

## Association Mapping for Drought Tolerance and Stem Rust Resistance in a Spring Wheat Panel

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**Abstract:** In an attempt to detect QTL loci conferring drought tolerance and stem rust resistance in wheat, a genome-wide association study was carried out on a panel of 300 spring wheat accessions from different origins genotyped using single nucleotide polymorphism (SNP). The studied lines were phenotyped in Egypt under well- watered and severe drought stressful conditions, while naturally exposed to several stem rust races. Several QTLs and drought tolerance genotypes were detected in this study. Under normal conditions, five SNPs on chromosome 2A, 6B, 6A, 5B and 4A were significantly associated with yield, six significant QTLs for plant height detected on chromosomes 6A and 2B and for stem rust resistance four significant QTLs on chromosomes 2B and 6A were identified. Under drought conditions six QTLs on chromosomes 6B, 5A, 2A, 5B, and 6A that were significantly associated with yield, eleven QTLs affecting plant height were detected on chromosomes 1B, 2B, 6A, , and 4A, while for stem rust resistance three QTLs on chromosomes 1B, 6A, and 4A were detected. QTLs identified in this research may be used for further studies as markers assisted selection in different breeding programs.

**Key words:** Wheat • Stem rust • Drought • Population structure • Association mapping

### INTRODUCTION

Various climatic changes in weather adversely affect cultivated crops; these changes are expected to affect the productivity of the cultivated land. Temperature is one of the main climatic factors which affected crop production, so its increasing or decreasing and its fluctuation from one season to another will lead to reduction in the productivity of some crops. [1]. Rise in temperature levels results in the formation of many droughts areas across the world countries as well as Egypt. Egypt has been expected to face drought problems in its cultivated area, which emphasizes the significance of continuous searching for useful drought tolerant crop species and even different varieties within cultivated plants. Different crops have specific mechanisms to withstand the drought stresses of the environment in arid and semi-arid zones. It is necessary to examine different genotypes and to highlight the most adapted genotypes for further selection and to be used as starting parental materials in breeding programs for drought tolerance [2].

Wheat is a widespread cultivated crop in many countries across the world due to its richness in carbohydrate. This crop suffers from serious fungal disease, such as stem rust disease [3]. Germs carried by air or known as windborne germs are very rapidly spread, especially when they find the appropriate conditions from heat and humidity. Early infection with stem rust, may lead to a large loss of yield, and sometimes completely loss occurred because of the total vegetative injury. Genetic differences between plants are one of the natural means available within the plants from which researchers can choose the best genotypes to overcome the different biotic stresses that face wheat crop. One of the tools that help to detect differences plant performance when faced by different diseases are molecular markers [4].

Drought tolerance traits are complex and controlled by a larger number of genes which are influenced by different environmental factors at different levels that can be temporary or permanent [5]. This kind of traits cannot be quantified in an easy way and their individuals cannot be classified in different groups. The effect of each gene on the drought tolerance has a minor effect and showing

additive and non-additive genetic consequence [6]. Due to its polygenic heritage and the genotype by environment interaction, drought tolerance classically has low heritability [7, 8].

Association mapping (AM) is an innovative way to link differences at the level of phenotypic traits with the differences at the level of DNA sequences and aimed to discover genetic markers linked with genes regulating the phenotypic complex traits such as drought tolerance. Many studies of AM with plants had used a large number of lines and covering the whole plant genome for a significant relationship between a panel of Single Nucleotide Polymorphic (SNP) and a specific phenotype [9]. Linking between phenotypic and genotypic variations can be done generally by following one of the two approaches [9]: (1) using the “biparental” mapping populations that are known as QTL-mapping and (2) using the genetically different lines from the diverse germplasm collections or natural populations that is named linkage disequilibrium mapping (LD mapping) or “association mapping”. Association mapping is currently used effectively with many crop species such as *Triticum aestivum*, *Hordeum* sp., soybean, and corn [10-12].

Genome-wide association study (GWAS) is a good tool for choice based on the success in sequencing technologies and the efficiency of SNP markers which is considered as high-throughput marker tool, it is aimed to explore the whole genome, it distinguishes SNPs and different variations in DNA related with specific traits such as water stress and other biotic and abiotic traits [12-15]. One undesirable approach of AM is that the genetic drift, underlying population stratification due to breeding history, selection, or founder effects can prompt inaccurate associations [16, 17]. This matter, however, can be abridged by accounting for population structure using the distance matrix among the lines or using the relationship matrix [18].

The pathogen of stem rust disease in wheat is considered to have a persistent ability to overcome the wheat resistance; it can migrate and attack different regions [19]. Resistant wheat cultivars have always been the suitable control of this disease worldwide. Historically, stem rust was recorded as the earliest diseases destructively affect the wheat plants ranging from small to large scale-cultivated areas with non-resistant varieties [20]. Response against the pathogen action depends mainly on the R genes represent a recognition zone between the specificity of the race and the host plant or rely alternatively on some minor genes

effects resulting in the resistance in adult plants. To discover genes linked to resistance as a source of durable resistance SNP marker is favorite tool using for the marker-assisted selection (MAS) [4]. This study aimed at identifying potential QTLs linked with drought tolerance and stem rust resistance in wheat, using a collection of 300 wheat genotypes from 107 countries around the world.

## MATERIALS AND METHODS

**Plant Material:** In 2016 a panel of 300 spring wheat genotypes from 107 countries around the world obtained from USDA-ARS were genotyped through the Triticae Coordinated Agriculture Project using the Illumina iSelect 9K wheat array at the USDA-ARS genotyping laboratory in Fargo, ND, USA. This panel was phenotyped at Elkhizan, Behaira, Egypt (31.093288, 30.503189). All the 300 wheat genotypes were arranged in a split-plot arrangement in an augmented randomized complete block design [21]. Water regimes applied in the main plot and genotypes in the subplot. Three check cultivars were used i.e. “Sids 13”, “Gemmiza 9”, and “Giza 168”, the check cultivars were planted in each incomplete block. All the genotypes, including the check cultivars, were planted in plots of four rows and 2.5 m long.

**Studied Traits:** Plant height, stem rust, and grain yield were recorded due to limited resources. Plant height was measured at maturity as the average height in cm from ground to the tip of the spike (awns excluded). All the genetic materials evaluated in the field trials were scored for reaction to stem rusts by visually estimating the percentage of a pustule infected leaf area at milky-wax maturity phases using modified Cobb Scale [22]. Grain yield was measured by harvesting all four rows of each plot then thrashing it after 2 to three days. For irrigation treatments, three irrigations were applied (in addition to seeding irrigation) at 30, 60 and 90 days after sowing (DAS) as a non-stress treatment. One irrigation was applied (in addition to the seeding irrigation) at 60 DAS as stress treatment.

**Marker Data:** All the plant materials used in this study were genotyped using array-scored SNPs on an Illumina Infinium assay (Illumina, San Diego, CA) [23] consisting of 9 K SNPs. Genotype scores were coded as  $x = \{-1, 0, 1\}$ , where -1 represented homozygous for the minor allele, 0 represented heterozygotes, and 1 represented homozygous for the major allele.

**Statistical Analysis:** The analysis of variance and phenotypic performance were estimated using PROC Mixed in SAS (9.2) as following:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + E_{ijk}$$

$\mu$  is a population mean;  $\alpha_i$  is the main effect of the water regimes (A);  $\beta_j$  is the main effect of wheat genotypes (B);  $(\alpha\beta)_{ij}$  is the interaction effect of A and B;  $E_{ijk}$  is the error. SNP calls were made using the Tassel Pipeline (<http://maizegenetics.net>), with modification for non-reference SNP calling by Poland *et al* [24].

The kinship matrix (K) was estimated using R/EMMA [25]. Before performing the association tests, markers with minor allele frequencies (MAF<0.05) will be removed. The adjusted phenotypic means across years and SNP markers will be subjected to association analysis using mixed linear model (MLM) in TASSEL software [26] publicly available at <http://www.maizegenetics.net/tassel>. The association analysis will be carried out by performing a linear mixed model association with restricted maximum likelihood estimates. The mixed model for K method was:

$$Y = \mu + Zu + Wm + e$$

where y is a vector of phenotype observation,  $\mu$  is a vector of intercepts, u is a  $n \times 1$  vector of random polygene background effects, e is a vector of random experimental errors with mean 0 and covariance matrix Var (e), Z is an incidence matrix relating y to u. In our case, since we do not have replication for each genotype, the Z design matrix of the model is the identity matrix with the size of number of observations. We have Var (u)= 2KVg, where K is a known  $n \times n$  matrix of kinship coefficients, Vg is the unknown genetic variance which is a scalar.

## RESULTS AND DISCUSSION

**Phenotypic Analysis of Grain Yield (GY), Plant Height (PH) and Stem rust (SR) Resistance:** Wide differences were found among the studied wheat genotypes for the three studied phenotypic traits (GY, PH and SR) during field evaluations under irrigated and drought conditions. Water deficit reduced full expression of those traits in drought treatments.

Analysis of variance of the studied traits based on augmented design was presented in (Table 1). Results indicated significant effects ( $p < 0.05$ ) for water levels on GY as well as difference among the studied genetic materials. Results of the present study are in concurrence

Table 1: The analysis of variance of the effect of drought and well-watered treatments for plant height (PH), grain yield (GY) and stem rust (SR) of panel of 300 spring wheat genotypes.

S.O.V	DF	Mean Squares		
		GY <sup>1</sup>	PH <sup>2</sup>	SR <sup>3</sup>
Replicates (REP)	1	12.3 <sup>ns</sup>	1.44491	257.2**
Stress	1	757681.5**	28749.5**	181.2**
REP* stress	1	9.0654 <sup>ns</sup>	14.31394 <sup>ns</sup>	72.8**
Line	299	90.0307**	64.2**	6.6**
line*stress	299	16.9 <sup>ns</sup>	13.80951 <sup>ns</sup>	2.98**

ns: non-significant \*\* and \* significant at  $P < 0.01$  and  $P < 0.05$  respectively. <sup>1,2</sup> and <sup>3</sup> referred to grain yield, plant height and stem rust.

with the results of studies [27, 28]. The effect of water stress during the filling stage of the kernels and on harvested grain resulted in the formation of small grain, also reduction in the total number of grains/ spike, grain weight/spike, and 1000- kernel weight was occurred [29].

Plant height of the 300 wheat genotypes showed significant effects ( $p < 0.05$ ) for both of the two water levels and differences among the genotypes under study. The results of the current study are compatible with results of other investigators who are reported that different irrigation treatments significantly influenced plant height [30 - 33]. A study showed that decrease in height of studied barely genotypes in response to drought stress may be due to a decrease in the relative hardness and dehydration of protoplasm, which is related with a loss of turgor pressure and reduced of cell division and expansion [34].

The 300 wheat genotypes used in the present study were showed significant differences in stem rust resistance among them. Similar diverse significant response in stem rust resistance among the different wheat genotypes were presented in pervious study for screening of sources of resistance to stem rust race Ug99 in wheat [35].

The interaction between genotypes and stress conditions were non significant in grain yield and plant height but it was highly significant in stem rust.

**Marker-trait Associations (MTAs):** The plant materials used in this study were genotyped using array-scored SNPs using the Illumina iSelect 9K wheat array at the USDA-ARS genotyping laboratory in Fargo, ND, USA. Markers showed LOD score greater than 3.0 for grain yield, plant height and stem rust under normal growth condition and drought stress conditions were presented in Table (2). A significance threshold level of 10-04 was deemed suitable, considering the deviation of the observed test statistics  $[-\log_{10}(p)]$  values from the

Table 2: The most significant markers for grain yield (GY), plant height (PH) and stem rust (SR) under normal growth condition and drought stress conditions.

Trait	SNP name	Allele	Chr	LOD	P-value	Position	Stress
GY	IWA3992	T/G	2A	7.88	1.318*10 <sup>-8</sup>	142.9	Normal
GY	IWA4825	A/G	6B	6.52	3.03*10 <sup>-7</sup>	50.7	Normal
GY	IWA3247	A/G	6A	4.57	2.69*10 <sup>-5</sup>	212.3	Normal
GY	IWA5079	T/G	5B	4.19	6.45*10 <sup>-5</sup>	49.6	Normal
GY	IWA7395	T/C	4A	4.00	0.0001	51.6	Normal
GY	IWA4825	A/G	6B	5.48	3.31*10 <sup>-6</sup>	50.8	Drought
GY	IWA3990	A/G	5A	5.28	5.24*10 <sup>-6</sup>	112.0	Drought
GY	IWA3992	T/G	2A	5.26	5.49*10 <sup>-6</sup>	142.9	Drought
GY	IWA5079	T/G	5B	5.05	8.91*10 <sup>-6</sup>	49.6	Drought
GY	IWA5086	T/C	5B	5.01	9.77*10 <sup>-6</sup>	59.3	Drought
GY	IWA3247	A/G	6A	4.23	5.89*10 <sup>-5</sup>	212.3	Drought
PH	IWA652	T/C	2B	9.22	6.03*10 <sup>-10</sup>	110.8	Normal
PH	IWA6505	A/G	2B	9.06	8.71*10 <sup>-10</sup>	148.7	Normal
PH	IWA2527	A/G	6A	9.04	9.12*10 <sup>-10</sup>	206.9	Normal
PH	IWA2538	A/G	6A	7.78	1.66*10 <sup>-8</sup>	177.6	Normal
PH	IWA2343	T/C	2B	5.46	3.47*10 <sup>-6</sup>	225.5	Normal
PH	IWA6453	T/C	2B	4.83	1.48*10 <sup>-10</sup>	165.9	Normal
PH	IWA6505	A/G	2B	10.31	2.69*10 <sup>-5</sup>	148.7	Drought
PH	IWA2527	A/G	6A	9.89	4.89*10 <sup>-11</sup>	206.9	Drought
PH	IWA652	T/C	2B	9.54	2.88*10 <sup>-10</sup>	110.8	Drought
PH	IWA2538	A/G	6A	9.26	5.49*10 <sup>-10</sup>	177.6	Drought
PH	IWA2343	T/C	2B	7.03	9.33*10 <sup>-8</sup>	225.5	Drought
PH	IWA131	T/C	1B	5.55	2.82*10 <sup>-6</sup>	30.5	Drought
PH	IWA3856	A/C	6A	5.15	7.079*10 <sup>-6</sup>	9.1	Drought
PH	IWA6453	T/C	2B	5.01	9.77*10 <sup>-6</sup>	165.9	Drought
PH	IWA4402	A/G	1B	4.68	2.089*10 <sup>-5</sup>	38.2	Drought
PH	IWA3443	A/G	1B	4.40	3.98*10 <sup>-5</sup>	22.9	Drought
PH	IWA558	A/G	4A	4.03	9.33*10 <sup>-5</sup>	154.3	Drought
SR	IWA6016	T/C	2B	6.13	7.41*10 <sup>-7</sup>	142.1	Normal
SR	IWA6026	A/C	2B	5.89	1.288*10 <sup>-6</sup>	63.2	Normal
SR	IWA6048	A/G	2B	5.63	2.34*10 <sup>-6</sup>	39.1	Normal
SR	IWA4929	T/C	6A	5.31	4.89*10 <sup>-6</sup>	115.8	Normal
SR	IWA3684	A/G	1B	3.29	5.13*10 <sup>-4</sup>	77.6	Drought
SR	IWA1391	A/G	6A	3.08	8.32*10 <sup>-4</sup>	217.7	Drought
SR	IWA2764	A/G	4A	3.05	2.91*10 <sup>-4</sup>	24.1	Drought

Chr; chromosome, LOD; values are the peak logarithm of odds score for the given QTL and Position of QTL located on chromosome: as cM distance from the top of each map.

expected test statistics values in the Q-Q plots [36]. Significant markers for the traits are shown in Table (2). Manhattan plots of the GWAS results are also shown in Figs. (1 and 2). Q Q plot presenting the most significant QTL linked with grain yield, plant height, and stem rust after correcting for the false discovery rate under normal growth conditions and drought stress is also shown in Figs. (3 and 4). Although several MTA were detected at  $P < 0.05$  for all traits, we are reporting only strong MTA ( $P < 0.001$ ) for normal and drought stress conditions. The p-values were corrected for false discovery rate.

Grain yield MTAs were detected on chromosomes 2A, 6B, 6A, 5B, 5A and 4A under normal and drought stress (Figs 1 and 2). Markers IWA3992, IWA4825, IWA3247, IWA5079 and IWA7395 were associated with grain yield under normal growth condition with LOD score ranged between 4.00 and 7.88 for marker IWA7395 and marker IWA3992 respectively (Table2), while markers

IWA4825, IWA3990, IWA3992, IWA5079, IWA5086 and IWA3247 were found to be the most significant markers associated with grain yield under the stressed condition with LOD score ranged from 4.23 and 5.48 for IWA3247 and IWA4825, respectively (Table 2). A number of studies have revealed QTLs for yield and yield component located on short arm of chromosome 6A in winter wheat as reported by several investigators [37, 38] and in spring wheat chromosome 5B is found to be a region which has multi traits that were significant for yield and yield components [39]. Furthermore, Pinto *et al* [40] reported that the chromosomes 3B and 5B has a robust QTL for yield. Additionally, some SNP markers in chromosomes 3B and 5A were reported by Assanga *et al*. [38] to be linked with grain yield that interestingly had highly consistence across stressed conditions of both heat and drought environments. Sukumaran *et al* [41] detected MTAs in chromosomes 3B, 5A, 5B, and 6A.

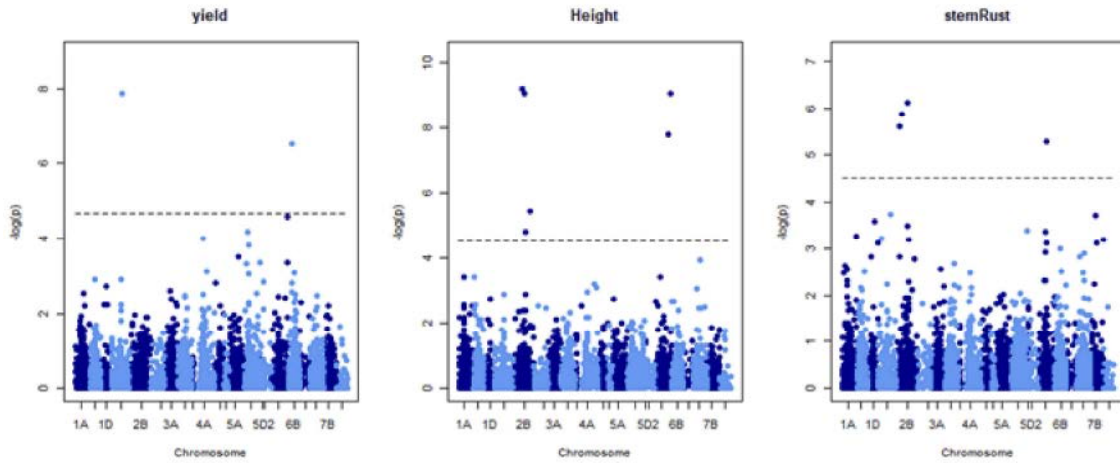


Fig. 1: Manhattan plot presenting the most significant QTL associated with grain yield, plant height and stem rust under normal growth conditions.

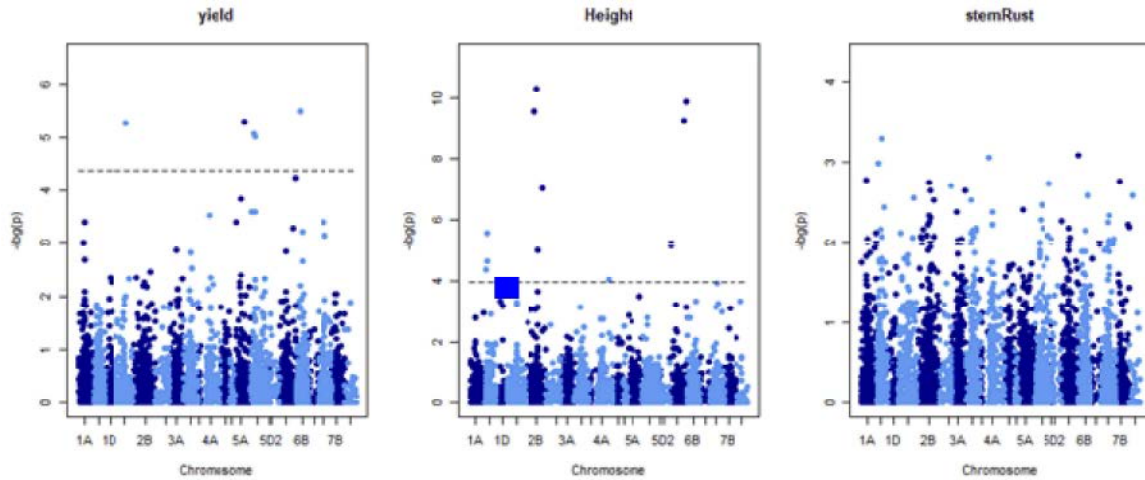


Fig. 2: Manhattan plot presenting the most significant QTL associated with grain yield, plant height and stem rust (drought stress conditions)

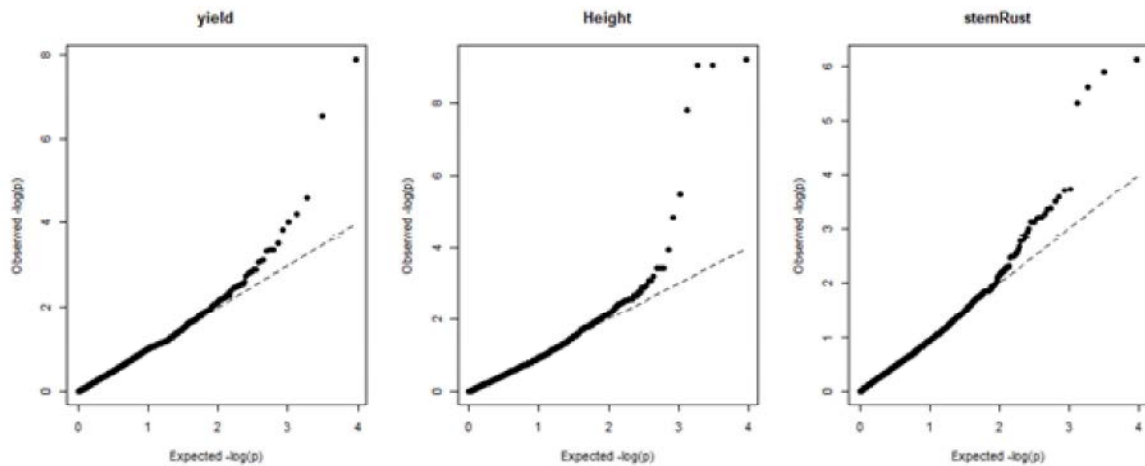


Fig. 3: Q Q plot presenting the most significant QTL linked with grain yield, plant height, and stem rust after correcting for the false discovery rate under normal growth conditions.

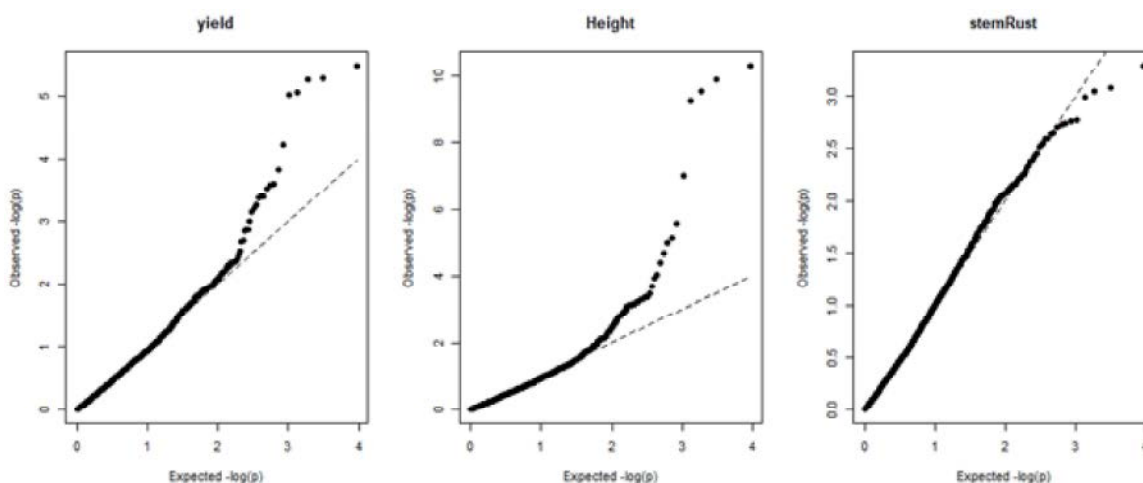


Fig. 4: Q Q plot presenting the most significant QTL linked with grain yield, plant height, and stem rust after correcting for the false discovery rate under drought stress conditions.

Marker- trait associations were found for plant height on chromosomes 2B, 6A, 1B and 4A under normal and drought stress where chromosomes 2B and 6A under normal condition (Figure.1) while, were 2B, 6A, 1B and 4A under drought stress (Figure.2). Markers for plant height under normal growth condition significantly linked with plant height were ranged between 4.83 and 9.22 for marker IWA6453 and IWA652 while under stress condition a high significant linkage between the markers and the trait with LOD score ranged between 4.03 and 10.31 for marker IWA558 and IWA6505, respectively (Table 2). Six QTLs; IWA652, IWA6505, IWA2527, IWA2538, IWA2343 and IWA6453 markers were linked with plant height under normal growth conditions (Table2). This study detected plant height MTA in the regions of previously reported QTL on chromosomes 6A, 1B and 6A and 4A, 2B and 5A [42, 39, 43].

For the stem rust traits under normal growth condition four significantly markers have LOD score ranged from 5.31 and 6.13 for markers IWA4929 and IWA6016 while, three markers are significantly linked with stem rusts resistance (IWA3684, IWA1391 and IWA2764) under drought stress condition (Table 2). These markers were ranged from LOD score of 3.05 and 3.29 for markers IWA2764 and IWA3684, respectively. These included QTL three on chromosomes 2B and one on 6A under normal condition (Figure1) while under drought condition the significant QTL were located on chromosomes 1B, 6A, and 4A (Figure 2). The obtained results are in agreement with the results of Letta *et al.* [44]. There are many QTL regions, which are significantly associated with resistance to stem rust at the seedling and adult plant stage in different germplasm, which are

sources of genes that can be used to resist stem rust in wheat [45 - 52]. Progress in genetic engineering has led to the identification of a number of stem- resistant genes (Sr) that have been incorporated into the wheat genome. Some of these, including Sr22, Sr25, Sr27, Sr32, Sr33, Sr35, Sr37, Sr39, Sr40, Sr44, Sr45, Sr46 and a few unnamed genes are still Resistant to Ug99 and its derivatives [53]. Adult plant resistance genes with minor but additive effects on stem rust and leaf rust are common in wheat germplasm [35, 54]. The use of multiple adult plant resistance genes fruition high levels of resistance to stem rust as achieved in leaf rust and stripe rust resistance [55]. QTL mapping using high- throughput simple sequence repeat (SSR), single nucleotide polymorphism (SNP) or Diversity Arrays Technology (DArT) markers gives the opportunity for genome-wide mapping [52]. Gene Sr58 is adult plant resistance it is mapped distally on 1BL and is independent of the seven QTLs on chromosome 1BS [56]. Based on mapping of significant SNP sequences to the wheat CSS and the published 9K map, Cavanagh *et al.* found [57] that the chromosomal locations of 2BL and 2BS were determined to be on the long arm and short arm of 2B, respectively. Chromosome 2B carries many QTLs that provides resistance to African stem rust races have been reported previously as summarized by the Ug99 resistance loci consensus map including QTL unpublished in CIMMYT [58]. Previous reports of QTL located on 4A that are significantly associated with resistance to African stem rust include the mapping studies by several investigators [48, 49]. There are three specific Sr genes in chromosome 6A resistant to stem rust (Sr26, Sr13, and Sr52) reported by several investigators [59, 60].

## CONCLUSION

In the present study, genome-wide association studies were applied on spring wheat panel to identify SNP markers linked to grain yield, plant height and stem rust resistance under normal and drought stress condition using the 9K SNP chip. Marker - trait association analysis identified 11 SNPs significantly associated with the grain yield (5 SNPs under normal conditions and 6 SNPs under drought stress). They were located on 6 chromosomes 2A, 6B, 6A, 5B, 5A, and 4A. For plant height, 17 SNPs were identified (6 SNPs associated with plant height under normal conditions and 11 SNPs associated with plant height under drought stress) located on 4 chromosomes (2B, 6A, 1B, and 4A). Seven SNPs were significantly associated with the stem rust resistance (4 SNPs under normal conditions and 3 SNPs under drought stress). They were located on 4 chromosomes 2B, 6A, 1B and 4A.

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