DISCUSSION

Wheat (*Triticum aestivum* L.) production is adversely affected by environmental stresses. Among these stresses, drought is the most serious abiotic stress leading to reduced crop yield (Ergen and Budak, 2009; Fleury *et al*., 2010) in 50% of the cultivated area in the developing, and 70% in the developed countries (Trethowan and Pfeiffer, 2000). The global warming has a significant impact on temperature and precipitation profiles, increasing the incidence and severity of drought (Mir *et al*., 2012). The water resources are also expected to decline in the coming decades (Zhao *et al*., 2008). Hence drought tolerant varieties are urgently needed. However, developing high yielding drought tolerant varieties is not easy due to a number of factors such as, i) the complex and quantitative nature of the trait with complicated environmental interactions (Budak *et al*., 2013), ii) its mode, timing, and severity of the dehydration stress and occurrence with other abiotic and biotic stress factors are significant (Reynolds, 2006), iii) field evaluation of the traits and genotype × environmental interaction (GEI) that play crucial role for screening tolerant varieties (Trethowan and Pfeiffer, 2000).

Therefore, development of varieties harboring quantitative trait loci (QTLs) from better and effective sources of drought tolerance is very crucial and a viable option to effectively handle this important abiotic stress. In order to accelerate the procedure of development of new varieties, new molecular marker approaches could be more beneficial because traits like drought tolerance are difficult to handle during field evaluation. The robust QTLs can be utilized through marker assisted selection (MAS) for the development of drought tolerant varieties.

In view of above facts, the present investigation was undertaken with following two important objectives:

1. Development of RIL population for drought tolerance.
2. Molecular mapping for drought tolerance in spring wheat.
5.1 Development of RIL population for drought tolerance

A 191 RILs derived from a cross K65 × HUW468. The variety K65 is drought tolerant while HUW 468 was released for irrigated environments. Mapping population were maintained and advanced from F3 to F6 during the year 2008 to 2010 using off-season nursery at IARI, regional station, Wellington F7 and F8 generations were utilized for the phenotyping in the year 2010-11 and 2011-12 at two locations; Rajiv Ghandi South Campus, Mirzapur and Main Campus, BHU, Varanasi. The F8 generation was used for genotyping.

5.2 Molecular mapping for drought tolerance in the cross K65 × HUW468

5.2.1 Phenotypic analysis of RILs

Phenotypic analysis was performed on 191 RILs in F7 and F8 generations during two consecutive years 2010-11 and 2011-12 at Agriculture Research Farm BHU (Varanasi) and RGSC, Barkachcha, Mirzapur. The mean value over the years of all 12 traits showed quantitative inheritance as indicated by their continuous distribution pattern across the population. Some lines showed transgressive segregation in both the directions suggesting that the two parents were diverse for the alleles of the genes contributing to drought tolerance. In the other procedure based on DSI, same results were obtained for all the traits. The findings of phenotypic analysis were similar to the earlier studies (Dasti et al., 2007, Maccaferri et al., 2011; Mysza et al., 2011; Lopes et al., 2013; Zhang et al., 2014).

5.2.2 Analysis of variation and correlation of traits of RILs

The analysis of variance for all 12 traits showed that significant variation was observed for genotype, environment, replication and genotype × environment. The significance of genotype × environment interaction suggested that the performance of genotypes was influenced by the environment. Similar results were reported by Peleg et al., (2009) and Lopes et al., (2013) for drought tolerance. The results showed that each RIL was genetically different. Similar significant results based on DSI of the
traits were observed for genotypes as well as environment component of variation in the present as well as previous studies (Dasti et al., 2007; Kirigwi et al., 2007; Wu et al., 2014).

Phenotypic correlation was estimated among 12 traits and yield showed significant correlation with all the traits. Highly significant positive correlation was observed between BM and GY suggesting that there was tight linkage between these two traits and was consistent with the earlier results (Mathews et al., 2008; Wu et al., 2014, Zhang et al., 2014). Moderate to low positive significant association were measured for PH, TGW, DM, Chlrg, SL, DH and GFD with GY respectively. Similar findings were reported by Peleg et al. (2009). This indicated that selection for higher grain yield is possible using these traits. Significant but negative correlations were also measured for CTvg, chlvg and CTrg with GY respectively indicating that their lower values will favour grain yield. For DSI, significant and positive correlation observed between DSIGY and DSIPH and DSIGFD indicated their possible role in selecting for grain yield under water stress. However, negative but significant correlation were also observed for DSIDH with DSIClrg, DSIGFD and DSIBM. This indicated that days to heading can be used to manipulate the grain fill duration and other traits.

5.2.3 QTL mapping for drought tolerance in K65 × HUW468

The earlier studies on drought tolerance indicate that wheat is exposed to drought stress during various developmental growth stages of life cycle. Current genetic studies and efforts facilitating the understanding the mechanism that improve drought tolerance in wheat using improved screening techniques or traditional plant breeding and, molecular and genomic approaches, also be conclude that drought tolerance traits operate through multiple genes/QTLs and are polygenic in nature. At gene level, plants tolerate water stress by expression of different sets of gene/QTLs at different developmental stages. For breeding drought tolerant varieties, diverse genetic stocks with different degrees of expression for drought tolerance, understanding of genetics, development and deployment of QTLs for drought tolerance traits have been suggested to be used. Progresses in new biotechnological
tools such as next generation sequencing technologies can also play important role for the enhancement of drought stress tolerance in crop plants.

Recently, the advent of next generation sequencing (NGS) has revolutionized genomic approaches; including new tools which are valuable for the discovering, sequencing, genotyping; not hundreds but thousands of markers across almost any genome of interest in a single step (Luikart et al., 2003) or even in the populations in which little genetic information is available (Davey et al., 2011). In comparison of low throughput genotyping approaches (RFLPs, RAPDs, AFLPs and SSRs), genotyping by sequencing (GBS) is recently developed high throughput NGS platform (Elshire et al., 2011; Poland et al., 2012a). This tool is a simple, robust, rapid, flexible, inexpensive and less laborious. It is sequenced based genotyping approach which utilizes enzyme based complexity reduction coupled with DNA bar-coded adopters for the production of multiplex libraries of a samples capable of producing tens of thousands to hundreds of thousands of molecular markers across genomes of interest. The GBS has removed the bottlenecks of traditional genotyping approaches where two steps process marker discovery followed by assay design and genotyping are involved.

GBS SNPs marker has become increasingly popular in plant breeding due to their abundance and dynamic nature. GBS SNPs has been used in a variety of applications in crop plants including; saturating an existing genetic map (Spindel et al., 2013), genomic characterization in wheat and barley (Poland et al., 2012), genetic ordering of a drift genome sequence in barley (Mayer et al., 2012; Mascher et al., 2013), characterization of germplasm diversity in maize and switchgrass (Romay et al., 2013; Lu et al., 2013) and basic and applied genomic studies in oat (Huang et al., 2014). In bread wheat, main advantage of GBS SNPs over molecular markers like RFLPs, RAPDs (Roder et al., 1998; Gupta and Varshney, 2000), SSRs and other class of molecular markers has been the detection of adequate genetic polymorphism where it is low naturally for traits of interest.

Considering all above facts and benefits in the mind, GBS SNP markers were exploited to detect the QTLs for drought tolerance in the RILs of the cross K65 ×
HUW468. GBS was employed for the exploitation of SNP markers throughout the whole genome in the RILs along with their parents. Overall 37186 bi-allelic SNPs were identified from sequence tags covering whole wheat genome. They were passed to other filtering steps and subsequently processed right through the mapping population for missing data (<20%) of individual SNP in each RIL as done by Poland et al., (2012a). Only 8880 GBS SNPs passed filtering step and these markers were exploited to detect the polymorphism between resistant (K65) and susceptible (HUW468) parents for drought tolerance. Among filtered 8880 SNPs markers, 3343 GBS SNPs displayed polymorphism for both the parents. The rate of polymorphism detected between the parents (37.67%) was consistent with the results of Kumar et al., (2009), Tiwari et al., (2013) and Srinivasa et al., (2014). All these marker loci segregated in the expected ratio 1:1 (P= 0.05) with few exceptions. The marker loci were randomly distributed right through the genome and used normally for the map construction since segregated marker loci do not affect the linkage map (McDanie et al., 2007).

The B genome was most polymorphic (50.88%) as compared to A and D genome with polymorphism of 33.71% and 15.41%, respectively. Similar reports were observed by Kumar et al., (2009), Paliwal et al., (2012) and Srinivasa et al., (2014). The D genome is less saturated with SNP markers and considered as least polymorphic compared to SSR markers in wheat (Ganal and Roder, 2007). Among the linkage groups, linkage group 2 (2A, 2B and 2D) was highly polymorphic and highest number of SNP markers (652) were mapped to this linkage group. Among all the chromosomes, 5B was highly saturated by SNP markers (383) mapped. Although, only 37.67% SNP markers were polymorphic, the gaps of certain region with the genomes were not covered. However, it is unlikely that some major QTLs remain undetected since the detected QTLs explained phenotypic variance (PVE or R²) ranging from 2.50% to 11.98% based on the DSI across the two environments.

For DSI of different traits, four QTLs were identified for DSIDH on the chromosomes 3D (1 QTL), 5B (1 QTL) and 6B (2 QTLs). Out of four QTLs, the susceptible parent HUW468 contributed the positive alleles for QDtdsidh.bhu-3D,
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QDtdsidh.bhu-6B.1 and QDtdsidh.bhu-6B.2, while positive alleles for QDtdsidh.bhu-5B were inherited from tolerant parent K65. The QTLs detected in chromosome 3D, 5B and 6B were in the close agreement with previous studies (Moustafa et al., 2014; Peleng et al., 2009; Mason et al., 2013; Lopes et al., 2014).

Three QTLs detected for DSICTvg, were located on the chromosomes 5A and 7A for enhanced drought tolerance and were derived from the tolerant parent K65 except the QTL located on chromosome 5A that was derived from the susceptible parent HUW468. The location of three of the two loci coincides with the previously mapped ones, QDtdsictvg.bhu-7A.1 (Pinto et al., 2010) and QDtdsictvg.bhu-7A.2 (Mason et al., 2013). The QTL (QDtdsictvg.bhu-5A) mapped on chromosome 5A has not been reported previously and inherited by susceptible parent HUW468 showing that positive alleles were dispersed across the parents; as a consequence transgressive segregation occurred.

With respect to QTLs of DSICTrg, the positive alleles for QDtdsictrg.bhu-1A was inherited from drought tolerant parent K65, while the drought susceptible parent HUW468 was contributed positive alleles for QDtdsictrg.bhu-2A detected on the chromosomes 1A and 2A, respectively; suggesting that both the parents carried few different alleles for this trait. The results of indentified QTLs on chromosomes 1A and 2A is in agreement with the earlier mapped population by Kumar et al., (2012) and Pinto et al., (2010), respectively.

The QTLs for chlorophyll content at two different stages i.e., vegetative and reproductive growth (Chlvg and Chlrg) were mapped on two different chromosomes 5D and 2A respectively but both the QTLs (QDtdsichlvg.bhu-5D and QDtdsichlvg.bhu-2A) inherited positive alleles from drought susceptible parent HUW468. This appear to prove that accumulation of chlorophyll content enhanced when intensity of stress increased and shared a common physiological mechanism for the expression, although different genetic mechanisms operate for the expression of QTLs at different growth stages. The location of these QTLs coincided with other mapped populations for chlorophyll content in drought condition (Zhang et al., 2009; Mysza et al., 2011; Kumar et al., 2012 and Yang et al., 2016).
One QTL was mapped for DSIPH on chromosome 2B. The positive alleles for \(QDtdsiph.bhu-2B\) were contributed from drought tolerant parent K65. This indicated that susceptible parent HUW468 carried narrow spectrum alleles which were not expressed in current population, although drought tolerant parent carried a wide range of alleles for plant height being positively inherited in drought condition. The same location of plant height QTLs were identified in other mapped populations for drought tolerance (Mathews et al., 2008; Maccafferri et al., 2011 and Lopes et al., 2013).

Likewise, one QTL was identified for DSISL on chromosome 3A. The \(QDtdsisl.bhu-3A\) inherited positive alleles from drought susceptible parent HUW468. The logarithm of odds (LOD) value of \(QDtdsisl.bhu-3A\) was 5.05 and explained phenotypic variance (PVE) up to 5.28. There was no reference for evidence in the previously mapped populations for spike length QTL location on chromosome 3A and therefore, \(QDtdsisl.bhu-3A\) filled the gap on the chromosome 3A.

Two QTLs (\(QDtdsidm.bhu-2A.1\) and \(QDtdsidm.bhu-2A.2\)) were mapped for DSIDM on chromosome 2A. The positive alleles for \(QDtdsidm.bhu-2A.1\) were inherited form drought susceptible parent HUW468, although, tolerant parent K65 contributed positive alleles for \(QDtdsidm.bhu-2A.2\), suggesting that both the parents carried few different alleles contributes additive effect for DM as result transgressive segregation occurred in the RILs. The locations of these identified QTLs were coinciding with earlier mapped population by Wang et al., (2009).

One QTL was detected on chromosome 1A for GFD on the basis of DSI. The drought susceptible parent HUW468 contributed positive alleles for \(QDtdsigfd.bhu-1A\). This suggested that susceptible parent also carried those alleles that are well expressed in drought condition and identified loci on chromosome 1A. This is well supported through earlier mapped populations for drought tolerance (Borner et al., 2002; Peleng et al., 2009 and Wang et al., 2009).

One QTL was mapped for DSIBM on chromosome 1D. The positive alleles were contributed for \(QDtdsibm.bhu-1D\) via drought susceptible parent HUW468. The QTL conferring plant biomass (BM) has not been reported earlier under drought
conditions. The D genome of wheat is less saturated by the molecular markers in previous populations studied for drought tolerance. Therefore the finding of \textit{QDtdsibm.bhu-1D} for biomass of wheat assumes significant importance.

Three QTLs were identified for DSIGY on chromosome 3B, 5A and 6B. The positive alleles were inherited for all three QTLs \textit{viz.}, \textit{QDtdsigy.bhu-3B}, \textit{QDtdsigy.bhu-5A} and \textit{QDtdsigy.bhu-6B} via drought susceptible parent HUW468. The QTL located on chromosome 3B has been mapped in earlier studies (Maccaferri \textit{et al.}, 2008 and 2011; Pinto \textit{et al.}, 2010; Golabadi \textit{et al.}, 2011; Mason \textit{et al.}, 2013; Zhang \textit{et al.}, 2014; Moustafa \textit{et al.}, 2014). Another QTL located on chromosome 5A was identified in earlier mapped populations (Quarrie \textit{et al.}, 2005; Mathews \textit{et al.}, 2008; Peleng \textit{et al.}, 2009; Maccaferri \textit{et al.}, 2011; Mysza \textit{et al.}, 2011; Lopes \textit{et al.}, 2013; Zhang \textit{et al.}, 2014; Moustafa \textit{et al.}, 2014). Likewise, the QTL mapped on chromosome 6B has been previously reported by some workers (Quarrie \textit{et al.}, 2005; Mathews \textit{et al.}, 2008; Maccaferri \textit{et al.}, 2011; Mysza \textit{et al.}, 2011; Lopes \textit{et al.}, 2013; Zhang \textit{et al.}, 2014).

Finally, in order to find QTLs for drought tolerance one QTL for DSITGW was detected on chromosome 7D. Positive alleles for \textit{QDtdsitgw.bhu-7D} were inherited from tolerant parent K65 suggesting that more positive alleles were segregated from K65 during recombination and the finding was in the close agreement with earlier mapped population for drought tolerance (Borner \textit{et al.}, 2002; Huang \textit{et al.}, 2003; Roder \textit{et al.}, 2008; Wang \textit{et al.}, 2009; Nezhad \textit{et al.}, 2012).

The D genome in the mapped RIL population is less saturated with molecular markers than the A and B genome, and contains several gaps. Therefore, it is quite possible that minor QTLs and their additive interaction remained undetected on genome D. It appears that a much larger population along with highly saturated map is required for the confirmation of the minor QTLs and their additive interactions associated with trait of interest.