ACHIEVEMENTS AND PROSPECTS OF MOLECULAR BREEDING FOR DROUGHT TOLERANCE IN SOYBEAN [*Glycine max* (L.) MERR.]

Mueen Alam KHAN

[1]Department of Plant Breeding and Genetics, University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur, Bahawalpur, Pakistan

Khan M. A. (2018*): Achievements and prospects of molecular breeding for drought tolerance in soybean [Glycine max (L.) merr.].-* Genetika, Vol 50, No.3, 1095-1109.
Drought stress is one of the most serious constraints of soybean production worldwide. While the drought tolerance is one of the most complex attributes for soybean breeders to deal with. Due to complex (quantitative) nature of drought tolerance trait, the progress to develop drought tolerant plants is slow. Molecular breeding thus emerged as a necessary approach in soybean breeding programs. A plethora of genetic information regarding the functional genomics and other molecular resources is available. The effective and astute use of these resources will certainly facilitate the breeders to develop soybean cultivars tolerant to drought.

Keywords: Drought stress, *Glycine max* L., molecular breeding, soybean.

INTRODUCTION

Cultivated soybean (*Glycine max* L.) is undoubtedly one of the most important legume crops providing a rich source of oil and protein to humans and livestock around the world. Its oil is also considered attractive as a future source of bio-diesel (HILL *et al.,* 2006; PIMENTEL and PATZEK 2008; CANDEIA *et al.,* 2009). With its ability to fix atmospheric nitrogen (BURRIS and ROBERTS, 1993), and hence requiring minimal nitrogen input in the form of fertilizers, makes it a profitable crop among the farmers. In 2017, the United States was the largest producer of soybean with 119.5 million metric tons (35%) production followed by Brazil and Argentina

_____

Corresponding author: Mueen Alam Khan, Department of Plant Breeding and Genetics, University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur, Bahawalpur, Pakistan, E-mail: mueen_1981@yahoo.com

producing about 113 (33%) and 47 million metric tons (14%) respectively (SOYSTATS, 2018: http://www.soystats.com).

Soybean growth and productivity is severely affected by the myriads of abiotic stresses, among which the drought is the major one responsible for significant yield reductions. Almost every growth stage of soybean is sensitive to drought (MANAVALAN *et al.,* 2009). Conventional breeding methodologies for improving drought tolerance in crop plants are primarily based on screening germplasm and an empirical selection for yield under different drought stress conditions over several years (MANAVALAN *et al.,* 2009; TRAN and NGUYEN, 2009; HAMWIEH *et al.,* 2013). To deal with this, traditionally, breeders perform tedious crossings (hybridizations) and selections of superior progenies. The selected ones thus moved to field evaluations before given to farmers for general cultivation. Because of the variability in drought patterns from year to year, and to overcome the low response to direct selection, substantial efforts have targeted the manipulation of morpho-physiological traits influencing drought resistance through escape, avoidance, and/or tolerance mechanisms (LUDLOW and MUCHOW, 1990; BLUM, 1996; HAMWIEH *et al.,* 2013)

Over the past decades, rapid progress in DNA markers and their linkage maps has facilitated the identification, localization, and dissection of loci conferring quantitative traits (referred to as QTL) (PATERSON, 1995; HYTEN et al., 2010). The use of marker assisted selection (MAS) and transgenic techniques have far reaching applications in bringing rapid genetic improvements beyond the limits of conventional breeding. Thus molecular breeding is simply the integration of these modern tools in to conventional system of crop genetic improvement leading to development and release of advanced and productive cultivars. Numerous drought stress-related QTL/genes have been identified and a number of them have also been transferred to commercially grown cultivated varieties. The purpose of the present review is therefore to describe the key developments in the field of molecular breeding including mapping and genomics highlighting the need of integrated approaches for drought stress tolerance in soybean along with the future directions.

ROLE OF QUANTITATIVE TRAIT LOCI (QTL) MAPPING AND MARKER ASSISTED SELECTION (MAS)

It is indeed ironic that most of the economically important breeding traits are complex. A complex trait is one that is controlled by a number of QTL/genes (in fact a QTL can carry multiple genes in itself). Drought tolerance is one such complex quantitative trait controlled by the minor (polygenes/QTL) with small but cumulative effect. Thus the identification of QTL is considered to be a great achievement in breeding. The stable and consistent expression of QTL across different environmental conditions is the most critical factor from breeding stand point. The application of DNA marker technology and QTL mapping has greatly facilitated our understanding of genetic basis underlying drought tolerance phenomenon (CUI *et al.,* 2008). The basic principle working behind is the tight linkage of DNA marker with the trait of interest so that even at the time of crossing over the two co-segregate together. A number of QTL associated with drought tolerance have been identified (Table 1). Mainly recombinant inbred lines (RILs), near-isogenic lines (NILs), backcross lines (BC), F2/F4 populations and doubled haploid lines (DH) are used as mapping populations to effectively map QTL.

DU *et al.,* (2009) detected a total of 40 QTL related to yield and drought tolerance using RILs population developed from a cross between Kefeng1 (drought tolerant) and Nannong1138-

2 (drought sensitive). Leaf pubescence density (PD) is considered to be an important component for the adaptation of soybean to drought-prone environment, DU *et al.,* (2009) did QTL mapping using RIL population derived from cross between cultivars Kefeng 1 and Nannong1138-2. Simple sequence repeats (SSR) markers were utilized for that purpose. They detected a total of 20 QTLs on molecular linkage groups (MLGs) A2, D1b, E, H, G and I. The QTLs for PD on MLG H were mapped to near *Ps* locus while the QTLs on MLG D1b were located near *Rsc-7*. Three genome regions for PD and water status traits on MLGs A2, D1b and H were found to be associated indicating the important role of leaf surface PD trait in the soybean drought tolerance. WANG *et al.,* (2012) using backcross inbred line (BIL) population to map QTL for drought tolerance at germination stage. Using SSR markers, they identified 17 QTLs with additive effects and/or additive × environment interaction effects, involved in drought tolerance of soybean in germination stage, were found on linkage group G2-A2, G10-D2, G11-E.

Table 1 Summary of QTLs identified for drought tolerance in soybean[a]

Trait(s)	Cross	Mapping population	Number of QTL detected	Type of DNA marker	Reference
Water use efficiency (WUE)	S-100 x Tokyo, 116	F2	2	SSR*	MIAN *et al.,* (1998)
Yield, maturity, and water use efficiency	Minsoy x Noir 1	RILs	3	SSR	SPECHT *et al.,* (2001)
Leaf wilting	Jackson x KS4895	RILs*	1	SSR	BHATNAGAR *et al.,* (2005)
Canopy wilting	KS4895 x Jackson	RILs	4	SSR	CHARLSON *et al.,* (2009)
Seed yield per plant (YP) and drought susceptibility index (DSI)	Kefeng 1 x Nannong1138-2	RILs	19(YP) 10(DSI)	SSR	DU *et al.,* (2009)
Water relative content (WRC)	Hongfeng 11 x Clark	BILs	8	SSR	CANDONG *et al.,* (2011)
Germination and seedling stages	Hongfeng11x Clark	BILs*	4	SSR	QIU *et al.,* (2011)
Canopy-wilting	PI 416937 x Benning	RILs	7	SSR	ABDEL-HALEEM *et al.,* (2012)
Shoot and root parameters	Essex x Forest	RILs	12	SSR	BRENSHA *et al.,* (2012)
Germination stage	Hongfeng 11 x Harosoy	BILs	18	SSR	ZHANG *et al.,* (2012)
Root architectural traits	Dunbar x PI 326582A	BILs	1	SNPs	MANAVALAN *et al.,* (2015)
Relative root and shoot lengths	M8206 x TongShan and ZengYang x M8206	NAM*	111	SNPLDB	KHAN *et al.,* (2018)

* RILs (Recombinant Inbred Lines), BILs, (Backcross Inbred Lines), SSR (Simple Sequence Repeats), SNP (Single nucleotide polymorphism), NAM (Nested association mapping).

ª Similar studies reported in text have not been mentioned in this Table.

YANG *et al.,* (2014) also used BIL population of soybean derived from the cross of drought tolerant wild soybean, "SNWS0048" as a recipient parent and drought-sensitive variety "Jinda73" as a donor parent was examined to identify the QTL and epistatic QTL and GE effects on drought-tolerant physiological traits. They detected six QTLs with additive effects and/or additive × environment interaction effects and three pairs of QTLs with additive × epistatic main and/or epistasis × environment interaction effects different water environment clearly indicating the affects of different water regimes on the expression of genes relevant to drought stress.

It can be inferred from the above discussion that a number of QTL/genes have been identified in soybean. However the procedure is not without some limitations too. For example,

i) The size of mapping population is critical. The larger the population size the better will be the mapping. However this is not always practical due to limited resources available.

ii) The phenotypic errors are sometimes large, as the accurate and precise phenotyping is the key to molecular mapping and can be affected by the non-availability of measurement techniques, and poor interpretation of field data etc.

iii) Moreover for the effective MAS deployment in to our breeding programs the precise stability of QTL across different genetic backgrounds is essential, however QTL x E and epistatic interactions unfortunately are the phenomena often misunderstood and due to this we are unable to achieve the anticipated benefits of MAS (XU and ZHU, 2012). Unstable QTLs give unreliable data when applied in MAS.

iv) A QTL will be of little importance if it fails to stabilize and improve the crop yield under stress (PRICE and COURTOIS, 1999).

ASSOCIATION MAPPING (AM) AND GENOME WIDE ASSOCIATION STUDIES (GWAS) IN SOYBEAN FOR DROUGHT TOLERANCE

The classical linkage mapping (QTL mapping) based on bi-parental crossing is effective in identifying large number of loci for drought tolerance. However identifying small effect QTL using the bi-parental population incurs a significant challenge for the breeders (HOLLAND, 2007). Moreover low heritabilities and high G x E interactions are some more significant demerits of bi-parental populations. In contrast to this the association mapping (AM) is another analytical approach for mapping QTL (NING *et al.,* 2016). AM (also called the linkage disequilibrium, LD mapping) refers to the significant association of marker with the phenotypic trait concerned (GUPTA *et al.,* 2005; SOTO-CERDA *et al.,* 2012). AM evaluates whether certain alleles are found within a population more frequently than expected (FLINT-GARCIA *et al.,* 2003). GWAS (based on LD) is a powerful approach to identify the genetic factors underlying the complex traits by telling the history of recombinant events occurred in any specific crop germplasm. GWAS typically use the high density single-nucleotide polymorphisms (SNPs) across the genome of a given species and determine the significant statistical marker trait associations (RAY *et al.,* 2015).

GWAS is fast becoming a standard tool for establishing marker trait associations. Regarding drought tolerance it has been performed in various crops like rice (WU *et al.,* 2015), wheat (EDAE *et al.,* 2014), maize (LIU *et al.,* 2013; XUE *et al.,* 2013; WANG *et al.,* 2016) and chick pea (THUDI *et al.,* 2014). Regarding soybean, DHANAPAL *et al.,* (2015) using GWAS identified 21 putative loci for carbon isotope ratio as a surrogate trait of water use efficiency (WUE). In

soybean, GWAS has also been performed for traits like plant height and maturity (SONAH *et al.,* 2015), yield and yield related components (HAO *et al.,* 2012; WEN *et al.,* 2015), protein and oil content (SONAH *et al.,* 2015; HWANG *et al.,* 2014), nitrogen related traits (DHANAPAL *et al.,* 2015; RAY *et al.,* 2015) and low-phosphorus stress (NING *et al.,* 2016).

The high rate of false positives due to spurious marker-trait associations is a main hurdle towards its wide range applicability. But because of the development of some powerful statistical tools, this issue has greatly been resolved (YU *et al.,* 2006). For instance, HE *et al.,* (2017) suggested an innovative restricted two-stage multi-locus GWAS procedure (RTM-GWAS) to resolve the encountered problems. In this method, tightly linked SNPs are grouped into SNP linkage disequilibrium blocks (SNPLDBs) to generate multiple haplotypes or alleles per block/locus to match the multiple-allele property of the natural population. Quite recently KHAN *et al.,* (2018) used this novel procedure and identified 73 and 38 QTLs with 174 and 88 alleles contributed main effect 40.43 and 26.11% to phenotypic variance (PV) and QTL–environment interaction (QEI) effect 24.64 and 10.35% to PV for relative root length and relative shoot length that served as drought tolerance indicators, respectively. However the best approach would therefore be to combine the two methods of QTL mapping (traditional linkage mapping and AM) to minimize each other's limitations with maximum accuracy and utilization of resources (MAHUKU *et al.* 2016). Keeping in view the wider applications of GWAS, it is expected that the use of this technique will substantially increase in the years to come.

TRANSGENIC APPROACHES

Transgenic technology has enabled the breeders to overcome the problem of direct gene transfers between entirely unrelated organisms. This approach carries two basic objectives; first to know the mechanisms involved in plant's innate response to stress factor and second to identify a transgene with a greatest possible impact on the stress itself and quickly being transferred from laboratory to the breeding programs designed to improve the cultivars (REYNOLDS *et al.,* 2005). As the regulatory networks underlying the abiotic stress factors are fully explored, more and more so-called candidate genes can be identified to be used to develop transgenic plants (BARNABÁS *et al.,* 2008; FLEURY *et al.,* 2010). The subtle examples depicting the power of transgenes in soybean for drought tolerance is shown in Table 2. Naturally plant are bestowed upon with stress sensor signals able to perceive the external stress and thereby activating the transcription factors leading to the activation of cascade of genes making the plant to withstand that particular stress factor. Transcription factors (TF) is an important class of genes that up or down-regulate other genes especially at the time of stress. Most important and well-studied class of transcription factors is drought responsive element binding (DREB) factors have been identified in regulating gene expression under drought, salt and cold stresses in *Arabidopsis* (YAMAGUCHI-SHINOZAKI and SHINOZAKI, 1994). GAO *et al.,* (2005) observed improvement in wheat against drought and salt stresses by expression of stress inducible TF *GmDREB* from soybean. Similarly CHEN *et al.* (2007) isolated a novel DREB homologous gene, *GmDREB2* from soybean and further found that the over-expression of *GmDREB2* activated expression in transgenic *Arabidopsis* and tobacco, resulted in enhanced tolerance to drought and high-salt stresses.

GUTTIKONDA *et al.* (2014) introduced the *Arabidopsis thaliana DREB1D* TF driven by the constitutive and ABA-inducible promoters into soybean through *Agrobacterium tumefaciens-*mediated gene transfer. Transgenic plants showed differential drought tolerance responses with a

significantly higher survival rate compared to non-transgenic plants when subjected to comparable severe water-deficit conditions. A novel basic leucine zipper (*bZIP)* TF gene, *GmbZIP1* was isolated from soybean cultivar Tiefeng 8 by GAO *et al.,* (2011). They found the expression of GmbZIP1 was highly induced by ABA, drought, high salt and low temperature in soybean roots, stems and leaves under different stress conditions. They also observed a greater tolerance to drought, salt and cold stresses due to *GmbZIP1*-overexpressing in transgenic A*rabidopsis*, tobacco and wheat plants. Similarly DE PAIVA ROLLA *et al.* (2014) transformed soybean with *AtDREB1A* for tolerance to drought. They also observed significant augmentation in yield components when the drought stress was introduced. Likewise, LI *et al.,* (2017) found over-expression of *GmFDL19*, also a bZIP TF in transgenic soybean leading to increased tolerance to salt and drought stresses.

Great progress has been made in developing transgenic lines over-expressing the transgenes. However mere over-expression has proven to be a futile attempt for durable and sustained tolerance. As a matter of fact, instead of using single gene, use of multiple genes is suggested for bigger and better impact (BAJAJ *et al.,* 1999) but is indeed a daunting undertaking. Nonetheless these transgenic plants can greatly help us in understanding and elucidating the responses of plant towards stress. But since the degree of drought significantly varies in field, the trangene expression may differ than what is observed in laboratory or green house conditions. Until recently FUGANTI-PAGLIARINI *et al.,* (2017) transformed soybean with DREB and ABA-responsive element binding (AREB) TFs and evaluated their performance in field conditions and quite encouraging results for drought tolerance were obtained. This further necessitates continuous and replicated field based evaluations over a range of environments and across different genetic backgrounds to clearly observe the effectiveness of this technology (NAKASHIMA *et al.,* 2014).

Table.2. List of transgenic lines developed in soybean for drought tolerance[a]

Gene	Encoded product	Donor organism	Transformation method	Trait(s) observed	Reference
P5CR	L-Δ^1-pyrroline-5-carboxylate reductase	*Arabidopsis*	*Agrobacterium* vacuum infiltration transformation procedure	The transgenic soybean plant were subjected to drought and heat stress. The sense plant showed mild symptoms of stress while the antisense were severely stressed	DE RONDE *et al.,* (2004)
GmMYB76, GmMYB92, and *GmMYB177*	MYB DNA-binding domain	Soybean	*Agrobacterium*-mediated gene transformation	The three *GmMYB* genes played a differential role to stress tolerance in transgenic *Arabidopsis* plants	LIAO *et al.,* (2008)
GmUBC2	Ubiquitin-conjugating enzyme	Soybean	*Agrobacterium*-mediated gene transformation	Transgenic *Arabidopsis* plants over-expressing the gene showed enhanced tolerance to drought and	ZHOU *et al.,* (2010)

salt

Gene	Protein/Domain	Source	Transformation method	Function	Reference
GmNAC11 and *GmNAC20*	Proteins localized to the nucleus and bound to the core DNA sequence CGT[G/A].	Soybean	*Agrobacterium rhizogenes-* mediated transformation	Over-expression of transcription factors promote abiotic stress tolerance and lateral root formation in transgenic tobacco and soybean plants	HAO *et al.,* (2011)
AtMYB44	*MYB* DNA-binding domain	*Arabidopsis*	*Agrobacterium tumefaciens*	Expression of gene conferred drought/salt stress tolerance in transgenic soybean	SEO *et al.,* (2012)
LOS5/ABA3	Molybdenum cofactor sulphurase	*Arabidopsis*	*Agrobacterium-* mediated gene transformation	Expression of LOS5/ABA3 was up-regulated by drought stress, which led to notable increase in ABA accumulation. Furthermore transgenic soybean under drought stress had reduced water loss by decreased stomatal aperture size and transpiration rate	LI *et al.,* (2013)
AtAREB1	Leucine zipper (bZIP) protein that regulates abscisic acid (ABA)-dependent stress-responsive gene expression	*Arabidopsis*	Biolistic transformations	Transgenic soybean plants with improved water stress tolerance were observed.	BARBOSA *et al.,* (2013)
GmPOI	Pollen_Ole_e_I conserved Domain	Soybean	*Agrobacterium-* mediated gene transformation	The soybean plants over-expressing *GmPOI* showed higher tolerance to drought stress than wild types	SONG *et al.,* (2013)
GmEXP1	Expansin proteins	Soybean	*Agrobacterium-* mediated gene transformation	Transgenic tobacco plants over-expressing the gene showed enhanced	LO *et al.,* (2015)

				tolerance to drought.	
AtAREB1	Leucine zipper (bZIP) protein that regulates abscisic acid (ABA)-dependent stress-responsive gene expression	*Arabidopsis*	Biolistic and *Agrobacterium*-mediated gene transformations	Results showed that the constitutive over-expression of TF *AtAREB1* lead to an improved capacity of the soybean crop to cope with drought with no yield losses.	MARINHO *et al.,* (2015)
BADH	Betaine aldehyde dehydrogenase catalyses the oxidation of betaine aldehyde to glycine betaine.	Atriplex canescens	*Agrobacterium*-mediated gene transformations	Transgenic soybeans expressing BADH showed increased drought tolerance	QIN *et al.,* (2017)

ᵃ Similar studies reported in text have not been mentioned in this Table.

POTENTIAL OF FUNCTIONAL GENOMICS

Plant functional genomics is a rapidly evolving discipline concerned with knowing the function of genes. Since the drought tolerance is complex, so it is almost imperative to have in depth knowledge of hundreds and thousands of up or down regulated genes or proteins involved in the process. Expressed sequence tags (ESTs) are random sequences of gene transcripts, considered to be novel genomic tool for gene identification. The generation of ESTs from cDNA libraries and complete genome sequence information in *Arabidopsis,* rice and soybean provide valuable information about gene discovery (SREENIVASULU *et al.,* 2007; SCHMUTZ *et al.,* 2010). These ESTs are crucial for accurate genome annotation; provide valuable information about gene structure, expression patterns and transcript abundance (SEKI *et al.,* 2002; UMEZAWA *et al.,* 2008). Full-length cDNAs (obtained by reverse transcribing the mRNA) are regarded as robust tools functionally analyzing the genes and thus serve as a precious source for the improvement of soybean drought stress tolerance. UMEZAWA *et al.,* (2008) obtained 6,570 new full-length sequences of soybean cDNAs derived from tissues treated with different abiotic stresses. With the advancement of DNA microarray technology, several hundred stress induced genes have been identified in plants and has indeed revolutionized the global gene expression profiling system (UMEZAWA *et al.,* 2006; VIJ and TYAGI, 2007). Another method being used to elucidate the gene function is antisense- and RNA interference (RNAi)-mediated transcriptional gene silencing (as reviewed by MANAVALAN *et al.,* 2009) and their involvement in drought tolerance in soybean was demonstrated by KULCHESKI *et al.,* (2011). A relatively cost effective high-throughput gene sequencing technique known as RNA- sequencing (RNA-seq) is in use has several advances over microarray technology considering the fact that RNA-seq does not require gene information and is capable of identifying novel transcripts that were previously unknown and also providing opportunities to analyze non-coding RNAs (DESHMUKH *et al.,* 2014). CHEN *et al.,* (2016) used Illumina RNA-seq transcriptome profiling to study differentially expressed

genes (DEGs) under drought and flooding conditions. A total of 2724 and 3498 DEGs were identified comprising of 289 TFs.

Similarly other functional genomics tools like serial analysis of gene expression (SAGE), array-based transcript profiling technologies and quantitative real time PCR (qRT-PCR) are in use to access the function of genes expressed in drought stress (SREENIVASULU *et al.,* 2007). Although we have not been able to realize the full potential of these tools, yet the availability of these functional genomics approaches coupled with the transgenic breeding opens a new exciting venture for soybean breeding leading us to a better understanding of the expression patterns of genes not only concerned with drought but also for other stresses too (TRAN and MOCHIDA, 2010).

THE WAY AHEAD

Due to climatic changes, there will be increased occurrences of drought spells during the crop growth periods, and global food production will continue to be challenged. In that respect the demand for food is expected to rise with increasing world population; and therefore improving crop productivity to ensure yield on sustainable basis is imperative. Legumes are rich in proteins and thus have a unique role in alleviating the hunger and poverty from the world and thus leading to a sustainable agriculture. Soybean being one of the most important legumes is globally recognized as an important oil and food crop while drought being the most significant environmental stress hampering its production. Drought tolerance in a complex trait thus poses a significant challenge for the breeding programs destined to develop the soybean cultivars tolerant to drought. Conventional plant breeding has been and will likely to a potential key player keeping in view the climatic scenarios and addressing the food security issues around the globe. Dawn of molecular age has laid down the solid foundation for molecular breeding that has now emerged as a promising tool to overcome the barriers imposed by the conventional breeding. Identification of drought related putative QTLs coupled with MAS hold a tremendous potential to breed for drought tolerance in soybean. In addition to QTL mapping derived from biparental population, association mapping (LD) including GWAS further accelerates the dissection of complex traits by careful screening of large number of soybean accessions carrying the genetic variability. Genetic engineering is also an effective approach to deal with the current scenario. A number of candidate genes for drought tolerance have been incorporated from different crops to soybean. However more extensive work is still needed to judge the stability of these transgenic lines in field conditions. The completion of soybean genome sequence and beginning of functional genomics prove an invaluable source of elucidating and dissecting the gene regulatory networks allowing us to decipher the riddle of plant resistance towards drought. These approaches will certain to provide impetus to our breeding programs mostly thriving on conventional means, to combat the global issues related to climatic changes. We conclude by saying that for the better and comprehensive understanding of soybean crop response towards drought, an interactive and holistic approach integrating the breeding, genomics and molecular biology disciplines, is required to a much greater extent than currently being implemented for envisioning the long standing goal of getting good quality, high yielding drought tolerant soybean.

REFERENCES

ABDEL-HALEEM, H., JR. CARTER, T.E. PURCELL, LC. KING, CA. RIES, LL., P. CHEN *et al.* (2012): Mapping of quantitative trait loci for canopy-wilting trait in soybean (*Glycine max* L. Merr). TAG, *125*(5): 837-846.

BAJAJ, S., J. TARGOLLI, LF. LIU, THD. HO, R.WU (1999): Transgenic approaches to increase dehydration-stress tolerance in plants. Mol. Breeding, *5*(6): 493-503.

BARBOSA, E.G.G., J.P. LEITE, S.R.R. MARIN, J.P. MARINHO, J.D.F.C., CARVALHO, R., FUGANTI-PAGLIARINI *et al.* (2013): Overexpression of the ABA-dependent AREB1 transcription factor from Arabidopsis thaliana improves soybean tolerance to water deficit. Plant Mol. Biol. Report., *31*(3): 719-730.

BARNABÁS B., K., JÄGER, A., FEHÉR (2008): The effect of drought and heat stress on reproductive processes in cereals. Plant Cell Environ., *31*: 11–38

BHATNAGAR, S., C.A., KING, L. PURCELL, J.D. RAY (2005): Identification and mapping of quantitative trait loci associated with crop responses to water deficit stress in soybean [*Glycine max* (L.) Merr.]. The ASACSSA-SSSA International annual meeting poster abstract, November 6–10.

BLUM, A. (1996): Crop responses to drought and the interpretation of adaptation. Plant Growth Regul., *20*:135–148.

BRENSHA, W.B., S.K. KANTARTZI, K. MEKSEM, I.V. GRIER, L. ROBERT, A. BARAKAT *et al. (*2012): Genetic analysis of root and shoot traits in the 'Essex'by 'Forrest' recombinant inbred line (RIL) population of soybean [*Glycine max* (L.) Merr.]. J. Plant Genome Sci., *1*:1–9.

BURRIS, R.H., GP. ROBERTS (1993): Biological nitrogen fixation. Ann. Rev. Nutr., *13*: 317 – 335.

CANDEIA, R., M. SILVA, J. CARVALHOFILHO, M. BRASILINO, T. BICUDO, I. SANTOS *et al.* (2009): Influence of soybean biodiesel content on basic properties of biodiesel– diesel blends. Fuel, *88*: 738–743.

CANDONG, L.I., M. XINGFEN, J. HONGWEI, G. TAI, W. ZHIXIN, W. XIUHONG *et al.* (2011): QTL Identification of WRC to Soybean in Drought Tolerance Selection Population. Chinese Agric. Sci. Bulletin, *27*(9):152-155.

CHARLSON, D.V., S. BHATNAGAR, C. ANDY KING, JD. RAY, CH. SNELLER, TE. CARTER, LC. PURCELL (2009): Polygenic inheritance of canopy wilting in soybean [*Glycine max* (L.) Merr.]. TAG, *19*:587–594.

CHEN, M., Q.Y. WANG, X.G. CHENG, Z.S. XU, L.C. LI, XG. YE *et al.* (2007): GmDREB2, a soybean DRE-binding transcription factor, conferred drought and high-salt tolerance in transgenic plants. Bioch. Biophys. Res. Comm., *353*(2): 299-305.

CHEN, W., Q. YAO, GB. PATIL, G. AGARWAL, R.K. DESHMUKH, L. LIN, B. WANG *et al.* (2016): Identification and Comparative Analysis of Differential Gene Expression in Soybean Leaf Tissue under Drought and Flooding Stress Revealed by RNA-Seq. Front. Plant Sci., *7*:1044.

CUI, K., J. HUANG, Y. XING, S. YU, C. XU, S. PENG (2008): Mapping QTLs for seedling characteristics under different water supply conditions in rice (*Oryza sativa*). Physiol. Plant., *132*: 53–68.

DE PAIVA ROLLA, A.A., J.D.F.C. CARVALHO, R. FUGANTI-PAGLIARINI, C. ENGELS, A. DO RIO, S.R.R. MARIN *et al.* (2014): Phenotyping soybean plants transformed with rd29A: AtDREB1A for drought tolerance in the greenhouse and field. Transgenic Res., *23*(1): 75-87.

DE RONDE, J.A., W.A. CRESS, G.H.J. KRÜGER, R.J. STRASSER, J. VAN STADEN (2004): Photosynthetic response of transgenic soybean plants, containing an Arabidopsis P5CR gene, during heat and drought stress. J. Plant Physiol., *161*(11): 1211-1224.

DESHMUKH, R., H. SONAH, G. PATIL, W. CHEN, S. PRINCE, R. MUTAVA *et al.* (2014): Integrating omic approaches for abiotic stress tolerance in soybean. Front. Plant Sci., *5*:244. doi: 10.3389/fpls.2014.00244.

DHANAPAL, AP., J.D. RAY, SK. SINGH, V. HOYOS-VILLEGAS, JR. SMITH, LC. PURCELL *et al.* (2015): Genome-wide association study (GWAS) of carbon isotope ratio (δ13C) in diverse soybean [*Glycine max* (L.) Merr.] genotypes. TAG, *128*(1): 73-91.

DHANAPAL, A.P., J.D. RAY, S.K. SINGH, V. HOYOS-VILLEGAS, J.R. SMITH, L.C. PURCELL *et al.* (2015): Genome-wide association analysis of diverse soybean genotypes reveals novel markers for nitrogen traits. The Plant Genome, *8*(3).

DU, L., C. CAI, S. WU, F. ZHANG, S. HOU, W. GUO (2016): Evaluation and exploration of favorable QTL alleles for salt stress related traits in cotton cultivars (*Gossiupium hirsutum* L.). PLoS One, *11*:e0151076

DU, W.J., S.X. FU, D.Y. YU (2009): Genetic analysis for the leaf pubescence density and water status traits in soybean [*Glycine max* (L.) Merr.]. Plant Breed., *128*(3): 259-265.

DU, W., M. WANG, S. FU, D. YU (2009): Mapping QTLs for seed yield and drought susceptibility index in soybean (Glycine max L.) across different environments. J. Genet. Genomics, *36*(12): 721-731.

DU, W., D. YU, S. FU (2009): Detection of quantitative trait loci for yield and drought tolerance traits in soybean using a recombinant inbred line population. J. Int. Plant Biol., *51*(9): 868-878.

EDAE, E.A., PF. BYRNE, SD. HALEY, MS. LOPES, MP. REYNOLDS (2014): Genome-wide association mapping of yield and yield components of spring wheat under contrasting moisture regimes. TAG, *127*(4): 791-807.

FLEURY, D., J.S. STEPHEN, H. KUCHEL, P. LANGRIDGE (2010): Genetic and genomic tools to improve drought tolerance in wheat. J. Exp. Bot., *61* (12): 3199–3210.

FLINT-GARCIA, S.A., J.M. THORNSBERRY, E.S. BUCKLER (2003): Structure of linkage disequilibrium in plants. Ann. Rev. Plant Biol., *54*:357–374.

FUGANTI-PAGLIARINI, R., L.C. FERREIRA, FA. RODRIGUES, H.B.C. MOLINARI, S.R.R. MARIN, M.D.C. MOLINARI, J. MARCOLINO-GOMES *et al.* (2017): Characterization of Soybean Genetically Modified for Drought Tolerance in Field Conditions. Front. Plant Sci., *8*:448.

GAO, S., H. XU, X. CHENG, M. CHEN *et al.* (2005): Improvement of wheat drought and salt tolerance by expression of a stress inducible transcription factor GmDREB of soybean (*Glycine max*). Chin. Sci. Bull., *50* (23): 2714—2723.

GAO, S.Q., M. CHEN, Z.S. XU, C.P. ZHAO, L. LI, H.J. XU, et al. (2011). The soybean GmbZIP1 transcription factor enhances multiple abiotic stress tolerances in transgenic plants. Plant Mol. Biol., *75*(6): 537-553.

GUO, W.Z., T.Z. ZHANG, Y.Z. DING, Y.C. ZHU, X.L. SHEN, X.F. ZHU (2005): Molecular marker assisted selection and pyramiding of two QTLs for fiber strength in Upland cotton. Acta Genet. Sin., *32*: 1275–1285.

GUPTA, P.K., S. RUSTGI, P.L. KULWAL (2005): Linkage disequilibrium and association studies in higher plants: present status and future prospects. Plant Mol. Biol., *57*(4): 461-485.

GUTTIKONDA, S.K., B. VALLIYODAN, A.K. NEELAKANDAN, L.S.P. TRAN, R. KUMAR, T.N. QUACH, *et al*. (2014): Overexpression of AtDREB1D transcription factor improves drought tolerance in soybean. Mol. Biol. Rep., *41*(12): 7995-8008.

HAMWIEH, A., M. IMTIAZ, R.S. MALHOTRA (2013): Multi-environment QTL analyses for drought-related traits in a recombinant inbred population of chickpea (*Cicer arientinum* L.). TAG, *126*(4): 1025-1038.

HAO, Y.J., W. WEI, Q.X. SONG, H.W. CHEN, Y.Q. ZHANG, F. WANG *et al.* (2011): Soybean NAC transcription factors promote abiotic stress tolerance and lateral root formation in transgenic plants. Plant J., *68*(2): 302-313.

HAO, D., H. CHENG, Z. YIN, S. CUI, D. ZHANG, H. WANG, D. YU (2012): Identification of single nucleotide polymorphisms and haplotypes associated with yield and yield components in soybean (Glycine max) landraces across multiple environments. TAG, *124*(3): 447-458.

HE, J., S. MENG, T. ZHAO, G. XING, S. YANG, Y. LI, R. GUAN, J. LU, Y. WANG, Q. XIA, B. YANG (2017): An innovative procedure of genome-wide association analysis fits studies on germplasm population and plant breeding. TAG, *130*:2327-2343.

HILL, J., E. NELSON, D. TILMAN, S. POLASKY, D. TIFFANY (2006): Environmental, economic, and energetic costs and benefits of biodiesel and ethanol biofuels. Proc. Natl. Acad. Sci. USA, *103*:11206–11210.

HYTEN, D.L., IY. CHOI, Q.J. SONG, J.E. SPECHT, T.E. CARTER (2010): A high density integrated denetic linkage map of soybean and the development of a 1536 universal soy linkage panel for quantitative trait locus mapping. Crop Sci., *50*:960–968.

HWANG, E.Y., Q. SONG, G. JIA, JE. SPECHT, DL. HYTEN, J. COSTA, P.B. CREGAN (2014): A genome-wide association study of seed protein and oil content in soybean. BMC Genomics, *15*(1): 1.

KHAN, M.A., F. TONG, W. WANG *et al.* (2018): Analysis of QTL–allele system conferring drought tolerance at seedling stage in a nested association mapping population of soybean [G*lycine max*(L.)Merr.] using a novel GWAS procedure. Planta, *248*(4): 947-962.

KULCHESKI, F.R., L.F.V. DE OLIVEIRA, L.G. MOLINA, M.P. ALMERA˜O, F.A. RODRIGUES *et al.* (2011): Identification of novel soybean microRNAs involved in abiotic and biotic stresses. BMC Genomics., *12*:307.

LI, Y., Q. CHEN, H. NAN, X. LI, S. LU, X. ZHAO *et al.* (2017): Overexpression of *GmFDL19* enhances tolerance to drought and salt stresses in soybean. PLoS One, *12*(6): e0179554.

LI, Y., J. ZHANG, J. ZHANG, L. HAO, J. HUA, L. DUAN *et al.* (2013): Expression of an Arabidopsis molybdenum cofactor sulphurase gene in soybean enhances drought tolerance and increases yield under field conditions. Plant Biotech. J., *11*(6): 747-758.

LIAO, Y., H.F. ZOU, H.W. WANG, W.K. ZHANG, B. MA *et al.* (2008). Soybean GmMYB76, GmMYB92, and GmMYB177 genes confer stress tolerance in transgenic *Arabidopsis* plants. Cell Res., *18*(10): 1047-1060.

LIU, S., X. WANG, H. WANG, H. XIN, X. YANG, J. YAN *et al.* (2013). Genome-wide analysis of ZmDREB genes and their association with natural variation in drought tolerance at seedling stage of *Zea mays* L. PLoS Genet., *9*(9): e1003790.

LO, T.S., H.D. LE, V.T.T. NGUYEN, H. HA (2015): Overexpression of a soybean expansin gene, GmEXP1, improves 1 drought tolerance in transgenic tobacco 2. Turk J Bot., *39*(6): 988-995.

LUDLOW, M.M., RC. MUCHOW (1990): A critical evaluation of traits for improving crop yields in water-limited environments. Adv. Agron., *43*:107–153.

MAHUKU, G., J. CHEN, R. SHRESTHA, L.A. NARRO, K.V.O. GUERRERO *et al.* (2016): Combined linkage and association mapping identifies a major QTL (qRtsc8-1), conferring tar spot complex resistance in maize. TAG: 1-13.

MANAVALAN, L.P., SK. GUTTIKONDA, L.S.P. TRAN, H.T. NGUYEN (2009): Physiological and molecular approaches to improve drought resistance in soybean. Plant Cell Physiol., *50*(7), 1260-1276.

MANAVALAN, L.P., SJ., PRINCE, TA., MUSKET, J., CHAKY, R., DESHMUKH *et al.* (2015): Identification of novel QTL governing root architectural traits in an interspecific soybean population. PLoS One, *10*(3): e0120490.

MARINHO, J.P., N. KANAMORI, L.C. FERREIRA, R. FUGANTI-PAGLIARINI *et al.* (2015): Characterization of molecular and physiological responses under water deficit of genetically modified soybean plants overexpressing the AtAREB1 Transcription Factor. Plant Mol. Biol. Rep., 1-17.

MIAN, M.A.R., D.A. ASHLE, H.R. BOERMA (1998): An additional QTL for water use efficiency in soybean. Crop Sci., *38*: 390–393.

MOOSE, S.P. and R.H. MUMM (2008): Molecular plant breeding as the foundation for 21st century crop improvement. Plant Physiol., *147*(3): 969-977.

NAKASHIMA, K., K. YAMAGUCHI-SHINOZAKI, K. SHINOZAKI (2014): The transcriptional regulatory network in the drought response and its crosstalk in abiotic stress responses including drought, cold, and heat. Front. Plant Sci., *5*:170.

NING, L., G. KAN, W. DU, S. GUO, Q. WANG, *et al*. (2016): Association analysis for detecting significant single nucleotide polymorphisms for phosphorus-deficiency tolerance at the seedling stage in soybean [*Glycine max* (L) Merr.]. Breeding Sci., *66*(2): 191-203.

PATERSON, A.H. (1995): Molecular dissection of quantitative traits: progress and prospects. Genome Res., *5*:321–333.

PIMENTEL, D., T. PATZEK (2008): Ethanol production using corn, switchgrass and wood; biodiesel production using soybean. In Biofuels, Solar and Wind as Renewable Energy Systems. Edited by Pimentel, D. pp. 373 – 394. Springer, New York.

PELEMAN, J.D., J.R. VAN DER VOORT (2003): Breeding by design. Trends Plant Sci., *8*: 330–334.

PRICE, A., B. COURTOIS (1999): Mapping QTLs associated with drought resistance in rice: progress, problems and prospects. Plant Growth Regul., *29*(1-2): 123-133.

QIN, D., C.L. ZHAO, X.Y. LIU, P.W. WANG (2017): Transgenic soybeans expressing betaine aldehyde dehydrogenase from Atriplex canescens show increased drought tolerance. Plant Breed., *136*:699–709.

QIU, P.C., WB. ZHANG, C.D. LI, H.W. JIANG, C.Y. LIU *et al.* (2011): Genetic overlap of drought-tolerance loci between germination stage and seedling stage analyzed using introgression lines in soybean. Acta Agron. Sin., *37*: 477-483.

RAY, J.D., AP. DHANAPAL, SK. SINGH, V. HOYOS-VILLEGAS *et al.* (2015): Genome-Wide Association Study of Ureide Concentration in Diverse Maturity Group IV Soybean [*Glycine max* (L.) Merr.] Accessions. G3: Genes Genom. Genet., g3-115.

REYNOLDS, M.P., A. MUJEEB-KAZI, M. SAWKINS (2005): Prospects for utilizing plant-adaptive mechanisms to improve wheat and other crops in drought-and salinity-prone environments. Ann. Appl. Biol., *146*(2): 239-259.

SCHMUTZ, J., SB. CANNON, J. SCHLUETER *et al.* (2010): Genome sequence of the palaeopolyploid soybean. Nature, *463*:178–183.

SEKI, M., M. NARUSAKA, A. KAMIYA *et al.* (2002): Functional annotation of a full-length Arabidopsis cDNA collection. Science, *296:*141–145.

SEO, J.S., HB. SOHN, K. NOH, C. JUNG, JH. AN *et al.* (2012): Expression of the Arabidopsis AtMYB44 gene confers drought/salt-stress tolerance in transgenic soybean. Mol. Breeding, *29*(3): 601-608.

SONAH, H., L. O'DONOUGHUE, E. COBER, I. RAJCAN, F. BELZILE (2015): Identification of loci governing eight agronomic traits using a GBS-GWAS approach and validation by QTL mapping in soya bean. Plant Biotech. J., *13*(2): 211-221.

SONG, W.W., F.M. DUAN, W.B. LI, Q. LIN, H.X. ZHOU, X. HAN, J.A. WANG (2013): GmPOI gene encoding a Pollen_Ole_e_I conserved domain is involved in response of soybean to various stresses. Biol. Plantarum, *57*(1): 85-90.

SOTO-CERDA, B.J., S. CLOUTIER (2012): Association mapping in plant genomes. INTECH Open Access Publisher.

SOYSTATS (2018): SoyStats, a reference guide to important soybean facts & figures. American Soybean Association. http://soystats.com.

SPECHT, J.E., K. CHASE, M. MACRANDER, G.L. GRAEF, J. CHUNG, J.P. MARKWELL *et al.* (2001): Soybean response to water: a QTL analysis of drought tolerance. Crop Sci., *41*: 493 – 509.

SREENIVASULU, N., S.K. SOPORY, P.B. KAVI KISHOR (2007): Deciphering the regulatory mechanisms of abiotic stress tolerance in plants by genomic approaches. Gene, *388*:1–13.

THUDI, M., H.D. UPADHYAYA, A. RATHORE, P.M. GAUR *et al.* (2014): Genetic dissection of drought and heat tolerance in chickpea through genome-wide and candidate gene-based association mapping approaches. PLoS One, *9*(5): e96758.

TRAN, L.S, HT. NGUYEN (2009): Future Biotechnology of Legumes. In: Emerich WD, Krishnan H (eds) Nitrogen fixation in crop production. ASA-CSA-SSSA, Madison, pp. 265–308.

TRAN, L.S.P., K. MOCHIDA (2010): Functional genomics of soybean for improvement of productivity in adverse conditions. Funct. Integr. Genomics, *10*(4): 447-462.

UMEZAWA, T., M. FUJITA, Y. FUJITA, K. YAMAGUCHI-SHINOZAKI, K. SHINOZAKI (2006): Engineering drought tolerance in plants: discovering and tailoring genes to unlock the future. Curr. Opin. Biotech., *17*: 113 – 122 .

UMEZAWA, T., T. SAKURAI, Y. TOTOKI, A. TOYODA, M. SEKI *et al.* (2008): Sequencing and analysis of approximately 40,000 soybean cDNA clones from a full-length-enriched cDNA library. DNA Res., *16*:1 – 14.

VIJ, S., A.K. TYAGI (2007): Emerging trends in the functional genomics of the abiotic stress response in crop plants. Plant Biotech. J., *5*: 361–380.

WANG, M., W.M. YANG, W.J. DU (2012): Construction of a molecular marker linkage map and its use for quantitative trait locus (QTLs) underlying drought tolerance at germination stage in soybean. African J. Biotech., *11*(65): 12830-12838.

WANG, N., Z.P. WANG, X.L. LIANG, J.F. WENG, X.L. LV *et al.* (2016): Identification of loci contributing to maize drought tolerance in a genome-wide association study. Euphytica, *210*: 165-179.

WANG J, H. LI, X. ZHANG, C. YIN, Y. LI, Y. MA *et al.* (2011): Molecular design breeding in crops in China. Acta Agron. Sin., *37*: 191–201.

WANG, J., X. WAN, H. LI, W.H. PFEIFFER, J. CROUCH, J. WAN (2007): Application of identified QTL-marker associations in rice quality improvement through a design-breeding approach. TAG, *115*: 87–100.

WEN, Z., J.F. BOYSE, Q. SONG, P.B. CREGAN, D. WANG (2015): Genomic consequences of selection and genome-wide association mapping in soybean. BMC Genomics, *16*(1): 1.

WU, J., F. FENG, X. LIAN, X. TENG, H. WEI, *et al.* (2015): Genome-wide Association Study (GWAS) of mesocotyl elongation based on re-sequencing approach in rice. BMC Plant Biology, *15*(1): 218.

XU, H., J. ZHU (2012): Statistical approaches in QTL mapping and molecular breeding for complex traits. Chin. Sci. Bull., *57*(21): 2637-2644.

XUE, Y., M.L. WARBURTON, M. SAWKINS, X. ZHANG, T. SETTER, Y. XU *et al.* (2013): Genome-wide association analysis for nine agronomic traits in maize under well-watered and water-stressed conditions. TAG, *126*(10): 2587-2596.

YAMAGUCHI-SHINOZAKI, K., K. SHINOZAKI (1994): A novel cis-acting element in an Arabidopsis gene is involved in responsiveness to drought, low-temperature, or high-salt stress. Plant Cell, *6*(2): 251-264.

YANG, W., M. WANG, A. YUE, J. WU, S. LI, G. LI, W. DU (2014): QTLs and epistasis for drought- tolerant physiological index in soybean (*Glycine max* L.) across different environments. Caryologia, *67* (1):72-78.

YU, J., G. PRESSOIR, W.H., BRIGGS, I.V. BI, M. YAMASAKI *et al.* (2006): A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. Nat. Genet., *38(*2): 203-208.

ZHANG, W.B., P.C. QIU, H.W. JIANG, C.Y. LIU, C.D. LI, G.H. HU, Q.S. CHEN (2012): Dissection of genetic overlap of drought and low-temperature tolerance QTLs at the germination stage using backcross introgression lines in soybean. Mol. Biol. Rep., *39*(5): 6087-6094.

ZHOU, G.A., R.Z. CHANG, L.J. QIU (2010): Overexpression of soybean ubiquitin-conjugating enzyme gene GmUBC2 confers enhanced drought and salt tolerance through modulating abiotic stress-responsive gene expression in Arabidopsis. Plant Mol. Biol., *72*(4-5): 357-367.

DOSTIGNUĆA I PERSPEKTIVE MOLEKULARNOG OPLEMENJIVANJA ZA TOLERANTNOST PREMA SUŠI KOD SOJE (*Glycine max* (L.) MERR.)

Mueen Alam KHAN

Departman za oplemenjivanje biljaka i genetiku, of Plant Breeding and Genetics, Univerzitetski koledž za poljoprivredu i životnu sredinu, Islamia Univerzitet Bahawalpur, Bahawalpur, Pakistan

Izvod

Stres stresa je jedno od najozbiljnijih ograničenja proizvodnje soje širom sveta, dok je tolerancija za sušu jedno od najsloženijih svojstava za oplemenjivače soje. Zbog kompleksne (kvantitativne) prirode osobine tolerancije za sušu, napredak u razvoju biljaka koji su tolerantni za sušu je spor. Molekularno oplemenjivanje stoga se pojavilo kao neophodan pristup u programima oplemenjivanja soje. Dostupan je veliki broj genetskih informacija u vezi sa funkcionalnom genomikom i drugim molekularnim resursima. Efikasna i upečatljiva upotreba ovih resursa svakako će olakšati oplemenjivačima da razvijaju sorte soje tolerantne na sušu.