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RESEARCH ARTICLE

Genetic Analysis and QTLs Identification of Some Agronomic Traits in Bread wheat (*Triticum aestivum* L.) under Drought Stress Conditions

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ABSTRACT: In order to study the genetic conditions of some agronomic traits in wheat, a cross was made between Gaspard and Kharchia varieties. F_2, F_3 and F_4 progenies with parents were evaluated under drought conditions. Three-parameter model [m d h] considered as the best fit for number of fertile tiller and flag leaf length using generations mean analysis method. For number of grain per spike and main spike grain weight three-parameter model [m d i] was used. For number of spikelet per spike, grain yield and plant height four-parameter model [m d h i] was used. The heritability values ranged from 56% for flag leaf length to 81% for grain yield. The F_3 generation with 100 individuals was used to construct a genetic linkage map. Using the method of composite interval mapping 3, 1, 5, 2, 2 and 1 QTLs were detected for plant height, grain yield, number of spikelet per spike, flag leaf length, main spike grain weight and number of fertile tiller respectively.

KEYWORDS: Bread wheat, generations mean analysis, drought stress, gene effects, Quantitative trait loci

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important nutritious crops in the world. Drought stress is one of the effective factors reducing the crop yield. Information of the type of gene action involved in the inheritance of a character is helpful to decide the breeding procedures for improving the plant characteristics [18]. Plant breeders and geneticists frequently use generation mean analysis to obtain information of gene action controlling the economic traits in wheat [1, 6, 7, 17]. The choice of breeding procedures for genetic improvement of wheat or any other crop is largely depending on the knowledge of type and relative amount of genetic components and the presence of non-allelic interactions for different characteristics in the plant material under investigation [27]. In the study on durum wheat by Fethi and Mohamed [8], dominance effects and dominance × dominance epistatic were found to be more important than additive effects and other epistatic components for grains per spike. Mostafavi et al [26] estimated weight per spike, grain number per spike, grain weight per spike, using generations mean analysis and concluded that all the genes effects, including mean, additive, dominance, epistatic effects, including additive × additive, additive × dominance and dominance × dominance can be effective on the inheritance of traits. Yadava, and coworkers in 1995 [36] in a study on six wheat cultivars and progenies from crosses of these cultivars used generation mean analysis and showed that additive and non-additive

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effects are significant for number of tiller per plant, number of grain per spike, grain weight and yield per plant. Singh et al [32] using generation mean analysis with six-parameter model evaluated heredity traits such as number of grains per spike and grain weight. In most of the crosses additive and additive × additive effects were significant. Dominance and dominance × dominance effects were observed in 33% of crosses but can be exploited because of the existence of epistatic effects. Forozanfar et al [9] determined that four- parameters model [m d h i] is the best fitted for non- fertile tiller. In QTL mapping, two types of data are required: genotype information and phenotype traits values [34]. Identification of associated molecular markers at a major locus contributing to drought tolerance would be useful for the indirect selection of wheat plants for drought tolerance [13]. The application of molecular marker techniques for quantitative trait locus (QTL) analysis has proved to be an effective approach to dissect complex quantitative traits in cereals [10]. Most QTLs for drought tolerance in wheat have been identified through yield and yield components measurements under water-limited conditions [21, 23, 24, 28, 35]. Borner et al [2] reported three major QTLs on chromosomes arms 2DS, 4AL and 6BL, for grain number and grain weight traits. Four major QTLs were detected for plant height on chromosome arms 1AS, 2DS, 4AL and 6AS [2]. Ibrahim et al [12] detected, six QTLs for grain yield on chromosomes 1D, 2D, 3B, 4D, 5B and 7B in the backcross population from the cross Triso × Syn084 with explained genetic variances ranging from 2.1% to 11.4%. One of the purposes of this study was to estimate the gene effects and heritability in a specific bread wheat cross, in order to apply the efficient breeding strategies in wheat breeding. The purpose of the second part of the research was to identify quantitative trait loci (QTL) controlling of evaluated traits under drought conditions.

MATERIALS AND METHODS

Gaspard (sensitive wheat cultivar to drought stress) and Kharchia (tolerant wheat cultivar to drought stress) were used in this study. Five generations, parent cultivars (P_1, P_2), F_2, F_3 and F_4 generations of crossing (Gaspard / Kharchia) were planted during the cropping season (2010-2011) in a Randomized Complete Block Design with two replications at research farm of Institute of Science and High Technology and Environmental Sciences, Graduate University of Advanced Technology, Kerman-Iran (570 N, 300 E, 1755 m above sea level). In each replication, there were two rows for each parent, 13 rows for each F_2, 150 rows for each F_3 and 100 rows for each F_4 generation. 10 seeds were planted in each row of 1m length. The distance between rows was 50 cm. All necessary cares were under consideration during the growth period. Drought stress was applied at the flowering stage by terminating irrigation until the end of growth. The assessed traits were: Grain yield, number of fertile tiller, plant height, flag leaf length, number of grain per spike, main spike grain weight and number of spikelet per spike. The mean values, standard errors and variances of the different generations for all traits were subjected using the joint scaling test [22]. To estimate the mean [m], additive[d], dominance [h], additive × additive [i], additive × dominance [j] and dominance × dominance [l] effects and values and the best model ($y=m+\alpha[d]+\beta[h]+\alpha^2[i]+2\alpha\beta[j]+\beta^2[l]$), for all traits were detected. It is indicated that y, m, d, h, i, j and l represent mean for one generation, mean of all generations, sum of additive effects, sum of dominance effects, sum of additive × additive, sum of additive × dominant and sum of dominant × dominant interactions, respectively. α, β, α^2, $2\alpha\beta$, β^2 are the coefficients for the additive, dominant effects and their interactions in the model, respectively. The best model was chosen with significant parameters and non-significant chi-square. Components within each model were evaluated for significance by t-test. Variance components (additive, dominance and environment) were estimated as described by Mather and Jinks [22] using the following equations:

$$V_{F2} = \frac{1}{2}D + \frac{1}{4}H + E_1$$

$$V_{F3} = \frac{1}{2}D + \frac{1}{16}H + E_2$$

$$\bar{V}_{F3} = \frac{1}{4}D + \frac{1}{8}H + E_1$$

$$E_1 = \frac{M_2}{r} \qquad E_2 = M_{11}$$

V stands for variance and the subscripts refer to generations. E, D and H are environment, additive and dominance effects, respectively.

Broad sense heritability (h^2_b) and narrow sense heritability (h^2_n) were calculated as follow:

$$h^2_{bs} = \frac{V_{F_2} - \sqrt{V_{P_1} \times V_{P_2}}}{V_{F_2}}, h^2_{ns} = \frac{\frac{1}{2}D}{V_{F_2}}$$

F_3 families were used for identification of QTLs of evaluated traits. The Phenotypic data for Grain yield, number of fertile tiller, plant height, flag leaf length, number of grain per spike, main spike grain weight and number of spikelet per spike were collected. DNA was extracted from young leaves of individual F_3 wheats to form bulk, according to the Delaporta DNA extraction protocol [30]. Polymerase chain reaction (PCR) was conducted following the procedure out lined by Roder et al [29]. 32 polymorphic SSR primers, used for genotyping the F_3 population using 100bp ladder. The PCR reaction mixture consisted of 4 µl gDNA in 50 $^{ng}/_{\mu L}$ concentration, 4.5 µl deionized water, 1 µl PCR buffer 10x, 1 µl dNTPs 2.5 mM, 1.5µl $Mgcl_2$ 50 mM ,1 unit Taq DNA polymerase in 5 $^{unit}/_{\mu L}$ concentration and 0.5 µl primer 2 µM. Template DNA was initially denatured at 94°C for 3 min, followed by 38 cycles of 94°C for 1 min, 50-58°C for 1 min and 72°C for 2 min, with a final extension at 72°C for 5 min. PCR products were separated on 8% (w/v) polyacrylamide gels (38:2 acrylamide: bisacrylamide) in TBE 10x buffer (Tris-Base, Acid Boric, EDTA, pH 7.6) at 225 V for 3h. QTLs were mapped by composite interval mapping (CIM) [37], which is a hybrid method, combining interval mapping with standard multiple regression methods[14,15,38]. The LOD score for declaring a QTL was 2.5 for this population. Mapping of the genes as QTLs was performed using Kosambi mapping function [19], with the MAPMANAGER-QTXb20 software.

RESULTS

Mean squares and mean values and their standard errors for the analyzed traits are presented in Table 1 and 2. Estimated results of gene action using generation mean analysis as genetic effects in five parameter model are presented in Table 3. The best fit model for number of fertile tiller and flag leaf length was additive -dominance single model. Three-parameter model [m d i] provided the best fit for number of grain per spike and main spike grain weight. The four parameters model, contain [m], [d], [h] and [i] was the best fit for number of spikelet per spike, grain yield and plant height. Kamboj et al [16] reported that the additive genetic effects were important for grain yield. But Akhtar and Chowdhdry [1] showed that dominance genetic effects were more important than additive gene action for grain yield per plant in wheat. Mehla et al [25] reported that types of epistatic additive × additive and dominance × dominance were important for grain yield per plant in wheat. The additive gene effects (d) were significant and either positive or negative for studied traits, such as grain yield, and main spike grain

Table 1. Mean squares of traits in Gaspard × Kharchyia cross

Source	df	Grain yield	Plant height	Number of fertile tillers	Number of grain per spike	Main spike grain weight	Flag leaf length	number of spikelet per spike
Replication	1	0.791*	19.45 ns	0.202 ns	78.776*	0.0026 ns	0.058 ns	0.786 ns
generation	4	0.333*	25.16*	3.826**	62.401*	0.0264*	4.52*	4.68*
error	4	0.0499	3.57	0.214	9.776	0.0041	0.665	0.685

ns,*, **: Respectively non-significant, significant in the level %5 and %1
df: degree of freedom

Table 2. Mean values and standard errors for different traits in Gaspard × Kharchyia cross

Generation	Grain yield	Plant height	Number of fertile tillers	Number of grain per spike	Main spike grain weight	Flag leaf length	Number of spikelet per spike
P_1	0.87± 0.12	32.17 ± 1.46	9.91 ± 0.78	12.81 ± 1.15	0.12 ± 0.023	10.13 ± 0.91	9.5 ± 0.93
P_2	1.89 ± 0.27	39.2 ± 3.9	15.9 ± 1.48	18.72 ± 1.89	0.45 ± 0.076	14.53 ± 1.24	12.3 ± 0.79
F_2	1.25±0.162	35.61 ± 1.07	13.43± 0.56	21.74 ± 1.74	0.39 ± 0.035	11.97 ± 0.40	14.58 ± 0.32
F_3	1.58 ± 0.08	40 ± 0.498	14.89± 0.25	23.6 ± 0.761	0.57 ± 0.016	14.3 ± 0.175	15.4±0.16
F_4	1.7 ± 0.102	40.61 ± 0.36	14.59± 0.29	25.34 ± 1.041	0.63 ± 0.021	13.5 ± 0.181	16.74 ± 0.16

Table 3. Estimates of genetic components for the traits in Gaspard × Kharchia cross

The traits of genetic components	m	[d]	[h]	[i]	[l]	χ^2
Grain yield	2.53 ± 0.054 **	-0.71 ± 0.177**	-0.3 ± 0.121 ns	-2.46 ± 0.348**	-	0.921
Number of fertile tillers	1.82 ± 0.325 **	0. 32 ± 0.201 ns	-1.21 ± 0.395**	-	-	0.864
Plant height	42.64 ± 0.839 **	3.51 ± 2.085 ns	-6.268 ± 1.65 **	-6.95 ± 2.24 **	-	2.063
Flag leaf length	14.76 ± 0.274**	0.725 ± 0.343 ns	-3.27 ± 1.115**	-	-	1.76
Number of grain per spike	23.92 ± 0.578 **	2.95 ± 1.87 ns	-	-8.15 ± 1.96**	-	3.643
Main spike grain weight	0.469 ± 0.012 **	0.112 ± 0.04*	-	-0.127 ± 0.04**	-	5.812
Number of spikelet per spike	16.16 ± 0.262 **	-0.727 ± 0.614 ns	-1.53 ± 0.491 **	-3.16 ± 0.668 **	-	0.094

ns,*, **: Respectively non-significant, significant in the level %5 and %1

Table 4. Estimates of variance components for evaluated traits

Traits	F2 Generation variance (V_{F_2})	F3 Generation means variance $(V_{\bar{F}3})$	F3 Generation variances mean $(\bar{V}_{F3})$	Additive variance (D)	Dominance variance (H)	Homogeneous entries generations mean Variance (E_1)	Homogeneous entries generations Variance mean (E_2)
Grain yield	1.65	0.206	1.236	12.089	2.356	0.472	1.748
Number of fertile tillers	31.51	1.4	2.311	17.164	36.3	13.359	10.99
plant height	217.195	0.747	42.63	224.232	88.356	104.83	73.979
Number of spikelet per spike	17.62	1.27	0.327	3.29	21.41	6.92	8.37
Flag leaf length	28.565	0.068	3.053	30.822	32.93	13.153	7.26
Number of grain per spike	130.546	50.54	104.081	150.906	84.63	55.092	139.005
Main spike grain weight	0.086	0.019	0.035	0.297	0.276	0.0377	0.0712

weight and were non-significant for other traits. It is suggested that for obtaining further to improvement of these traits be done selection method of their progenies. The dominance gene effects (h) were found to be highly significant for number of fertile tillers, plant height, flag leaf length and number of spikelet per spike. Therefore, suggesting that hybridization method is a useful breeding program for improving these traits. Degree of dominance, in controlling this trait was estimated over dominance (Table 5). Erkul et al [5] determined that additive-dominance model was sufficient to explain genetic variation for number of fertile tiller. In controlling the inheritance of number of spikelet per spike, both additive and dominance effects and also additive × additive epistatic are involved. The additive effect was non-significant. Singh et al [31] reported additive gene action for number of spikelet per spike that does not agree with the obtained results. Erkul et al [5] reported that additive and dominance effects made the major contributions in the inheritance of spikelet per spike and dominance effects were negative and higher than additive effects. For all traits except number of fertile tiller and flag leaf length, additive × additive epistatic effect was significant, indicating the importance of this component in the inheri-

Table 5. Broad-sense (h^2_{bs}), narrow-sense (h^2_{ns}) heritability and degree of dominance for evaluated traits

Traits	Broad-sense heritability (h^2_{bs})	Narrow-sense heritability (h^2_{ns})	Degree of dominance $\frac{h}{d}$
Grain yield	0.81	0.71	0.56
Number of fertile tillers	0.61	0.57	-4.09
Plant height	0.71	0.51	-1.78
Flag leaf length	0.56	0.53	4.51
Number of grain per spike	0.65	0.57	0.37
Main spike grain weight	0.77	0.56	-0.139
Number of spikelet per spike	0.64	0.6	-2.1

Table 6: Position, additive effects and explained phenotypic variance of the QTLs detected on different chromosomes

Traits	QTL	Flanking marker	Chromosome	LOD	Position	Additive effect	R^2
Plant height	Q.PH-7D.a	XGWM350-XGWM44	7D	2.7	146	15.2	0.28
	Q.PH-7D.b	XGWM350-XGWM437	7D	3.53	218	-13.64	0.29
	Q.PH-7D-c	XGWM437-XGWM458	7D	2.69	37.2	-6.02	0.29
Grain yield	Q.YLD-2B.a	XGWM257-XGWM148	2B	3.83	16	-0.72	0.11
Number of spikelet per spike	Q.NSS-2D.a	XGWM102-XGWM539	2D	2.15	60	20.3	0.098
	Q.NSS-2D.b	XGWM102-XGWM539	2D	2.56	62	19.18	0.11
	Q.NSS-2D.c	XGWM102-XGWM539	2D	2.9	64	16.5	0.13
	Q.NSS-2D.d	XGWM102-XGWM539	2D	3.15	66	12.7	0.14
	Q.NSS-2D.e	XGWM102-XGWM539	2D	3.32	68	8.2	0.147
Flag leaf length	Q.FLL-2D.a	XGWM320-XGWM429	2D	2.99	261	0.31	0.14
	Q.FLL-4B.b	XGWM251-XGWM6	4B	2.72	140	2.22	0.25
Main spike grain weight	Q.MSGW-3B.a	XGWM285-XGWM114	3B	2.9	28	-0.167	0.11
	Q.MSGW-4D.b	XGWM194-XGWM609	4D	4.42	36	0.008	0.24
Number of fertile tiller	Q.NFT-2D.a	XGWM261-XGWM484	2D	3.48	28	0.11	0.16

1. Trait abbreviations: plant height (PH); grain yield (YLD); number of spikelet per spike (NSS); flag leaf length (FLL); main spike grain weight (MSGW), number of fertile tiller (NFT).
2. QTL names consist of the qualifier "Q", the trait abbreviation, the chromosomal location and consecutive character to discriminate two or more QTLs per chromosome

tance of studied traits. These results are in agreement with Singh and Singh [33], and Yadava et al [36]. Akhtar and Chowdhry [1] reported the additive and additive × additive gene effects for number of grains per spike. Dominance effect and additive × additive epistatic were more important than additive effects and other epistatic components in most of the traits. The estimates of the different variance components, narrow-sense (h^2_{ns}) and Broad-sense (h^2_{bs}) heritability are presented in Table 4 and 5 respectively. The dominance variance (H) was higher than additive variance (D) for number of fertile tiller and number of spikelet per spike. But the additive variance (D) was higher than dominance variance (H) for the other traits. The highest (81%) and lowest (56%) Broad-sense heritability was obtained for grain yield and flag leaf length respectively. Degree of dominance in some of the characters was more than one, showing the importance of the dominance effect for these traits. Positive degree of dominance for the studied traits had happened in case of parents with higher average and negative degree of dominance had happened in case of parents with smaller average. Thirty-two SSR markers were found to be polymorphic between parents and thus were used for marker analysis. These markers were located on chromosome number 13 in wheat. A total of 14 QTLs were identified on 6 chromosomes 2D, 4D, 7D, 2B, 3B and 4B, for the 6 evaluated traits with the coefficient of determination ranging from 9.8% to 29% of the total

variation. A total of 3 QTLs were found for plant height which were located on 7D chromosome, explaining 28%, 28% and 29% of phenotypic variation respectively. Therefore, assisting to major effect this QTL, the flanking markers can use to marker-assisted selection. For number of spikelet per spike, five QTLs on chromosome 2D were identified, which explains 9.8% to 14.7% of phenotypic variations. The grain yield is determined by one QTL on 2B chromosome in 16cM position. Ibrahim et al [12] using backcross population from the cross Triso × Syn084 under both well-watered and drought-stress, reported seven QTLs. Main effects were associated with grain yield on chromosomes 1D, 2D, 3B, 4D, 5B and 7B. The exotic allele at QYld.T84-2D.a was associated with an increase of grain yield under both conditions. Huang et al [11] in order to study QTLs in agriculture and quality traits on the wheat doubled haploid, detected 50 QTLs out of which 24 QTLs are associated with agriculture and 26 are associated with quality traits among which 3 QTLs are reported for grain yield. Two QTLs were detected for the flag leaf length. These QTLs explained 39% of phenotypic variations. 2 QTLs are determined for the main spike grain weight. These QTLs were detected on one chromosome 3B, of which Q.MSGW-3B.a, Q.MSGW-4D.b were detected putative QTLs with main additive effect and explained 11 and 24% of phenotypic variation. Number of fertile tiller QTL (Q.NFT-2D.a) was detected on the 2D chromosome with a LOD of 3.48.This QTL peaked at Xgwm261 (28 cM) in the Xgwm261-Xgwm484 interval that explained 16% of the phenotypic variation in drought stress. Three QTL for grain weight by Kumar et al [20] was identified in wheat on the chromosome arms 2BS, 1AS and 7AS. Borner et al [2] reported major QTLs for grain number and grain weight in wheat. Elouafi and Nachit [4]in a study of a durum × *Triticum dicoccoides* backcross population reported some QTLs on chromosomes 2B, 2D, 6B, 3A, 5A, 1B, 4D ,6A and 7D for kernel weight in bread wheat.

DISCUSSION

Generation mean analysis of the data revealed additive and non-additive types of gene effects in all traits. Based on the evaluated genetic parameters, selection might be effective for grain yield, plant height, main spike grain weight and flag leaf length. For other traits hybridization method is a useful breeding program to improve these traits. Additive × additive interaction was significant for some traits and is a stabilized genetic component. The highest number of QTLs was detected for number of spikelet per spike. For total studied traits there was positive additive effect, therefore the alleles inherited from Gaspard parent on chromosomes of 2D, 7B and 7D increased all studied traits. Most of the identified QTLs were located on 7B genome that shows the importance of this genome in drought tolerance. Chromosome 7B can be introduced as high density gene rich region, containing QTLs detected mainly under drought stress. In conclusion the results of this research showed that there are some drought tolerant or resistant genes in Gaspard parent (which can be transferred to sensitive genotypes, using marker assisted selection. The selection of the cross, population structure and size, number of measured replications, environment and density of markers may affect the outcome of a QTL analysis [3].

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تجزیه و تحلیل ژنتیکی و شناسایی QTLهای برخی صفات زراعی گندم نان (*Triticum aestivum* L.) تحت شرایط تنش خشکی

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چکیده

به منظور مطالعه وضعیت ژنتیکی برخی صفات زراعی گندم، ارقام گاسپارد و خارچیا به عنوان والدین به همراه نسل های دوم ، سوم و چهارم در شرایط تنش خشکی مورد ارزیابی قرار گرفتند. برای صفات تعداد پنجه های بارور و طول برگ پرچم مدل سه پارامتری مشتمل براثرات افزایشی و غالبیت به عنوان بهترین مدل برازش داده شد. برای صفات تعداد دانه در خوشه و وزن دانه خوشه اصلی مدل سه پارامتری مشتمل بر اثرات افزایشی و افزایشی در افزایشی مشخص گردید. مدل چهار پارامتری مشتمل بر اثرات افزایشی ، اثرات غالبیت و اثرات افزایشی در افزایشی ، برای صفات تعداد سنبلچه در سنبله ، عملکرد دانه وارتفاع بوته برازش داده شد. وراثت پذیری صفات در محدوده ۵۶٪ برای طول برگ پرچم تا۸۱٪ برای عملکرد دانه قرارداشت.نسل سوم با ۱۰۰لاین برای تهیه نقشه ژنتیکی مورد استفاده قرار گرفت.برای صفات ارتفاع بوته ، عملکرد دانه ، تعداد سنبلچه در سنبله ، طول برگ پرچم ،وزن دانه در خوشه اصلی و تعداد پنجه های بارور به ترتیب ۳ ،۱ ، ۵ ، ۲ ، ۲ و۱ QTL تشخیص داده شد.

کلمات کلیدی: گندم نان ، تجزیه میانگین نسل ها ، تنش خشکی ، اثر ژن ، جایگاه کنترل کننده صفت کمی