

## QUANTITATIVE TRAIT LOCI FOR AGRONOMIC CHARACTERS IN A SEGREGATING CHINESE SPRING x *T. SPELTA* WHEAT POPULATION UNDER EGYPT ENVIRONMENT

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### ABSTRACT

*Sixty-six recombinant inbred lines derived from the cross between *Triticum aestivum* c.v. Chinese spring and *Triticum spelta* var duhamelianum developed at Kihara Institute for Biological Research, Japan, were evaluated at Assiut, Egypt for important QTLs for some agronomic traits. Twenty-one QTLs have been detected for heading date, plant height, spike length, number of spikes, spike weight and grain yield/plant. QTLs were distributed on ten chromosomes of 1A, 2B, 2D, 3A, 4A, 5A, 5D, 7A, 7B and 7D. Many of those QTLs were not consistent with those detected at Yokohama, Japan. The QTLs in 2D chromosome were uniquely associated with yield and yield components and offer the greatest potential for marker-assisted selection, in breeding for wheat improvement.*

Key words: *QTL, Hexaploid wheat, Environment*

### INTRODUCTION

Wheat is one of the most important food crops in the world. Most agronomically important traits of wheat are controlled by many genes and are highly affected by the environment. The genetic basis of grain yield and related agronomic traits was first established in 1980s by using molecular markers to construct genetic linkage maps in segregating populations such as F<sub>2</sub>, RILs, double haploid .....etc (Tanksley *et al* 1982, Edwards *et al* 1987, Lander and Botstein 1989 and Stuber *et al* 1987).

The QTL (Quantitative Trait Loci) analysis is the system which can find the association between the agronomic data collected from the experimental field and segregation of the molecular markers in a genetic linkage map. Thus QTL analysis allows a better understanding of complex agronomic traits by identifying and measuring the relative impact of alleles at a mapped chromosomal regions.

Marker Assisted Selection (MAS) is a wonderful application utilizing QTL analysis to increase breeding efficiency (Dudley 1993). Usually, plant breeders give much care about the stability of their improved

lines across different environments. Eberhart and Russell (1966) studied the concept of stability analysis to select environmentally stable lines with high agronomic performance. Recently, plant breeders attempt to select environmentally stable lines as new cultivars released by utilizing MAS. In such cases, it is important to study QTL x environment interaction by comparing QTLs detected in different environments. Selection of environmentally stable QTLs to identify and select certain QTLs for using them in MAS. The stability of lines within a mapping population has been studied statistically by separating and comparing lines based on marker (QTL) genotype (Lin *et al* 1986 and Kang 1993). Tinker *et al* (1996) were able to detect considerable QTLs x environment interaction for seven agronomic traits in two barley crosses. They found that many of the detected QTLs were highly consistent across environments. Yan *et al* (1999) could detect significant QTL x environment interaction associated with common QTLs for plant type traits in a doubled haploid (DH) rice population in two different environments. QTLs for yield and yield components of wheat had been studied in a range of environments including different types of stresses (Quarrie *et al* 2005).

The objective of the present study was to compare the QTLs detected under Egypt environment for some agronomic traits with those previously detected in Japan using same series of RILs population (Ahmed *et al* 2000a, b).

## MATERIALS AND METHODS

### Plant materials

Sixty six recombinant inbred lines (RILs) derived from the cross between *Triticum aestivum* c.v. Chinese spring (CS) and *Triticum spelta* var duhamelianum (Sp) were developed at Kihara Institute for Biological Research, Yokohama City University, Japan. RILs were grown in the experimental farm of the Faculty of Agriculture, Assiut University, Assiut, Egypt in a randomized complete block design (RCBD) with two replications for two successive seasons 2003/2004 and 2004/2005. Genotypes were sown on 25 Nov., and 29 Nov.. in 2003/2004 and 2004/2005, respectively. Plot size was 5 m<sup>2</sup>. All agricultural practices were done according to the recommendations of wheat production in Egypt.

Five plants per replication were used to measure the following traits: heading date (HD), recorded as the number of days from planting date till spikes were fully emerged from 50 % of the plants in a plot, plant height (PH), measured from ground level to the tip of the tallest three tillers (cm),

spike length (SL), length of three tallest spikes of each plant, spikes number (SN), number of spikes were counted for each plant, spike weight (SW), weight of total spikes of each plant (g) and grain yield/plant (GY), the weight of grain harvested (g). Analysis of variance (ANOVA) for each character was carried out and estimates of genotypic and phenotypic variances were computed from the expected mean squares.

### Molecular marker and QTL analysis

Details of the DNA probes used in this study as well as the techniques for DNA extraction, digestion, electrophoresis, plotting and southern hybridization were previously described by Ahmed *et al* (2000a). Linkage analysis of the loci was performed using the computer software, MAPMAKER ver. 2.0 of Macintosh (Lander *et al* 1987). The mapping function of Kosambi (Kosambi 1944) was used to calculate map distance.

### QTL analysis

The QTL analysis was performed as a single marker analysis using Q gene computer software (Nelson 1997) using average mean of the two growing seasons. This program was used to determine the association between individual marker loci and putative QTLs by using individual marker-genotype groups as class variables for the detection of linkage between markers and putative QTLs. Whenever the F-test exceeded a value necessary for probability value less than 0.02, the QTLs were considered to be significant. This significance level was chosen to maximize the identification of putative QTLs. The phenotypic effect was calculated as the difference between the class means of CS and Sp.

## RESULTS AND DISCUSSION

### Phenotypic variation of the RILs under Assiut environment

Significant differences were observed among the recombinant inbred lines for all studied traits (Table 1).

Table 1. Analysis of variance for the studied traits in RILs at Assiut, Egypt.

S.O.V.	df	Mean squares					
		HD	PH	SL	SN	SW	GY
Year	1	10360.4**	9358.8**	91.6**	1162.4	7117.6	1309.5
Rep/year	2	1.51	8.22	0.03	95.6	1591.7	209.4
Lines	67	288.1**	1024.5**	42.9**	1143.1**	7356.4**	3022.4**
Line x year	67	1.98	2.66	0.10	2.87	16.44	7.84
error	134	2.36	27.80	0.69	11.99	125.2	32.2

The abbreviations of characters are the same as those indicated in materials and methods. \*\*, highly significant at the 1 % level.

The grand mean of RILs was lower than the means of both parents for heading date and spike length, and higher for plant height. Means of number of spikes, spike weight and grain yield exceeded CS parent but were lower than Sp parent (Table 2 and Fig. 1).

**Table 2. Mean, genotypic, and phenotypic variance of the studied traits in the RIL population.**

Trait	HD	PH	SL	SN	SW	GY
<b>Mean</b>						
RIL	122.2	117.4	11.6	40.3	98.8	41.9
CS	124.9	112.8	13.8	29.7	83.6	31.9
Sp	145.8	99.0	15.4	46.2	110.0	47.9
<b>Variance</b>						
$\delta^2_G$	71.5	255.5	10.7	285.1	1834.9	753.6
$\delta^2_E$	2.4	27.8	0.7	11.9	125.2	32.2
$\delta^2_P$	73.9	283.3	11.4	297.1	1960.2	785.8

The abbreviations of characters are the same as those indicated in materials and methods.

The genotypic variance for all the studied traits was higher than that of environmental variance. Pooled phenotypic correlations were calculated to identify associations among all studied traits. As expected, grain yield had high positive correlation with number of spikes and spike weight but negative correlation with heading date. Hence earlier headed plants tended to have higher grain yield and yield components such as number of spikes and spike weight. Plant height and spike length showed low positive correlations with grain yield. The high correlations of grain yield, number of spikes and spike weight indicate either pleiotropy of common controlling genes or linkage of genes controlling these traits (Table 3).

**Table 3. Correlation coefficient among studied traits in the RIL population.**

Trait	HD	PH	SL	SN	SW
PH	-0.208				
SL	0.174	0.424**			
SN	-0.045	0.104	0.095		
SW	-0.127	0.150	0.064	0.903**	
GY	-0.222	0.094	0.026	0.729**	0.865**

The abbreviations of characters are the same as those indicated in materials and methods.

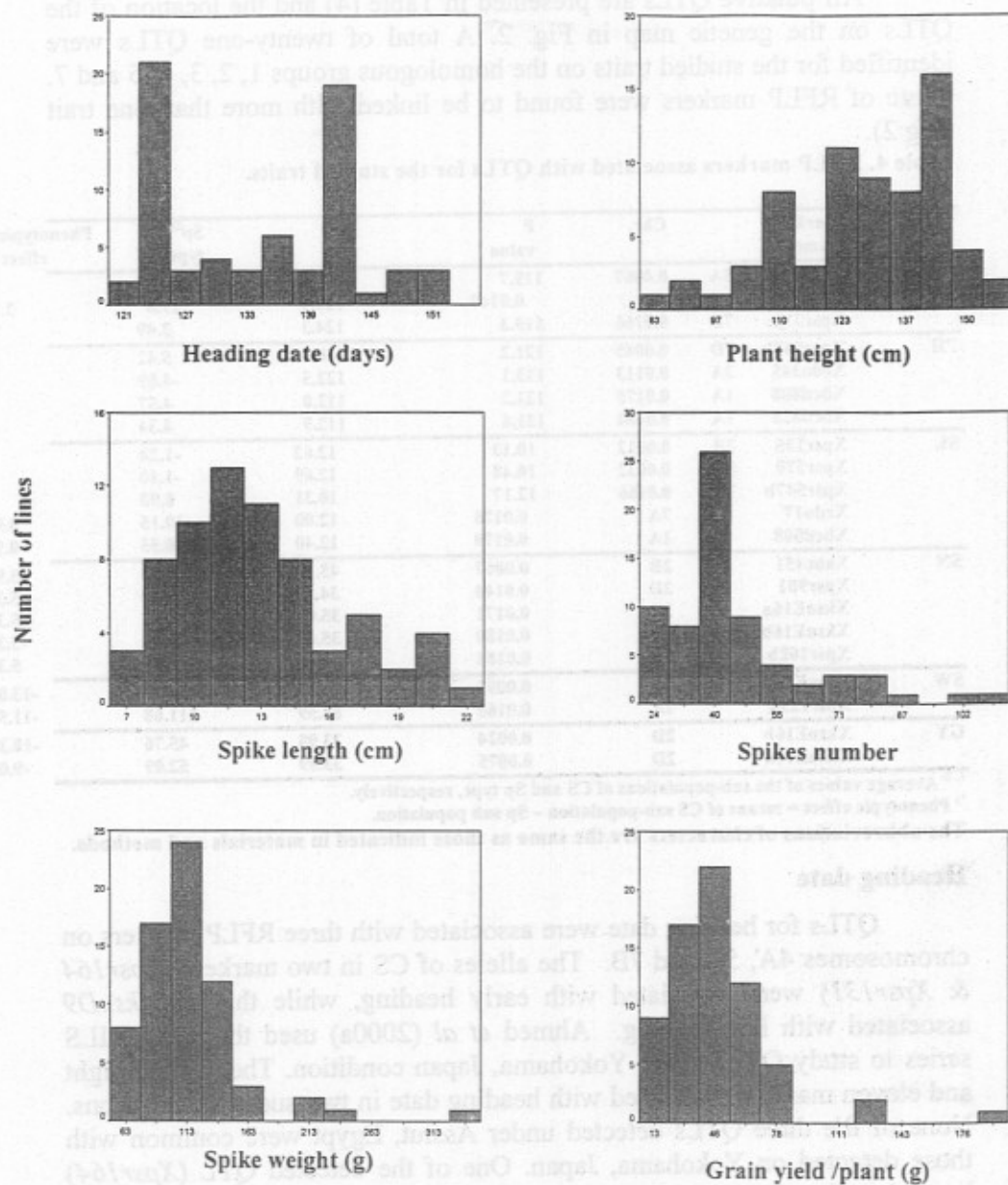


Fig. 1: Distribution of studied traits in recombinant inbred lines.

## Quantitative trait loci detection

All putative QTLs are presented in Table (4) and the location of the QTLs on the genetic map in Fig. 2. A total of twenty-one QTLs were identified for the studied traits on the homologous groups 1, 2, 3, 4, 5 and 7. Some of RFLP markers were found to be linked with more than one trait (Fig 2).

Table 4. RFLP markers associated with QTLs for the studied traits.

Trait	Marker Name	Chr.	P value	CS <sup>1)</sup> type	Sp <sup>2)</sup> type	Phenotypic effect
HD	Xpsr164	5A	0.0067	119.7	125.2	-2.72
	XksuD9	4A	0.0140	124.9	119.8	2.2
	Xpsr311	7B	0.0166	119.3	124.3	2.49
PH	Xpsr547b	7D	0.0045	121.2	110.4	5.42
	Xedo345	3A	0.0113	113.1	122.5	-4.69
	Xbcd808	1A	0.0175	121.2	112.0	4.57
	Xbcd828	1A	0.0184	121.6	112.9	4.34
SL	Xpsr135	2B	0.0012	10.13	12.62	-1.24
	Xpsr370	5D	0.0032	10.48	12.69	-1.10
	Xpsr547b	7D	0.0166	12.17	10.31	0.93
	Xedo17	7A	0.0178	12.00	10.15	0.9
	Xbcd808	1A	0.0179	12.40	10.55	0.9
SN	Xabc451	2B	0.0067	45.94	31.13	5.9
	Xpsr901	2D	0.0144	34.18	45.90	-5.3
	XksuE16a	2D	0.0171	35.03	45.67	-5.3
	XksuE16b	2D	0.0180	35.69	46.38	-5.3
	Xpsr102b	2B	0.0184	45.64	34.91	5.3
SW	XksuE16b	2D	0.0092	88.72	114.85	-13.0
	Xpsr129a	7A	0.0168	88.59	111.68	-11.5
GY	XksuE16b	2D	0.0024	33.98	45.76	-10.3
	XksuE16a	2D	0.0075	33.89	52.09	-9.0

<sup>1,2)</sup> Average values of the sub-populations of CS and Sp type, respectively.

<sup>3)</sup> Phenotypic effect = means of CS sub-population - Sp sub population.

The abbreviations of characters are the same as those indicated in materials and methods.

### Heading date

QTLs for heading date were associated with three RFLP markers on chromosomes 4A, 5A and 7B. The alleles of CS in two markers (*Xpsr164* & *Xpsr131*) were associated with early heading, while that of *XksuD9* associated with late heading. Ahmed *et al* (2000a) used the same RILS series to study QTLs under Yokohama, Japan condition. They found eight and eleven markers associated with heading date in two successive seasons. None of the three QTLs detected under Assiut, Egypt were common with those detected on Yokohama, Japan. One of the detected QTL (*Xpsr164*) located on 5A chromosome was also detected in Yokohama along with three QTLs detected on 5A chromosome (Ahmed *et al* 2000a). The other two QTLs (*XksuD9* & *Xpsr311*) were detected on 4A, and 7B chromosomes.

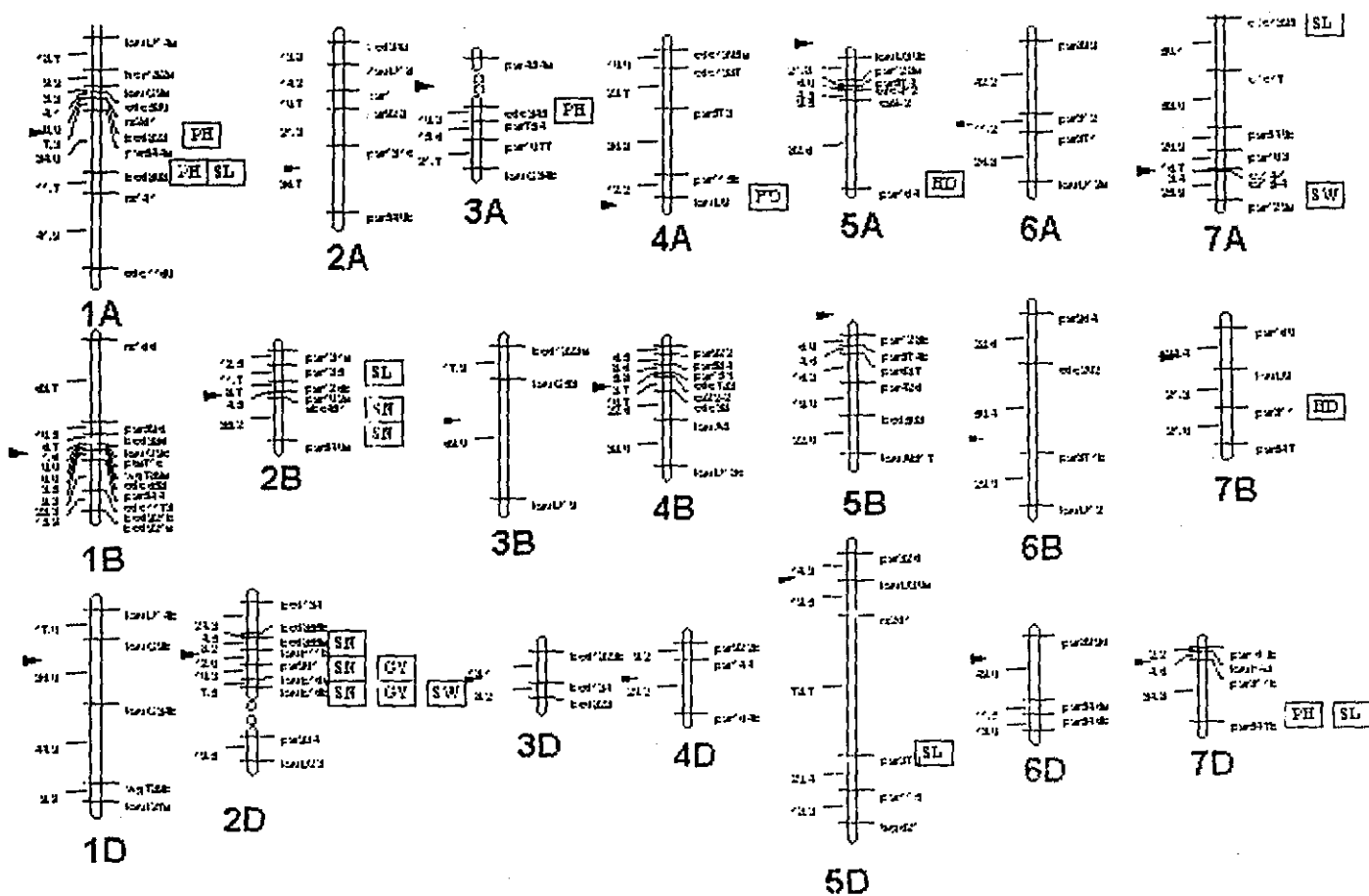


Fig. 2: RFLP linkage map of hexaploid wheat chromosomes concerning QTLs for the studied traits. The abbreviations of the traits are the same as those indicated in materials and methods. The name of markers and their map positions (in cM based on Kosambi function) are shown in the left and the right sides of chromosomes, respectively. The approximate position of the centromeres indicated by arrows, was deduced from published wheat maps.

Heading date is affected by environment, since wheat heading date is considered to be controlled by three major groups of genes: photoperiod response genes, vernalization response genes, and earliness *per se* genes (Bullrich *et al* 2002 and Shindo *et al* 2003). Yokohama and Assiut have large differences in climates. In general RILs tended to head very late under Assiut conditions compared to common local Egyptian wheat. We assume that delayed heading was due to the lack of vernalization requirements as well as to photoperiodic differences.

### **Plant height**

Four QTLs were detected for plant height, one on chromosome 3A, one on chromosome 7D and two on chromosome 1A. Plant height was influenced by seven QTLs under Yokohama, one of which was located on chromosome 1A (Ahmed *et al* 2000b). The CS allele on 1A and 7D chromosomes increased plant height, but the Sp allele on 3A chromosome reduced it (Table 4). The effect of group 1 chromosomes on plant height has been revealed using nullisomic lines (Seares 1954) and RFLP makers (Cadalen *et al* 1998). Keller *et al* (1999) found 11 QTLs affect plant height under three environments, two of which were located on 1A and 7D chromosomes.

### **Quantitative traits for spike and yield traits**

Five QTLs were identified for spike length on 1A, 2B, 5D, 7A and 7D. Those in 1A, 7A and 7D chromosomes showed a positive effect from the CS allele, whereas QTLs on 2B and 2D chromosomes showed a negative effect. Two QTLs (*Xpsr547b* and *Xbcd808*) were common with those detected for plant height. This finding may account for the fact that the plant height showed highly significant positive correlation with spike length (Table 3). Marza *et al* (2005) identified ten QTLs for spike length on 1A, 1B, 2B, 3B, 4B, 5B, 7A and 7B. Three of our QTLs on 1A, 2B and 7A chromosomes were consistent with results previously reported (Sourdille *et al* 2000, Jantasuriyarat *et al* 2004 and Marza *et al* 2005).

Five QTLs were found to affect number of spikes, two of them were located on 2B chromosome and three on 2D chromosome. The two RFLP markers linked with number of spikes on 2B chromosome are in order with 4.5 cM map distance. Therefore it could be one gene in that area controlling number of spikes. Similar finding could be found on 2D chromosome. The three QTLs came in order on the long arm of the 2D chromosome. QTLs for number of spikes were reported to be identified on 1B and 3B chromosomes (Huang *et al* 2004 and Marza *et al* 2005).



Two QTLs were detected for spike weight on 2D and 7A chromosomes, whereas two QTLs could be identified for grain yield/plant. One of them on 2D chromosome (*XksuE16b*) was affecting both of the two traits. The four QTLs detected for both spike weight and grain yield/plant, the Sp allele increased the spike weight and grain yield/plant. The strong association between yield and spike number resulted in three common QTLs, one between spike number and spike weight (*XksuE16b*) and two between spike number and grain yield/plant (*XksuE16a* & *XksuE16b*). Marza *et al.* (2005) detected QTL for spike weight on 2D chromosome.

Grain yield QTLs were mapped in the same positions as those for both number of spikes and spike weight on 2D chromosome, suggesting that number of spikes and spike weight may directly contribute to yield in that genomic region.

Coincidence of QTLs among different traits (plant height and spike length) on 1A and 7D chromosomes, and number of spikes and both of spike weight and grain yield/plant may indicate either single QTL with pleiotropic effect or that the genome regions associated with these QTLs harbor a cluster of linked genes associated with those traits.

In conclusion, we could detect QTLs for some agronomically important traits under Assiut, Egypt, which is different from the environment of origin of the RILs population (Yokohama, Japan). Many of those QTLs were not consistently detected on Yokohama, Japan. Shah *et al.* (1999b) detected QTLs for grain yield in individual environments, but individual QTLs were not consistently detected across environments. The inconsistent QTL detection in wheat was also reported earlier by Araki *et al.* (1999) and Kato *et al.* (2000).

The detected QTLs for the studied traits are distributed over ten chromosomes. The QTLs in 2D chromosome were uniquely associated with yield and yield components and offer the greatest potential for marker-assisted selection, which can be used for wheat improvement in breeding programs.

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## مواقع الصفات الكمية للصفات المحصولية فى عشيرة انعزالية من القمح

### تحت ظروف مصر

طلعت عبد الفتاح أحمد - عادل محمد محمود

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تم زراعة ست و ستين سلالة مشتقة من عشيرة انعزالية ناتجة عن التهجين بين سلالتين من القمح *Chinese spring x T. spelta* تم الحصول عليها من معهد كيهارا للابحاث البيولوجية باليابان وذلك لتقييمهم تحت ظروف أسيوط - مصر، لدراسة المواقع الوراثية لبعض الصفات المحصولية المهمة.

تم التعرف على احدى و عشرين موقعا لصفات كمية (QTL) خاصة بصفات ميعاد التزهير ، طول النبات ، طول السنبله ، عدد السنابل ، وزن السنابل و محصول الحبوب/ نبات. وقد توزعت تلك المواقع على عشرة كروموسومات هي 1A, 2B, 2D, 3A, 4A, 5A, 5D, 7A, 7B و 7D.

أوضحت الدراسة أن كثيرا من تلك المواقع تختلف عن المواقع التى سبق تحديدها تحت ظروف يوكوهاما باليابان .

و تشير الدراسة الى ان مواقع الصفات الكمية على الكروموسوم 2D تعتبر فريدة بالنسبة لمحصول الحبوب و مكوناته مما يعطيها أهمية بالغة لاستخدامها فى الانتخاب عن طريق المسابير الجزيئية *Molecular markers* فى برامج تحسين القمح بمصر.

المجلة المصرية لتربية النبات: ١٠ (١): ٢٧٧-٢٨٨ (٢٠٠٦)