WFL Publisher
Science and Technology
Meri-Rastilantie 3 B, FI-00980
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Journal of Food, Agriculture & Environment Vol.11 (3&4):1386-1394. 2013 *www.world-food.net*

Evaluation and selection of barley genotypes under optimum salt stress condition using tissue culture techniques and SDS-PAGE gel electrophoresis

Ehab Mohamed Rabei Metwali [1, 2*], Michael Paul Fuller [3], Salah Mohamed Hassan Gowayed [1, 2], Omar Abdel-Hakeim Almaghrabi [1] and Yahia Yosef Mosleh [1, 4]

*[1] Biological Science Department, Faculty of Science, North Jeddah, King Abdul Aziz University, P. O. Box number 11508, Jeddah 21463, Saudi Arabia. [2] Botany Department, Faculty of Agriculture, Suez Canal University, Ismailia 41522, Egypt. [3] Department of Biomedical and Biological Sciences, Faculty of Science and Technology, Plymouth University, Plymouth, PL4 8AA, UK. [4] Department of Aquatic Environment, Faculty of Fish Resources, Suez University, Suez, 43511, Egypt. *e-mail: ehab_25@hotmail.com*

Received 22 July 2013, accepted 28 October 2013.

Abstract

Genetic analysis was studied in a 5 × 5 diallel cross following Hayman's diallel analysis approach to obtain additive and dominance components of genetic variance using a mature embryo culture protocol. Different concentrations of NaCl at 5 and 10 g L^{-1} were added to each medium. Callus fresh weight (g), shoot length (cm), root length (cm), proline (µg g^{-1}), sodium (mg g^{-1}) and potassium (mg g^{-1}) content were determined. Also, screening for non-water-soluble protein polymorphism was carried out using leaves derived via callus culture and analysis using SDS-PAGE. Results on the genetic behaviour for previously traits, indicating the differences among the genotypes were significant ($p \leq 0.05$) and highly significant ($p \leq 0.01$). Wr/Vr graph in diallel analysis and average degree of dominance together with narrow-sense heritability values revealed additive gene effect for shoot length under control and saline treatments and callus fresh weight under control, while dominance gene action was detected for proline content under all treatments, K content under control and 5 g L^{-1}, callus fresh weight under 5 g $L^{-1}$and root length under 10 g L^{-1}NaCl. The order of array along the regression line showed that G.123 followed by G.124 are more stable than other parents and could be recommended for use in the production of a hybrid breeding programme for salt tolerance. Based on SDS-PAGE, approximately 36 bands which arranged between 124 kDa to 12 kDa were exhibited in most of parents and their hybrids under different treatments. Out of these 36 bands, 8 bands were polymorphic and one of these bands at 34 kDa was absent only in sensitive parent (Beecher) under salt treatments, while another band at 42 kDa appeared in most of the parents and their hybrids under salt treatments.

Key words: Barley (*Hordeum vulgare* L.), embryo culture, salinity, diallel analysis, hierarchical cluster analysis.

Introduction

Genetic information about the nature of various relationships between different gene alleles, and genetic behaviour of most growth characters in barley under saline stress would be useful to breeders in revealing the genetic potentialities of recommendable genotypes and organizing an effective genetic improvement program [1, 2]. The success of a barley breeding program for the development of abiotic stress tolerant varieties depends on precise estimates of genetic variation components of traits of interest and their dominant, additive and non-allelic interaction effects [3]. In quantitative genetics diallel methodology is a systematic experimental approach that is useful in providing a unique opportunity to obtain a rapid and overall picture of genetic control of a set of parents and identifying potential crosses for best selection in early generations [4].

The improvement of barley varieties suitable for growing under salt conditions by plant breeding requires genetically pure lines either to be used as parents for mating in breeding processes or to be distributed as new cultivars [5]. The selection of these pure lines by conventional breeding practice is time consuming. One of the solutions to this problem is the improvement of genotypes via *in-vitro* culture [6]. Studies at a cellular level provide better knowledge to understand the mechanisms of salt tolerance, since they require little space and less time for selection, as well as being carried out in a controlled environment which reduces environment effects [7, 8]. In barley species, different explant sources such as immature or mature embryos have been used for callus induction and plant regeneration [9]. Mature embryos were found to be a better choice in comparison to immature embryos, and are available through the year and can either be dissected or used directly [10].

NaCl has been used to simulate salt stress either *in-vivo* or *in-vitro* in barley [11-13]. Application of NaCl during the callus formation and/or regeneration processes constitutes a convenient way to study the effect of salinity and selective pressure can be applied[14]. Under NaCl stress, Babu *et al.* [15] and Naseer *et al.* [16] reported that callus growth, root length and shoot length all decreased with increasing NaCl concentration in the medium. On the other hand, genotypic variation in metabolic processes such as proline accumulation [17, 18] and nutrient uptake of elements such as Na and K [19] in plants under salt stress have been observed. Increased proline accumulation under salt stress supplies energy for growth and survival [20] as well as acting as a compatible solute to help protect membranes and macromolecules during dehydration stress. Wei *et al.*[21] found that high salt stress disrupts the homeostasis of ion distribution at both the cellular and the whole plant levels.

Electrophoretic techniques have been found to be a useful tool for the detailed studies of callus proteins [22]. Electrophoretic banding patterns of proteins have been found to be useful for the identification and characterization of particular genotypes and also establishing the predominance of one or the other parent in the hybrids [23]. This technique could also help to detect not only the "qualitative variability" through the presence or absence of bands, but also "quantitative" variation in band intensities among genotypes [24]. Here, we were interested in utilizing SDS-PAGE to examine barley non-water soluble protein and their relationship with salt tolerance. This relation could be used by the breeder in establishing strategies for selecting early generation materials in developmental programs of varieties.

The objective of this investigation was made to: (1) estimate the genetic components of variance and heritability of some growth and biochemical traits; (2) develop biochemical genetic markers such as protein electrophoresis profiles of non-water soluble protein to discriminate between these parental genotypes and their hybrids under normal and saline conditions using a mature embryo culture protocol; (3) assess some selection criteria for identifying salt tolerance in barley genotypes which can be recommended for subsequent plant breeding projects.

Materials and Methods

Five barley (*Hordeum vulgare* L.) genotypes differing in tolerance to salinity were obtained from the Barley Department, Agriculture Research Center, Giza, Egypt, for use in this study. The origin and pedigree of these genotypes are presented in Table 1. One set of diallel crosses was made during the growing season in 2009 - 2010, by crossing the five parents in all possible combination to obtain a total of 10 F_1 (First generation) hybrids. During years 2011 and 2012, mature embryo culture procedures were used to evaluate these genotypes under salinity stress *in vitro* at the Tissue Culture Unit, Division of Genomic and Biotechnology, Biological Science Department, Faculty of Science-North Jeddah, King Abdul-Aziz University, Saudi Arabia.

Callus induction: Mature grains of each parents and F_1 hybrids were surface sterilized by first washing with tap water three times, soaked in 70% ethanol for one minute, and then washed thoroughly with sterilized distilled water. Grains were then transferred into 30% "Clorox", a commercial bleach containing 1% sodium hypochlorite, plus 5 drops of Tween-20 as a surfactant material and kept on shaker for 10 min. Surface sterilized grains were rinsed three time in sterilized double distilled water under aseptic condition in a laminar air-flow hood and then soaked in sterilized distilled water for 20 h to facilitate imbibition prior to embryo isolation.

Mature embryos were dissected and separated from the imbibed grains by a simple surgical treatment under aseptic conditions. Four mature embryos plus scutellum of each of the parents and their hybrids were cultured in jars (ca. 50 mL) containing 25 mL of agar solidified basal MS medium [25] supplemented with 100 mg L^{-1} Myo-insitol, 1.00 mg L^{-1} thiamine-HCl, 2.00 mg L^{-1} 2,4-D, 30 g L^{-1} sucrose and different concentration of NaCl (5 and 10 g L^{-1}). The pH was adjusted to 5.7 by either 1M NaOH or HCl, prior to autoclaving at 121°C and 15 psi for 20 min. The cultures were incubated in the dark at 25±1°C for 5 weeks to encourage callus initiation and induction.

Analysis of salt resistance: Actively growing calli were employed for analysis the salt resistance of all 15 genotypes. Four friable callus pieces were placed in 3 replicate jar containing 25 mL sterile MS basal medium plus 1.0 mg L^{-1} BA and 0.5 mg L^{-1} IAA and supplemented with NaCl at concentrations of 5 and 10 g L^{-1}. Cultures were kept in an incubator at a constant temperature of 25±1°C. After 5 weeks regenerated shoots were transferred into rooting medium containing MS basal medium supplemented with 1.0 mg L^{-1} BA and 0.05 mg L^{-1} NAA in addition to different levels of NaCl (5 and 10 g L^{-1}). After root initiation, complete plantlets obtained by *in vitro* culture were washed under running tap water to remove agar for 2 - 3 min and soaked in a fungicide solution (2.0 g L^{-1} Benlate) for 5 min. Plantlets were then grown on in plastic pots 20 cm in depth and filled with peat-based compost, in a completely randomized design with three replications, and five plantlets were planted in each pot. To keep the high humidity (initially 90%) constant, the pots were shaded with polyethylene bags, which were gradually removed over the course of one week.

Analysis of salt resistance was determined by measurement of the following at 5 week from treatment:

1. Growth traits: 1.1. Callus fresh weight (g); 1.2. Shoot and root length (cm)
2. Biochemical traits: 2.1. Proline content (μg g^{-1}) by acid ninhydrine method [26]. 2.2. Sodium and potassium content (mg g^{-1}) by flame photometry [27].
3. Non-soluble protein: SDS-PAGE was used to compare the cultivars under different salt treatments by their protein finger prints of non-water soluble protein according to Laemmli [28] .

Protein sample extraction: Of leaf 0.2 g was taken and ground in a cold pestle mortar with liquid nitrogen and mixed with 2 mL extraction buffer containing 1M Tris HCl, pH 8.8, 0.25 M EDTA. Samples were transferred to eppendorf tubes and left in the refrigerator overnight, then vortexed for 15 s and centrifuged at 12,000 rpm at 4°C for 20 min. The pellets were collected and the steps above were repeated twice. After that 1 mL non-water soluble protein buffer (10% SDS, Glycerol, 1M Tris HCl, 0.25M EDTA) was added to each pellet and mixed well and left in the refrigerator overnight. The sample was then vortexed and centrifuged at 12,000 rpm for 15 min under cooling. The supernatant, considered as the non-water soluble protein extractant, was transferred to new Eppendorf tube. Protein concentration was estimated using Bradford's method [29] by measuring absorbance at 595 nm using a

Table 1. Pedigree, origin and degree of tolerance to salinity of the parental line and varieties.

Code	Genotype name's	Pedigree	Origin	Degree of salt tolerance
1	Giza 123	Giza117/FAO 86(Giza117 = Baladi 16/Palestine 100	Egypt	Tolerant
2	Giza 124	Giza 117/Bahteem52/Giza118/FAO86	Egypt	Tolerant
3	Arar	Perga/Sekitorisai	Syria	Mid-Tolerant
4	Mari	Bouns X Ray-mutant	Syria	Moderate
5	Beecher	Atlas/Vaughan	Syria	Sensitive

spectrophotometer and expressed as µg g^{-1} fresh weight. A standard curve for protein was prepared with bovine serum albumin.

Separation of protein samples: A volume of 50 µL of non-water soluble protein fraction was added to10 µL of 2-Mercaptoethanol and boiled in a water bath for 10 min, then 10 µL Bromophenol blue was added to each tube before sample loading. A volume of 15- 20 µL, depending on the concentration of protein in the sample were applied to each well by micropipette and control wells were loaded with protein standards (Amersham Biosciences-Sweden). Gels were stained using silver staining as described by Blum *et al.* [30] and after bands becomes clear, the gels were photographed and electrophoregrams for each entry under different treatments were scored depending on the presence (1) and absence (0) of bands. A cluster diagram was constructed based on unweighted pair-group method with arithmetic average (UPGMA). The similarity matrix, genetic distances and dendrogram analysis were computed using Numerical Taxonomy and Multivariate Analysis System, Version 2.1(NTSYS-PC [31]).

Statistical analysis: Estimation of the components of variation and determination of the nature of gene action in the studied characters were carried out using the diallel biometric approach as outlined by Hayman [32]. The development of an effective plant breeding program and the efficiency of selection largely depend upon the magnitude of genetic variability existing in the plant material under study; therefore, analysis of variance and components of variation were applied.

Results and Discussion

Genetic analysis of salt stress: Selection of plants combining good plant traits is the main goal of plant breeding and most of the genetic advances which have been made through intercrossing existing varieties have led to improvement in crops [33]. For effective selection of plants, especially under abiotic stress, variability in growth, physiological and biochemical traits must be genetically based. In the present investigation, the genetic materials were subjected to different doses of NaCl at (5 and 10 g L^{-1}) at the beginning of mature embryo culture under *in vitro* conditions (Fig.1). In previous reports, salinity response in barley[1, 34], rice[35] and wheat [7, 36] have been assessed using the same procedure, and therefore, the present methods provide a reliable evaluation of barley breeding material for enhanced salinity tolerance

In this study, results of analysis of variance (Tables 2 and 3) revealed that there were significant differences ($p \leq 0.05$) and highly significant differences ($p \leq 0.01$) among genotypes in terms of the 6 traits measured. This indicated the presence of genotypic variability for these traits and implied that these barley genotypes would respond positively to selection. This result is similar to the report of Sharifi [35] that showed highly significant differences among genotypes for all of the traits.In our study, the genetic analysis revealed the presence of genotypic variability for the traits under study and implied that these barley genotypes would respond positively to selection. Uniformity of Wr and Vr test (t^2) revealed the absence of non-allelic interactions showing that the data is adequate for the additive-dominance model for most of the traits with the exception of shoot length and Na content under 5 and 10 g L^{-1}, respectively, which would indicate that the

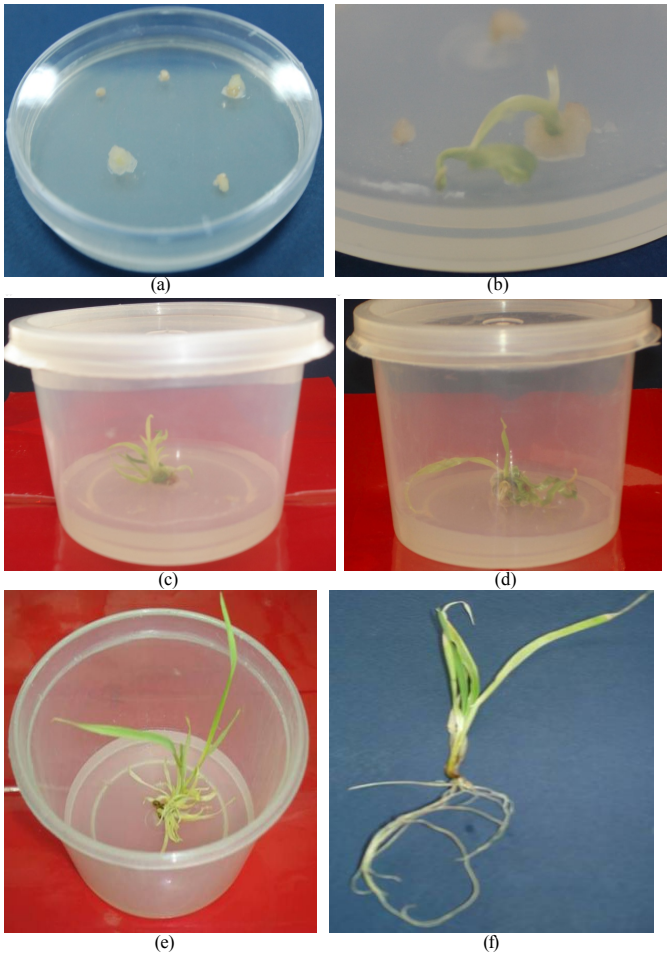

Figure 1. *In vitro* tissue culture mature embryos photography: (a) callus induction at 4 weeks and (b) shoot production from callus at 6 weeks (c) and (d) shoot regeneration after 6 weeks; (e) complete shoot regeneration after 8 weeks; (f) root regeneration after 10 weeks.

hypothesis was not upheld. However, as pointed out by Hayman[32] even if a trait exhibits a partial failure of assumption, analysis could be carried out for such traits.

The estimates of genetic components of variation (Tables 4-6) showed that both additive (D) and dominance (H1) were significant and highly significant for Na content under all treatments and root length under control and 5 g L^{-1} NaCl, which indicated the importance of both additive and dominance effects in the inheritance of these traits under this condition. However, the proline content under all treatments, K content under control and 5 g L^{-1} NaCl, callus fresh weight under 5 g L^{-1} NaCl, and root length under 10 g L^{-1} NaCl were controlled mainly by a dominance gene effect. On the other hand, shoot length under all treatment and callus fresh weight under control treatment were controlled mainly by an additive gene effect, thus the selection for these traits can be done in early generations selection.

The estimates of average value of dominance in loci having unequal positive and negative allelic frequencies (H2) were insignificant for most of the traits except root length under 5 g L^{-1} NaCl, proline content under control and 5 g L^{-1}, callus fresh weight under 5 g L^{-1} and sodium content under all treatments, this indicates the presence of dominance with asymmetrical gene distribution in the parents for these traits.

Dominance and significant value of (h^2) was appeared for root

Table 2. Analysis of variances and tested of validity for some growth traits in F_1 diallel crosses for the five parents under control and three salt concentration.

Source of variation	df	Treatments (NaCl)								
		0 g L^{-1}(Control)			5 g L^{-1}			10 g L^{-1}		
		Callus fresh weight	Shoot length	Root length	Callus fresh weight	Shoot length	Root length	Callus fresh weight	Shoot length	Root length
Replication	2	0.0029	3.274	1.344	0.0002	3.278	0.604	0.003	0.979	0.125
Genotypes	14	0.0076	15.29**	29.61**	0.0017**	15.484*	8.873**	0.0010**	16.064**	8.567**
Error	28	0.0046	3.595	10.021	0.0002	5.671	2.013	0.0001	2.659	3.994
t^2		2.66	0.712	0.007	0.183	31.27**	0.019	0.009	0.302	0.096
bWr/Vr		0.73 ± 0.13	0.41 ± 0.31	0.85 ± 0.25	1.02 ± 0.21	1.6 ± 0.159	0.59 ± 0.41	0.87 ± 0.24	0.95 ± 0.39	0.51 ± 0.40
H_0:b = 0		5.545*	1.302	3.434*	4.652	10.22**	1.428	3.503*	2.384	1.294
H_0:b = 1		2.124	1.867	0.571	-0.097	-3.970	0.959	0.5002	0.125	1.199

Table 3. Analysis of variances and tests of validity for some biochemical traits in F_1 diallel crosses for the five parents under control and three salt concentration.

Source of variation	df	Treatments (NaCl)								
		0 g L^{-1}(Control)			5 g L^{-1}			10 g L^{-1}		
		Proline	Na	K	Proline	Na	K	Proline	Na	K
Replication	2	0.0063	0.174	0.3203	0.0183	0.3148	0.3882*	0.1959	0.935	0.0908
Genotypes	14	5.882**	6.550**	1.046**	40.944	25.131**	1.869**	78.384**	37.563**	1.998
Error	28	0.036	0.460	0.151	0.075	0.387	0.092	0.112	0.252	0.077
t^2		0.093	0.039	0.050	2.673	3.503	0.101	1.422	19.032**	1.234
bWr/Vr		0.17 ± 0.47	0.88 ± 0.34	0.13 ± 0.65	0.03 ± 0.24	0.60 ± 0.15	0.19 ± 0.47	0.15 ± 0.29	0.43 ± 0.09	0.33 ± 0.28
H_0:b = 0		-0.379	2.545	0.199	0.145	4.009**	-0.411	0.516	4.8327*	1.173
H_0:b = 1		0.019	0.342	1.334	3.874*	2.613	2.536	2.841	6.2667	2.339

Where : * and ** significant at 0.05 and 0.01 level, respectively.

Table 4. The components of variation and their relative proportions for shoot length and root length under different treatments of NaCl in F_1 diallel crosses (± = s.e.).

Components of variation and parameters	Treatments (NaCl)					
	0 g L^{-1}(Control)		5 g L^{-1}		10 g L^{-1}	
	Shoot length	Root length	Shoot length	Root length	Shoot length	Root length
D ± SE (D)	5.16 ± 1.16*	14.14 ± 2.76**	11.18 ± 1.12**	3.14 ± 0.64*	8.91 ± 1.27**	1.73 ± 1.003
F ± SE (F)	-2.06 ± 2.89	14.06 ± 6.91	7.91 ± 2.81*	0.369 ± 1.61	3.70 ± 3.17	3.06 ± 2.50
H_1 ± SE (H_1)	4.625 ± 3.13	23.09 ± 7.47*	5.53 ± 3.039	5.17 ± 1.74*	6.96 ± 3.43	11.59 ± 2.70*
H_2 ± SE (H_2)	3.69 ± 2.84	19.13 ± 6.78	4.32 ± 2.75	4.86 ± 1.58*	4.82 ± 3.11	7.26 ± 2.45
h^2 ± SE (h^2)	-0.204 ± 1.9	20.23 ± 4.57*	-0.852 ± 1.86	2.99 ± 1.06	0.805 ± 2.10	0.821 ± 1.658
E ± SE (E)	1.191 ± 0.47	3.35 ±1.13	1.837 ± 0.459*	0.639 ± 0.263	0.849 ± 0.519	0.315 ± 0.409
$(H1/D)^{1/2}$	0.94655	1.2775	0.7033	1.2829	0.8842	2.5879
$H_2/4H_1$	0.1996	0.207	0.1955	0.2348	0.1733	0.1565
KD/KR	0.6517	2.273	3.0253	1.0959	1.6144	2.0387
h^2 (ns)	0.6585	0.199	0.4337	0.454	0.6410	0.4132

Table 5. The components of variation and their relative proportions for number of leaves and proline content under different treatments of NaCl in F_1 diallel crosses (± = s.e.).

Components of variation and parameters	Treatments (NaCl)					
	0 g L^{-1}(Control)		5 g L^{-1}		10 g L^{-1}	
	Callus fresh weight	Proline	Callus fresh weight	Proline	Callus fresh weight	Proline
D ± SE (D)	0.0029 ± 0.0005*	1.24 ± 1.04	0.0005 ± 4.53	5.13 ± 4.14	0.0003 ± 2.56	9.113 ± 6.106
F ± SE (F)	0.0027 ± 0.0013	2.44 ± 2.59	-0.0003 ± 0.0001	-4.06 ± 10.33	-7.86 ± 6.39	-14.99 ± 15.25
H_1 ± SE (H_1)	0.0034 ± 0.0013	10.06 ± 2.81*	0.0005 ± 0.00012*	40.48 ± 11.17*	0.0006 ± 6.91	59.11 ± 16.49*
H_2 ± SE (H_2)	0.0027 ± 0.0012	8.08 ± 2.54*	0.0005 ± 0.00011*	35.037 ± 10.13*	0.0006 ± 6.27	50.37 ± 14.958
h^2 ± SE (h^2)	0.0084 ± 0.0008**	1.48 ± 1.72	0.0019 ± 7.84	38.05 ± 10.13*	0.0014 ± 4.23	109.60 ± 10.09*
E ± SE (E)	0.0015 ± 0.0002**	0.011 ± 0.42	8.69 ± 1.85*	0.024 ± 1.69	4.058 ± 1.045*	0.0389 ± 2.493
$(H1/D)^{1/2}$	1.0813	2.8391	1.030	2.8098	1.5955	2.547
$H_2/4H_1$	0.2005	0.2009	0.2578	0.2163	0.2501	0.2130
KD/KR	2.5028	2.056	0.6244	0.7529	0.831	0.5118
h^2 (ns)	0.1685	0.159	0.6181	0.4545	0.4502	0.5651

Table 6. The components of variation and their relative proportions for sodium and potassium under different treatments of NaCl in F_1 diallel crosses (± = s.e.).

Components of variation and parameters	Treatments (NaCl)					
	0 g L^{-1}(Control)		5 g L^{-1}		10 g L^{-1}	
	Sodium content	Potassium content	Sodium content	Potassium content	Sodium content	Potassium content
D ± SE (D)	3.27 ± 0.55**	0.600 ± 0.148	5.15 ± 1.21*	0588 ± 0.266	9.08 ± 2.13*	0.673 ± 0.175
F ± SE (F)	2.022 ± 1.39	0.73 ± 0.37	-1.76 ± 3.03	0.739 ± 0.664	-6.84 ± 5.325	-0.301 ± 0.44
H_1 ± SE (H_1)	6.04 ± 1.51*	1.18 ± 0.399*	20.25 ± 3.27**	2.37 ± 0.718*	21.89 ± 5.75*	1.03 ± 0.473
H_2 ± SE (H_2)	5.50 ± 1.36*	0.841 ± 0.362	18.18 ± 2.97**	1.90 ± 0.651	18.61 ± 5.22*	0.900 ± 0.423
h^2 ± SE (h^2)	-0.088 ± 0.93	-0.012 ± 0.25	29.45 ± 2.002*	1.64 ± 0.439*	26.20 ± 3.53**	0.0011 ± 0.29
E ± SE (E)	0.147 ± 0.227	0.054 ± 0.061	0.127 ± 0.494	0.037 ± 0.109	0.099 ± 0.871	0.026 ± 0.072
$(H1/D)^{1/2}$	1.359	1.404	1.981	2.008	1.553	1.232
$H_2/4H_1$	0.2276	0.1778	0.2244	0.2005	0.2125	0.2201
KD/KR	1.588	2.514	0.8412	1.912	0.6094	0.6931
h^2 (ns)	0.370	0.289	0.490	0.236	0.669	0.686

D: additive genetic variance; H1 and H2: dominance genetic variance and corrected dominance genetic variance; F: product of additive by dominance; E: expected environmental variance; $(H1/D)^{1/2}$: average of degree dominance; KD/KR: proporation of dominance genes; h^2 (ns): heritability for diallel in a narrow sense.

length and callus fresh weight under control, proline and sodium content under 5 and 10 g L^{-1}NaCl and K content under 5 g L^{-1} NaCl, these results indicating that dominance effect for these traits coincides with heterozygosity and dominance seems to be acting in positive direction.

The mean estimates of co-variance of additive and dominance effect over the array (F) was positive and significant for shoot length under 5 g L^{-1} NaCl, suggesting that dominants were more frequent than recessive among parental genotypes. However, the F values were positive and insignificant for remaining the traits, which indicated the equality of the relative frequencies of dominance and recessive alleles in the parents.

The mean degree of dominance $(H1/D)^{1/2}$ was more than unity for all traits under study except shoot length under 5 and 10 gL^{-1} NaCl and callus fresh weight under control and 5 g L^{-1} NaCl. These clearly suggested that over dominance is controlling the allelic interaction in all loci of dominance gene effects of these traits. The proportion of genes having positive and negative effects ($H_2/4H_1$) which deviated largely from one quarter (0.25) was apparent only for shoot length under all treatments and root length under 10 g L^{-1} NaCl suggesting some sort of asymmetry at loci showing dominance effect for these traits. While the ($H_2/4H_1$) value of the remaining traits was nearly equal to one quarter; this indicated that negative and positive alleles were equally distributed among the parents. Clearly, this result appeared to be in good agreement to previous reports by Yitmaz and Konak [37] and Dornelles *et al.* [38]. In the case of $(H1/D)^{1/2}$ for shoot length under 5 and 10 g L^{-1}, callus fresh weight under control and 5 g L^{-1}, genes acting accumulatively were predominant as $(H1/D)^{1/2}$ was smaller than 1. Therefore, for improvement of these traits showing predominat additive gene effects, early generation selection may be effective. It had similarly been reported that genes acting accumulatively were predominant for callus fresh weight and shoot length when grown under different treatments of sea salt and additive gene action will increase the selection success in a breeding programme[39].

The value $(4DH_1)^{0.5} + F/(4DH_1)^{0.5}$-F which reflects the proportion of dominance and recessive genes in the parents (Dom./Rec.) was less than one for shoot length under control, callus fresh weight, Protein content and Na content under 5 and 10 g L^{-1} and for K content under 10 g L^{-1}. These results show that recessive genes had higher frequencies than dominant gene in the parents for these traits. However, this proportion exceeded one for the rest of the traits, which means that the parents are probably carrying more dominant genes for these traits.

Low narrow sense heritability estimates (h^2 ns) were obtained for root length, callus fresh weight, proline, Na and K content under control treatments. Therefore, improvement of the salt tolerance might be positive by simultaneous exploitation of both additive and dominance genetic components; this could be achieved by adapting biparental mating at F_2. High (h^2 ns) were detected for all the remaining growth and biochemical traits under 5 and 10 g L^{-1} NaCl. Heritability is often used by plant breeders as a measure of the precision of a trial for standardization of their selection units [40]. Akbar [41] stated that high value of h^2 ns shows the important role of additive gene effects in the inheritance of these traits under salt stress. Also, this led to the effective of selection of these traits in early generations under saline conditions.

The graphical presentation of Wr/Vr confirmed the above results on the mean degree of dominance (Fig. 2). The regression line intersects the Wr axis below the origin for shoot length under 5 g L^{-1} NaCl, root length under control and 10 g L^{-1}, callus fresh weight under saline and non-saline treatments and Na content under control and 5 g L^{-1} NaCl, indicating the presence of over-dominance for these traits, while it passed above the origin for the other remaining traits, suggesting the presence of partial or a small degree of dominance for these traits. The parabola were narrow for the proline content under all treatments, K content under control and 5 g L^{-1} NaCl, callus fresh weight under 5 g L^{-1} NaCl and Na content under 10 g L^{-1} NaCl, indicating the preveilence of dominance gene effects in the genetic control of these traits; these results are agreement with the above results (Tables 4- 6). The order of the array along the regression line showed that G.123 had maximum number of dominant genes for most of the traits followed by G.124, Arar and Mari, in contrast, Beecher was located in middle position of the regression lines, suggesting equal to dominant and recessive genes for all traits. These results confirmed the importance of parents G.123 followed by G.124 in breeding programmes for salt tolerant hybrids production, because it had the most dominant resistance genes and they are more stable than other parental genotypes under saline and non-saline treatments.

SDS-PAGE polymorphism of non-water soluble protein in callus: In an attempt to understand the molecular basis of salt tolerance and identify the biodiversity among different genotypes of barley, SDS-PAGE was analyzed to identify water non-soluble protein

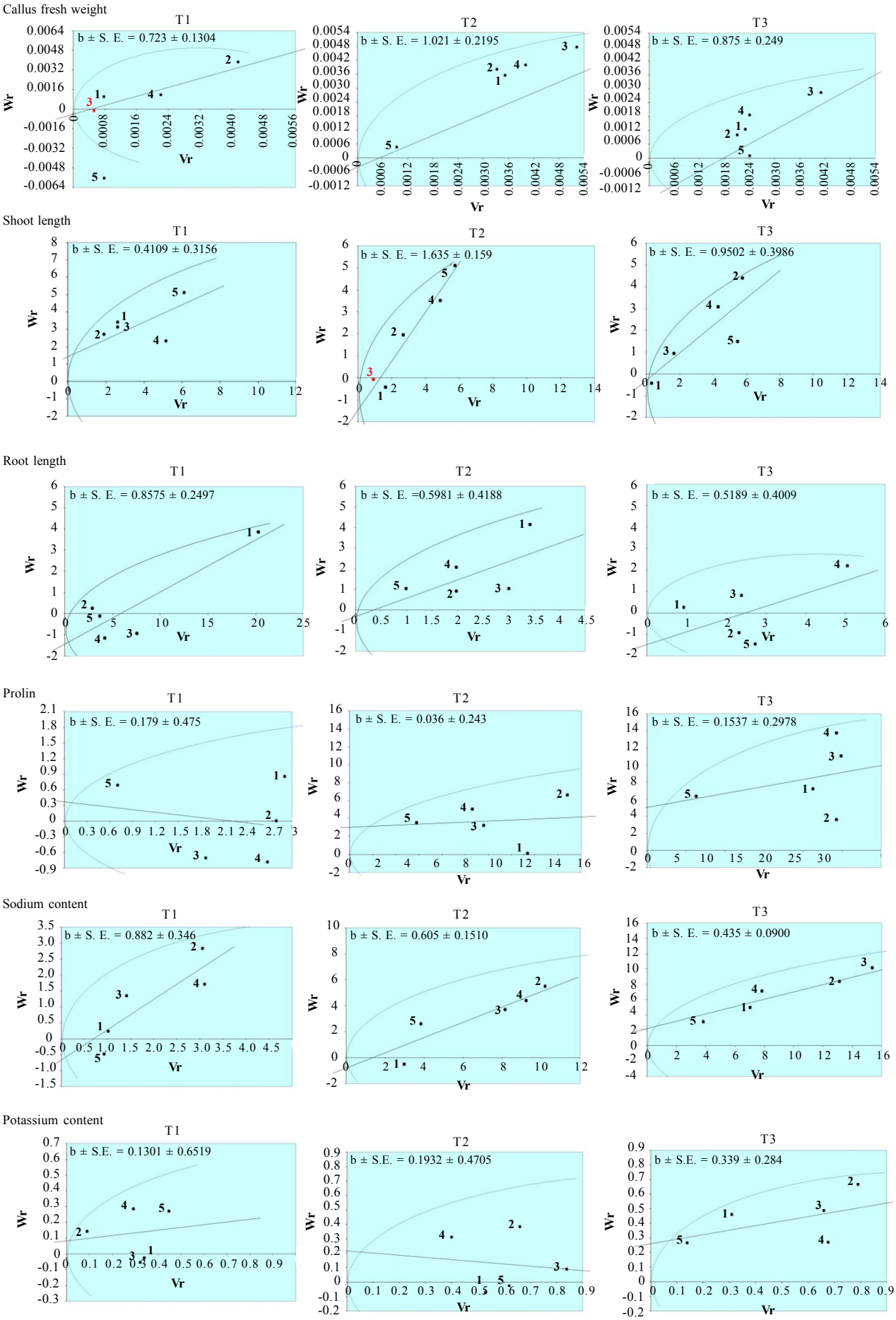

Figure 2. Wr and Vr values and regression line for callus fresh weight, shoot length, root length, proline, sodium and potassium content under T1 control (0 g L^{-1}), T2 (5 g L^{-1} NaCl), T3 (10 g L^{-1} NaCl) of five parents: 1) G123, 2) G124, 3) Arar, 4) Mari and 5) Beecher.

pattern involved in salt stress response in 15 barley genotypes. El-Rabey [42] and Haddad *et al.* [43] indicated that the electrophoresis separation of proteins of barley genotypes can be used as powerful evidence for phylogenic studies.

The protein patterns (Fig. 3) showed that there were a maximum of 36 protein bands which ranged between 124 and 12 kDa and most were exhibited in the parents and their hybrids. Regarding these 36 bands the 5 parents and their hybrids under control and salt stress were characterized by 28 common monomorphic bands at size fragments of (124, 110, 107, 103,100, 96, 94, 88, 84, 76, 74, 60, 56, 41, 40,28, 27, 26, 25, 24, 19, 18, 17, 15, 14, 13, 12 kDa. The sensitive parent Beecher exhibited the same number of bands under control and 10 g L^{-1} NaCl.

The present study demonstrated the absence of a 34 kDa protein in the sensitive genotype (Beecher) under salt treatment when compared with the control. One possible explanation is that the gene responsible to generate this protein was inhibited or not expressed as a result of the stress. This result is supported by previous results [44] which indicated that a 32 kDa protein was salt enhanced in sensitive barley genotypes. On the other hand, a 42 kDa protein occurred only in the 5 and 10 g L^{-1} NaCl treated plants in both tolerant parents (G.123 and G.124), the salt sensitive parent (Beecher) and also in most of hybrids whilst it disappeared in both hybrids Arar/Beecher and Mari/Arar under 5 g L^{-1} NaCl. The appearance of a newly synthesized protein at 42 kDa indicated that the salt stress induced the encoding gene to produce this salt inducible protein. The non-water soluble protein electrophoresis system suggested that the hybrids Arar/G.123 and Arar/G.124 were more adapted to the environmental stress of salinity, and these two hybrids had the greatest number and highest intensity of bands while others hybrids gave lower number of bands. This result confirmed the induction of some salt reactive bands after salt treatment, which may be interpreted as differential expression of some proteins due to the effect of salt [45]. There also appeared to have quantitative differences for non-water soluble proteins under salinity stress compared to the control. All

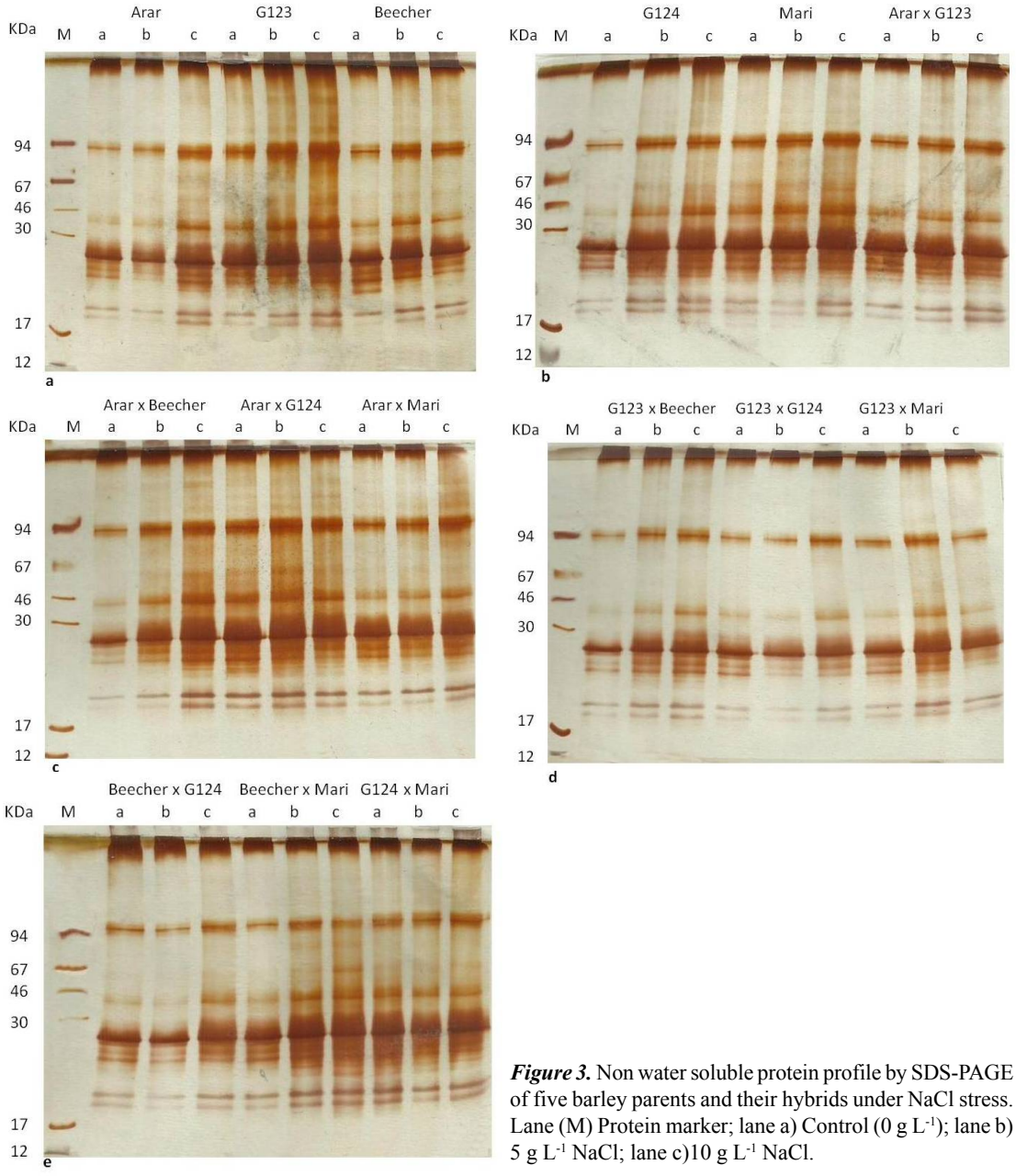

Figure 3. Non water soluble protein profile by SDS-PAGE of five barley parents and their hybrids under NaCl stress. Lane (M) Protein marker; lane a) Control (0 g L^{-1}); lane b) 5 g L^{-1} NaCl; lane c)10 g L^{-1} NaCl.

genotypes exhibited higher intensity in the appearance of many bands under salt stress whereas they were faint in the control treatment, this difference in band intensity is related to protein concentration and suggests differential gene expression patterns. William [46] pointed out that salt stress caused profound alterations in cellular metabolism such as protein function and variation in the synthesis of protein.

In order to identify the genetic relationship among the barley genotypes based on non-water soluble proteins, a hierarchical cluster method was applied. The barley genotypes and their hybrids genotypes were distributed in two main clusters (Fig. 4). Cluster number 1 consisting of the 5 parental genotypes and most of the hybrids, while cluster number 2 consisted of three hybrids only Beecher x G124, Beecher x Mari and G124 x Mari. It is interesting to note that the two salt tolerant genotypes G.123 and G.124 were grouped in a single sub-cluster which may be due to similarity in their genetic structure and common selection history. Also, most of the hybrids, G.13 x Arar, G.124 x Arar, G.123 x G.124 produced by salt tolerant parents were also grouped together under this sub-group. This classification was in agreement with the previous studies of the same genotypes but based on RAPD-PCR [12]. This result proved that SDS-PAGE of barley proteins was a good tool for testing genetic diversity and may be used as a substitute for RAPD-PCR, but more tests must be carried out to fully confirm this.

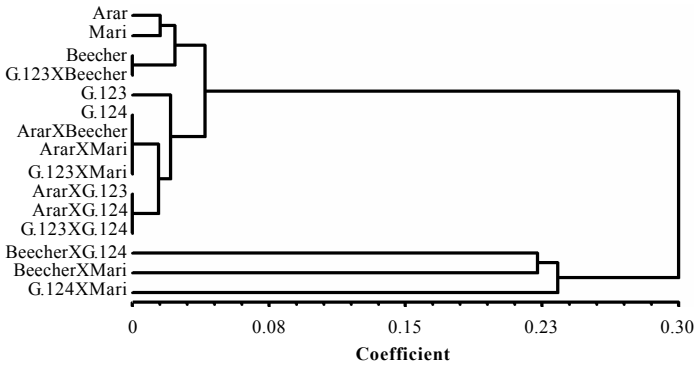

Figure 4. A UPGMA cluster dendrogram showing the genetic relationship among 10 barley hybrids and their parents based on similarity coefficient (Nei and Li [47]) using the presence and absence of polypeptide bands detected by SDS-PAGE of non-water soluble protein under control and salinity treatments.

Conclusions

Considering data obtained on callus fresh weigh, shoot length, root length, proline, Na and K content as growth and biochemical index of salinity stress tolerance, it is clear that additive effects formed the major part of variability for these traits under salt treatments and revealed the importance to breeding programs for improving these characters under stress conditions. Breeding programmes for salt tolerant hybrid production are recommended to use G.123 and G.124 as parental genotypes in the hybridization since they were shown to be stable and have most of the dominant resistance genes. Finally, the results proved that SDS-PAGE for non-water soluble protein was a good method for testing genetic variability between the different genotypes of barley and could be using as alternative marker tool for RAPD-PCR.

Acknowledgements

This work was funded by the Deanship of Scientific Research (DSR), King Abdul-Aziz University, Jeddah, KSA under grant No. (965-003-D1433). The authors, therefore, acknowledge with thanks DSR technical and financial support.

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Abbreviations

2,4-D = 2,4-dichlorophenoxy acetic acid, BA = 6-benzyladenine, IAA = indol-3-acetic acid, NAA = naphthaleneacetic acid, MS = Murashige and Skoog, h^2 = heritability, b = regression coefficient, D =additive gene, H = dominance gene.