not necessary for most plants, however, it can partially replace potassium ions in some functions. High concentrations of sodium ions in the soil are toxic to the plant except for halophytes. It becomes a toxic ion when its concentration exceeds the threshold of 20-30 mM. The family HKT (High Affinity K+ Transport), consisting of seven to nine members (genes) which allow sodium ion permeability (as well as potassium ion for some) in these functions, and thus play important roles in regulating the ionic balance of sodium and potassium [8]. Among the members of this family is the TaHKT2;2 gene which can be involved in the regulation of Na+ from the root to the sheath or maintaining K+ levels under conditions of salt stress or without it [9]. [10] demonstrated that Na+ competes with K+ for uptake by plant roots, implying that K+ transporters are also the gates for Na+ entry. Although the first K+ transporters were cloned a decade ago, however, the identity of the major pathway(s) for Na+ uptake has remained elusive. Transporters of the HAK-KT-KUP family (which includes the high-affinity K+-uptake transporter HAK, K+ transporter KT, and K+-uptake transporter KUP) are highly selective for K+ (Km 10-50 _M) but can also transport Na+, albeit with much lower affinity. HAK-KT-KUP proteins are thought to be K+/H+ symporters (moving both molecules in the same direction), permitting a concentration of K+ against its electrochemical gradient, to ensure K+ nutrition [11]. Another family of transporters, the HKT-TRK proteins (which includes the high-affinity K+ transporter HKT and its homologs), shows mixed ion selectivity when expressed in heterologous systems. The wheat TaHKT1, for example, functions as a Na+ K+ symporter at micromolar Na+ concentrations but as a Na+ uniporter (transporting only this single type of ion) at millimolar Na+ concentrations [12], while the Arabidopsis AtHKT1 transports only Na+. Rice has both types of transporter: OsHKT1 is a Na+ [13]. Transporter like AtHKT1 but OsHKT2 behaves as a symporter or uniporter as does TaHKT1 [14]. Transcripts of the OsHKT genes accumulated under low K+ concentrations and diminish in high external Na+; together with the nature of the transporters, these data suggest that HKT proteins might mediate substantial Na+ uptake [14].

A Genetic screen for SOS (salt overly sensitive gene) mutants yielded 28 new alleles of sos1, nine mutant alleles of a newly identified locus, SOS2, and one allele of a third salt tolerance locus, SOS3. The sos2 mutations, which are recessive, were mapped to the lower arm of chromosome V. Growth measurements demonstrated that sos2 mutants are specifically hypersensitive to inhibition by Na or Li
2. Materials and methods

A trial was carried out at research field, Agriculture College, Al-Muthanna university during the winter season 2018 - 2019, (9m Altitude, 31.3188° N, 452806° E). Seeds were purchased from Agricultural Directory of Al-Muthanna, Ministry of Agriculture. Rasheed, IBA99, Bhooth 22 and Abu Graib are well approved wheat cultivars and highly recommended by grower and researchers.

2.1 Experimental Design

Factorial Randomized Complete Block Design (F-RCBD) was adopted for this trial, where factor A represented by irrigating water qualities: Euphrates river water of 2.4 ds.m-1 electrical conductivity (a1), river water + 400 ppm of K+ (a2), saline water of 5 ds.m-1EC (a3), and saline water+ 400 Ppm of K+ (a4). Whereas, B factor was represented by Rasheed (b1), IBA99 (b2), Bhooth 22, and Abu-Graib (b4) cultivars. Therefore, 16 treatments were included in this experiment, each was replicated four times and each replicate possessed two 30 cm pot diameters.

2.2 Cultural practices

Water salinity was practically measured in the field where EC reached 2.5 ds.m-1 and pH = 7.2. Soil samples were taken at a depth of 0-30 cm from different sites of the field. Their chemical and physical properties were analysed after mixing with each other for homogenization then were softened and passed from 2 mm sieve (table, 1). Seeds of the four wheat cultivars (IBA 99, Bhooth 22, Rasheed and Abu Graib) were sown in 10 kg pots on 28 Nov. 2018 according to the previously chosen design. Irrigation, fertilization and other cultural practices were practiced according to those recommended for wheat growing [17].
Table 1. The chemical and physical properties of trial field

| Unit       | Value | Trait  |
|------------|-------|--------|
| -          | 7.3   | PH     |
| ds.m-1     | 3.4   | EC     |
| gm/Kg      | 22    | Clay   |
|            | 15    | loam   |
|            | 63    | sand   |
| Sandy clay loam |       | Soil texture |

NPK and urea fertilizers were added twice after thinning and month later, while weeds were eradicated manually whenever they appeared. During cool months, pots were irrigated weekly, whereas, irrigation was applied twice a week during hot months, since pots were arranged in plastic house and rain protected by thick polyethylene.

2.3 Molecular analysis

Molecular analysis starts with the extraction of DNA in the laboratories of the Biotechnology Research Center– Al-Nahrain University using the Hamorabi extraction kit (provided by the Institute of Genetic Engineering - University of Baghdad) and then conducting a PCR thermal profile. The primers sequence of the TaHKT2;2 genes was detailed in table 2. PCR product was migrated on the agarose gel to confirm the presence of targeted gene Along with …ladder.

Finally, (On 3 April 2019) plants were harvested and kept in paper bags to measure the investigated parameters, data were analyzed by 9.4 SAS software according to 0.05 level of significance and Duncan test.

Table 2. The primers of TaHKT2;2 gene sequences used in this study

| Primer name  | Type    | Primer sequence 5’- 3’       |
|--------------|---------|-------------------------------|
| TaHKT2,2     | Forward | GATCCACTCAACTTCTCCAC          |
| TaHKT2,2     | Reverse | TCATACCTTCCAGGATTTAC          |

3. Results and discussion

3.1 Wheat growth and yield responses to Na+: K+ antagonisms

Saline enriched with potassium showed significant superiority over other treatments in terms of plant height (72.25 cm) and spike length not significant (13.23 cm). River water supplemented by potassium was next, as it differed significantly in leaf area (28.286 cm²) and gave insignificant difference with salty water enriched by calcium in plant height. Furthermore, insignificant differences were detected among other water types in all other investigated parameters (table, 3), similar results were obtained by [18]. The obtained results confirming the antagonizing role of K+ against Na+, since both cations are resembling, besides the competing between both cations at the rhizospheres. K application profoundly increased photosynthetic rate, percentage of filled spikelet, yield and K content in rice straw. It significantly reduced Na and Mg content, and improved the K/Na, K/Mg and K/Ca ratios. The analysis of grain, straw and roots of rice for Na, K, Ca and Mg at harvest revealed significant differences in mineral composition of straw with K and Na under salinity stress, Na content was found to increase, and K declined [6].
Table 3. Wheat growth and yield responses to varying water qualities antagonized by K+ (*), (**)  

| W type   | Pl.he | 1000 S.wt | Spike no. | Spike L | Grains/S | Leaf area | Tiller no |
|----------|-------|-----------|-----------|---------|-----------|-----------|-----------|
| Rw       | 63.833b | 30.417a   | 2.6667a   | 11.5833a | 44.708a   | 21.867C   | 3.4167a   |
| R+K+     | 70.708ab | 30.25a    | 3a        | 11.9167a | 48.45a    | 28.286a   | 3.1667a   |
| Sal      | 71.083ab | 29.883a   | 2.75a     | 11.625a  | 41.383a   | 26.521ab  | 3.25a     |
| Sal+K+   | 72.25a  | 29.5a     | 2.6667a   | 13.2333a | 43.767a   | 24.407b   | 3a        |

(*), Figures of unshared trait are significant at 0.05 level Duncan test  
(**), W type = water type, Rw = River Water, R+K = River water enriched by 400 ppm K, Sal = Salty water, Sal+K = Salty water enriched by 400 ppm L, Pl he = Plant height (cm), 1000 Sw = Weight of 1000 grains (g), Spike no = Spike number per plant, Grains/S = Grains per spike (g), Leaf a = leaf area (cm).

3.2 Growth and yield responses of four wheat cultivars  
IBA99 appeared to be the most potent wheat cultivar (table, 4), it substantially exceeded other cultivars in plant height (70.375 cm), the weight of 1000 seeds (32.583 g), and grain number per spike (55.475), then Rasheed cultivar, as it differed significantly from others in plant height (74.417 cm), the weight of 1000 grains (33.33 g) and spike length (14.816 cm). Abu Graib was categorized in the third order, owing to its plant height (67.91 cm), and leaf area 25.852 cm 2. Bhooth was the worst cultivar, it only showed the highest leaf area (27.249 cm2. Subsequently, investigated wheat cultivar can be ordered according to their significances as below: IBA99 > Rasheed > Abu Graib>Bhooth. This categorization was mainly depended upon weight of 1000 grains to express the quality and grains number per spike for quantity, since our main objective was the production, where other detected traits are relevant for agronomists. Differences in cultivar responses are obvious in any comparative studies, where all workers attributed such variation to the capability of given cultivar to express its genome within the environment that it grows in. Very closed results were observed trilby [19] in barley and [20] in rice.

Plants express a gene encoding a Na+: K+ cotransporter (HKT1) has implications for understanding sodium toxicity in plants, which occurs on saline soils, because HKT1 could be a pathway for Na+ entry into plant tissues (Rubio, et al., 1995).HKT1-mediated Na+-K+ co-uptake that suggests this protein has distinct sites for high affinity K+ and Na+ binding. Random mutations have been identified that reduce Na+ uptake at toxic external NaCl concentrations, thus conferring yeast cells with salt tolerance when these HKT1 mutants are expressed in K+ uptake-deficient yeast cells [21].

Table 4. Growth and yield responses of four wheat varieties (*), (**)  

| Varieties | pl.he | 1000 S.w | Spike no | Spike L | Grains/S | Leaf area | Tiller no |
|-----------|-------|----------|----------|---------|----------|-----------|-----------|
| Rasheed   | 74.417a | 33.333a  | 2.75a    | 14.9167a | 39.033b  | 23.548b   | 3.0833a   |
| IBA99     | 70.375ab| 32.583a  | 2.8333a  | 12.625b  | 55.475a  | 24.432b   | 3a        |
| Bhooth22  | 65.167b | 27.133b  | 2.6667a  | 10.2333c | 45.325b  | 27.249a   | 3.6667a   |
| Abu Graib | 67.917ab| 27b      | 2.8333a  | 10.5833c | 38.475B  | 25.852Ab  | 3.0833a   |

(*), Figures of unshared trait are significant at 0.05 level Duncan test  
(**), Pl he = Plant height (cm), 1000 Sw = Weight of 1000 grains (g), Spike no = Spike number per plant, Grains/S = Grains per spike (g), Leaf a = leaf area (cm).

3.3 Cultivar responses to varying Na+: K+ antagonisms  
Rasheed wheat cultivar irrigated with river water enriched by 400 mg. l-1 was the most effective dual treatment as it differed significantly with most other dual treatments in the weight of 1000 grains (37.33 g), then followed by IBA99 irrigated by salty water supplemented with 400 mg. l-1, as it gave the highest grain numbers per spike (61.367). However, the worst dual treatment was Rasheed.
irrigated with salty water, as it showed the lowest grain number per spike (29.167), followed by Abu Graib irrigated with river water enriched by 400 mg. l-1 (21g). These results suggested that the most potent cultivars for instance IBA99 and Rasheed can their salt tolerance be well improved by potassium, since the latter capability in ameliorating salt adversity are well established. A study on the interactive effect of macronutrients (N, P, K+, Ca2+, Mg2+, and S) and salinity on the growth of hydroponically-grown wheat by [22] showed that, for a given salinity level, increases in nutrient supply improved plant growth in the deficient treatment only, and not in those treatments with optimal nutrient supply. It was found that concentration of macronutrients in wheat roots was increased from 0.04 to 0.2 x HS. In contrast to leaves and stems, mineral concentrations in grain were slightly increased (Na, Cl) or decreased (Ca, Mg, K) or were not affected at all (K) by salinity except at high salinity and low macronutrient level [22].

Table 5. Cultivar growth and yield responses to varying water qualities antagonized by K+

| Treatments | Pl.he | 1000 S.wt | Spike no. | Spike L | Grains/S | Leaf area | Tiller no. |
|------------|-------|-----------|-----------|---------|----------|-----------|-----------|
| River Wa   | Rasheed | 66.67ab  | 33.33ab   | 2.6667a | 15Ab     | 38.87c-e | 24.542cd  | 3.667ab   |
|            | IBA99 | 72.333a | 32abc  | 3a     | 13.3a-d  | 52.87a-c | 25.005cd  | 2.3333b   |
|            | Bhooth22 | 53.333b | 27.67abc | 2.3333a | 8.667E   | 52.33a-c | 16.118e   | 4a        |
|            | Abu Graib | 63ab   | 28.67abc | 2.6667a | 9.333de  | 34.8c-e | 21.830d   | 3.667ab   |
| RV + K+    | Rasheed | 78.667a | 37.333a | 2.6667a | 12.7b-E | 43.03b-e | 35.055a   | 2.667ab   |
|            | IBA99 | 67.17ab | 35.333ab | 3.3333a | 13b-e     | 58.67ab | 27.297cd | 3.667ab   |
|            | Bhooth22 | 70a   | 25.87abc | 3a     | 10.3c-e  | 51.4a-c | 25.017cd  | 3.3333b   |
|            | Abu Graib | 67AB | 21c   | 3a     | 11.7b-e  | 40.67B-E | 25.77cd  | 3Ab       |
| Salty water | Rasheed | 78.333a | 35.333a | 3a     | 14.7a-c  | 29.167e | 25.380cd | 3.333ab   |
|            | IBA99 | 66.33ab | 29.67abc | 2.3333a | 12b-e   | 49a-d  | 22.999d | 3ab       |
|            | Bhooth22 | 70a | 30.67abc | 2.3333a | 10.8b-e | 41.77b-e | 24.003cd | 3.667ab   |
|            | Abu Graib | 69.667a | 25.333bc | 3.3333a | 9de     | 45.6a-e | 33.788ab | 3ab       |
| Salty+ K+  | Rasheed | 74a | 27.33 abc | 2.6667a | 17.333a | 45.07a-e | 24.098C | 2.667ab   |
|            | IBA99 | 75.667a | 33.333ab | 2.6667a | 12.2b-e | 61.367a | 22.436d | 3ab       |
|            | Bhooth22 | 67.33ab | 24.333bc | 3a     | 11.1b-e | 35.8c-e | 29.054cb | 3.667ab   |
|            | Abu Graib | 72a | 33ab | 2.3333a | 12.3b-e | 32.87de | 25.77cd | 2.667ab   |

3.4 Molecular analysis to confirm the presence of TaHKT2;2 in cultivar genomes

TaHK2;2 is a member of HKT responsible for controlling sodium absorption and balancing its concentration with potassium ions. The impact of potassium supplement of both river and salty water were obviously reflected on the genome of most investigated cultivars, which confirmed by the appearance of gel electrophoresis denoted to TaHK2;2. The bands of TaHK2;2 can be obviously seen with a molecular size of approximately 500 bp in all extracted DNAs that were isolated from plant leaves subjected to different qualities of irrigation water (Figure 1). TaHK2;2 was observed in IBA99, Bhooth and particularly in Rasheed cv. subjected to salty water enriched by potassium. Salt resistance and ion antagonisms can be referred to HKT1 gen, since HKT1 belongs to a superfamily of symporters found in plant, fungal, and bacterial genomes whose primary structure and function is conserved among many different organisms [23]. These symporters appear to be mainly involved in potassium uptake across the plasma membrane in a diverse range of organisms [24]. It has been suggested that there might be an evolutionary link between the structure of K+ channels and this superfamily of K+ symporters based on sequence comparison between members of this symporter family and the bacterial K+ channel, KcsA from Streptomyces lividans [25]. It is worthy to mention that HKT1 is a high affinity K1 symporter expressed in wheat root cortical cells and in leaf cells bordering the vasculature [26].
Figure 1. the TaHKT2;2 gene bands with a size of 500 bp clearly shown in (1): represent the Bhooth22 cultivar with salt water irrigation, (2) Bhooth22 cultivar with river water irrigation (3) Bhooth 22 cultivar with salt water irrigation + potassium ion. (4) IBA99 represents with salt water irrigation, (5) IBA99 with river water irrigation, (6) IBA99 with salt water irrigation + potassium ion, and very clearly in (7) represents a Rasheed cultivar with salt water irrigation, (8) Rasheed cultivar with river water irrigation, (9) Rasheed cultivar with salt water irrigation + potassium ion. (10) Abu Graib cultivar.

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