PAPER DETAILS

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AUTHORS: Cengiz Toker,John Gorham,M Ilhan Çagirgan

PAGES: 162-169

ORIGINAL PDF URL: https://dergipark.org.tr/tr/download/article-file/158802

TURKISH J. OF FIELD CROPS 14(2): 162 – 169 2009

CERTAIN ION ACCUMULATIONS IN BARLEY MUTANTS EXPOSED TO DROUGHT AND SALINITY

Cengiz TOKER[1] John GORHAM[2] M. İlhan ÇAĞIRGAN[1*]

[1] Department of Field Crops, Faculty of Agriculture, Akdeniz University, TR-07059 Antalya, TURKEY
[2] School of Biological Sciences, University of Wales, Bangor, Memorial Building, Bangor LL 57 2UW, Gwynedd, UK

*Corresponding author: email: cagirgan@akdeniz.edu.tr

ABSTRACT

A total of twelve barley genotypes, of which mutants (M-K 23, M-K 24, M-K 25, M-K 35, M-K 55, M-K 63, M-Q 54 and M-Q 76) and their parents (Kaya and Quantum), and two checks (Chevron, salt sensitive and CM 67, salt tolerant), were grown to assess their tolerance to drought (irrigated with 15 min. per week) and salinity (irrigated 15 min. per day with 150 mol m^{-3} NaCl + 7.7 mol m^{-3} $CaCl_2$). For ion analysis, the fully emerged flag leaf of genotypes was used and the followings were measured Nitrate (NO_3), Malate, Sulphate (SO_4), Magnesium (Mg), Sodium (Na) and Potassium (K) and (K/Na) rate was computed. All of the ion accumulation in the leaves was generally higher in drought than those in saline and control plants. Although different responses to drought and salinity were found among the barley mutants, Quantum, M-Q 76, M-K 23 and M-Q 54 had the highest K/Na concentrations in the external saline treatments. These genotypes have performed as well as a salt tolerant genotype, CM 67. It was concluded that high K/Na rate could be used as a selection criterion to determine the level of tolerance to salinity of barley genotypes.

INTRODUCTION

It was indicated that salt affected soils covered nearly 10% of the total land surface with a total of 954.8 million ha in the world (Szabolcs, 1994). Photosynthetic machinery of plants is adversely affected *via* salinity and net CO_2 assimilation is decreased through its effect on stomata (Farquhar et al., 1987; Sharkey et al., 1990; Toker et al. 1999). Inhibition of cell expansion and the reduction of photosynthetic area for assimilation thus took place to be more important limitations to growth than the reduction in assimilation activity *per se*. It was pointed out that there are some similarities between effects of salinity and drought stresses on growth of plants. Gorham et al. (1985) divided the hazards of salinity on plants into three groups: (i) water stress arising from the more negative water potential of the rooting medium, (ii) specific ion toxicity generally associated with either excessive chloride or sodium intake, and (iii) nutrient ion imbalance when the excess of sodium or chloride leads to diminished uptake of potassium, nitrate or phosphate or to impaired internal distribution of one or another of these ions. In general, crop cultivars within a species, which tend to exclude Na^+ are more salt tolerant and do not exhibite the damaging effects of Na^+ as much as cultivars which tend to accumulate Na^+ are susceptible to salinity (Lauchli, 1986; Hajibagheri et

al., 1987; Schachtman and Muns, 1992). Gorham (1992) reported that there was a net exchange of Na^+ for K^+ in a mature cell of a plant. K/Na rate is increased in salt tolerant cultivars within species under salinity conditions.

The primary focus of the present study was to determine whether there were differences among ten barley genotypes subjected to salinity and drought stresses and control treatment, comparing with two checks. Another objective was to evaluate genotypes for certain ion accumulations and whether K/Na rate could be used a reliable indicator as a selection criterion when barley genotypes were subjected to drought and salinity stresses.

MATERIALS AND METHODS

Twelve barley (*Hordeum vulgare* L.) genotypes, including two parents (Kaya and Quantum) and their mutant lines (M-K 23, M-K 24, M-K 25, M-K 35, M-K 55, M-K 63, M-Q 54 and M-Q 76) and two checks (Chevron, salt sensitive and CM 67, salt tolerant), were used to examine the effects of drought and salinity stresses. A list of barley mutants and their important features were given in elsewhere (Toker et al. 1999; Çağırgan and Yıldırım 1990; Çağırgan et al. 1995; Gorham et al. 2000).

The experiment was carried out in a greenhouse at the University of Wales, Bangor, Gwynedd, Wales, UK under controlled conditions with a minimum temperature of 15 ^oC and a photoperiod of 16 hours, consisting of natural light supplemented with sodium vapour lamps. Two seeds were sown in each 1.6 l square pot filled with John Innes Compost and germinated in a flood bench system at about 20 ^oC. With a nutrient solution containing 1 g l^{-1} of phostrogen (pH 5.6), 0.5 ml l^{-1} of micronutrients and 0.1 ml l^{-1} of K silicate in the pots, in the control and saline treatments the pots were irrigated daily for 15 min., but in the drought stress the pots were watered weekly for 15 min. Completely Randomised Design was used with eight replications for drought and salinity and four replication for control. The salinity treatments were 150 mol m^{-3} NaCl and 7.5 mol m^{-3} $CaCl_2$ in addition to the nutrients, giving a solution with an electrical conductivity of 172 dSm^{-1} and a pH of 6.2. Stress was continued for 34 days between 21 October and 24 December 1997.

At the beginning of the flowering stage, the fully emerged flag leaf was placed in a microcentrifuge tube and frozen and than used for ion analyses. Sap was extracted from thawed and crushed leaves by centrifugation. Inorganic ion analyses were performed on a Dionex ion chromatograph using the procedures described by Gorham et al. (1987).

Results were analysed using the MINITAB (DESCRIBE, ANOVA and GLM functions) software package to assess statically significant difference among the genotypes. Mean values of genotypes were compared by Duncan's multiple range tests in MSTATC statistical package program (Freed et al., 1989).

RESULTS

According to ANOVA results, generally it was found that there were statically significant differences among genotypes at $p< 0.05$ (Table 1, 2 and 3). Genotype effects, however, were not significant for nitrate (NO_3), magnesium (Mg) and K/Na discrimination in drought treatments and for K/Na discrimination in control treatment (at $p< 0.05$.) Means within the columns that do not have a common letter are significantly different by Duncan's multiple range tests at $p< 0.05$.

Nitrate accumulations of genotypes were found between 26-157 mol m^{-3}, 75-171 mol m^{-3} and 6-60 mol m^{-3} for control, drought and salinity stresses, respectively. External saline conditions reduced nitrate accumulations, while drought stress increased nitrate concentrations. No exception, it was shown that salt tolerant genotype, CM 67, had the highest mean values for nitrate concentration in saline, drought and control treatments, whereas salt susceptible genotype, Chevron, had the lowest. Salinity decreased malate accumulations in the same pattern as the decrease in nitrate. In dry treatment, M-Q 54 had the highest with M-K 25 for malate accumulation, while M-K 24, M-K 25, M-K 23, M-Q 54, M-K 63 and CM 67 had the highest in salinity (Table 1).

Table 1. Means of genetypes, Duncan test and F values of nitrate (NO_3) and malate concentration (mol m^{-3} expressed sap) in fully expanded leaves after flowering stage of barley genotypes subjected to drought (irrigated 15 min. per a week) and salinity (irrigated 15 min. per a day and plus 150 mol m^{-3} NaCl and 7.5 mol m^{-3} $CaCl_2$) with control.

Genotypes	NO_3			Malate		
	Control	Drought	Salinity	Control	Drought	Salinity
Chevron	26 b	75 b	6 d	42 a	37 c	11 c
CM 67	157 a	171 a	60 a	17 b	38 c	19 abc
Kaya	59 b	87 b	38 abcd	31 ab	68 abc	18 bc
M-K 23	56 b	119 ab	50 abc	15 b	46 bc	28 ab
M-K 24	32 b	110 ab	54 ab	21 ab	49 bc	31 a
M-K 25	53 b	87 b	29 abcd	25 ab	85 a	29 ab
M-K 35	49 b	91 b	19 cd	22 ab	44 bc	11 c
M-K 55	64 b	110 ab	8 d	44 a	44 bc	10 c
M-K 63	78 b	92 b	28 abcd	21 ab	66 abc	19 abc
Quantum	42 b	76 b	13 d	43 a	76 ab	15 c
M-Q 54	35 b	104 b	22 bcd	44 a	88 a	21 abc
M-Q 76	54 b	85 b	20 cd	37 ab	62 abc	11 c
F values	3.82**	1.46^{ns}	2.91*	2.21*	2.67*	3.77**

- and **; there were statically significant differences among genotypic effects at $p< 0.05$ and $p< 0.01$, respectively.

Mean values of sulphate and magnesium accumulations increased in drought and control treatments, but reduced with salinity. Range in sulphate accumulations of

genotypes was 5-45 mol m^{-3}, 30-68 mol m^{-3} and 2-12 mol m^{-3} for control, drought and salinity treatments, respectively. In the salt treatments, M-Q 54 had the lowest value for sulphate accumulation. M-K 25 had the highest magnesium accumulation in external salinity (Table 2).

Calcium accumulation of genotypes ranged from 4 mol m^{-3} (M-K 24) to 43 mol m^{-3} (Quantum) for control and from 19 mol m^{-3} (M-Q 54) to 87 mol m^{-3} (M-K 25) for salinity. On the other hand, genotypic effect for calcium was not statically significant when plants subjected to dry stresses. Salinity had a larger effect than drought and control treatments for K/Na rate. When the genotypes exposed to salinity stresses, K/Na rate of genotypes changed from 1.28 mol m^{-3} to 3.78 mol m^{-3}. The highest rates for K/Na in the salinity treatment were Quantum, CM 67, M-Q 76, M-K 23 and M-Q 54. Duncan's multiple range tests Quantum, its mutants M-Q 76 and M-Q 54 and one mutants of Kaya, M-K 23, were clustered in same groups with salt tolerant genotype, CM 67, having the highest K/Na rate (Table 3).

Table 2. Means of genotypes, Duncan and F values of sulphate (SO_4) and magnesium (Mg) concentration (mol m^{-3} expressed sap) in fully expanded leaves after flowering stage of barley genotypes subjected to drought (irrigated 15 min. per a week) and salinity (irrigated 15 min. per a day and plus 150 mol m^{-3} NaCl and 7.5 mol m^{-3} $CaCl_2$) with control.

Genotypes	SO_4			Mg		
	Control	Drought	Salinity	Control	Drought	Salinity
Chevron	45 a	41 b	7 abc	24 ab	27 bc	18 bcd
CM 67	14 bcde	68 a	11 a	16 bcd	43 ab	25 bc
Kaya	24 b	50 ab	11 a	24 ab	42 ab	28 b
M-K 23	7 de	30 b	12 a	10 d	35 abc	22 bcd
M-K 24	5 e	42 b	10 ab	9 d	41 ab	21 bcd
M-K 25	11 bcde	47 b	11 a	12 cd	52 a	43 a
M-K 35	10 de	33 b	7 abc	12 cd	21 c	13 d
M-K 55	22 bc	33 b	4 bc	21 abc	31 bc	14 d
M-K 63	17 bcde	44 b	10 ab	15 bcd	31 bc	16 cd
Quantum	20 bcd	39 b	6 abc	26 a	36 abc	17 cd
M-Q 54	8 de	31 b	2 c	12 cd	30 bc	12 d
M-Q 76	9 cde	42 b	7 abc	15 bcd	33 bc	18 bcd
F değeri	7.03**	2.30*	2.31*	3.62*	1.86^{ns}	7.25**

* and **; there were statically significant differences among genotypic effects at $p< 0.05$ and $p< 0.01$, respectively.

Table 3. Means of genotypes, Duncan and F values of nitrate (K/Na) and calcium (Ca) concentration (mol m^{-3} expressed sap) in fully expanded leaves after flowering stage of barley genotypes subjected to drought (irrigated 15 min. per a week) and salinity (irrigated 15 min. per a day and plus 150 mol m^{-3} NaCl and 7.5 mol m^{-3} $CaCl_2$) with control.

Genotypes	Ca			K/Na		
	Control	Drought	Salinity	Control	Drought	Salinity
Chevron	30 abc	40 ab	37 bc	48 ab	50 ab	1.95 bc
CM 67	39 ab	73 a	58 b	35 ab	39 ab	3.61 a
Kaya	42 ab	50 ab	42 bc	28 b	43 ab	1.43 c
M-K 23	13 cd	52 ab	36 bc	40 ab	45 ab	2.56 abc
M-K 24	4 d	67 ab	42 bc	39 ab	32 b	2.16 bc
M-K 25	13 cd	71 a	87 a	50 a	48 ab	2.01 bc
M-K 35	22 abcd	29 b	21 c	36 ab	51 ab	1.36 c
M-K 55	36 abc	37 ab	20 c	37 ab	53 a	1.28 c
M-K 63	23 abcd	66 ab	41 bc	43 ab	37 ab	1.85 bc
Quantum	43 a	42 ab	30 bc	36 ab	40 ab	3.78 a
M-Q 54	11 cd	39 ab	19 c	41 ab	43 ab	2.52 abc
M-Q 76	17 bcd	63 ab	38 bc	47 ab	42 ab	2.81 ab
F değeri	2.73*	1.53^{ns}	4.14**	0.98^{ns}	1.06^{ns}	3.58**

* and **; there were statically significant differences among genotypic effects at $p< 0.05$ and $p< 0.01$, respectively.

DISCUSSION

Salinity tolerance in crops is mainly ascribed to high K/Na rate in the shoot of rye, triticale, *Aegilops* species carrying D genome and synthetic hexaploid wheats (Gorham, 1990a; Gorham, 1990b; Gorham, 1990c). Similar conclusions for K/Na were also obtained in the present study due to fact that K/Na rate was high in CM 67, salt tolerant genotype. That is, the check varieties behaved as expected (Wolf and Jesckke, 1986). According to Duncan's multiple range tests Quantum, its mutants M-Q 76 and M-Q 54 and one mutants of Kaya, M-K 23, were clustered in same groups with CM 67. Mutant of Quantum, M-Q 54, was registered by Cagirgan et al. (1998) as drought tolerant under field conditions comparing with the parent. It was reported that larger leaf growth reductions in response to salinity in *Sorghum bicolour* were associated with higher tissue levels of Na and Cl. *S. halepense* had a lower Na/K ratio in the leaves (Yang et al., 1990a). Also, Yang et al. (1990b) pointed out that Na/K ratios in salinized callus could be used as an indicator of whole plant salt tolerance in *Sorghum.* Barley is not only tolerance to salinity (Shannon, 1985), but also it is considered drought tolerance (Ceccarelli, 1996) and it has high K/Na ratio as a selection criterion when the plants were exposed to salinity stress. In contrast, K/Na discrimination in barley with 40 samples was found as low as drum wheats (Gorham et al. 1990). In diploid wheats, low

K/Na rate was found by Pecetti and Gorham, (1996). RLFP (Restriction Length Fragment Polymorphism) analysis that a trait for enhanced K/Na discrimination in wheat was governed by a single gene (*Kna1*) which was linked to five markers on the distal third of the long arm of 4D (Gorham et al., 1991). In addition, Gorham et al. (1997) outlined that sodium accumulation in leaves of *Triticum monococcum, T. boeticum, T. urartu, Aegilops squarrosa, Hordeum vulgare* and *T. aestivum* low and potassium concentrations remained high, but sodium accumulation in *Ae. sharonensis, T. durum* and *T. diccoccoides* was high. Ansari et al., (1990) reported salinity resistance accompanied by restricted uptake of Na and Ca and preferential accumulation of K and NO_3 in barley and wheat. Nevertheless, Alberico and Cramer (1993) concluded that Na^+ exclusion from the shoot was not related with and was an unreliable indicator of salt tolerance in maize.

CONCLUSIONS

It was concluded that there were statically significant differences for genotypic effect when genotypes were subjected to drought and salinity stresses. The high K/Na rate as a selection criterion was a reliable indicator of salt tolerance in barley, but not in dry stress. In addition, the present study demonstrated the necessity of using both salt tolerant and salt susceptible checks in order to assess of test lines. In the areas which were effected by salt and drought, barley cultivation is preferred to wheat cultivation. Consequently, these salt tolerant mutants can be useful to develop adapted genotypes to the areas where barley production dominates with its cultivation.

ACKNOWLEDGEMENTS

The authors would like to acknowledge to The British Council for supporting the present study through the AKDENIZ/WALES-Bangor bilateral link programme, and Akdeniz University Research Projects Unit. Special thanks extend to Mr. J. Bridges for technical assistance. The publication costs were provided by the International Atomic Energy Agency, IAEA-TUR-RC-13001 under the CRP on “Pyramiding for Drought and Salinity”.

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